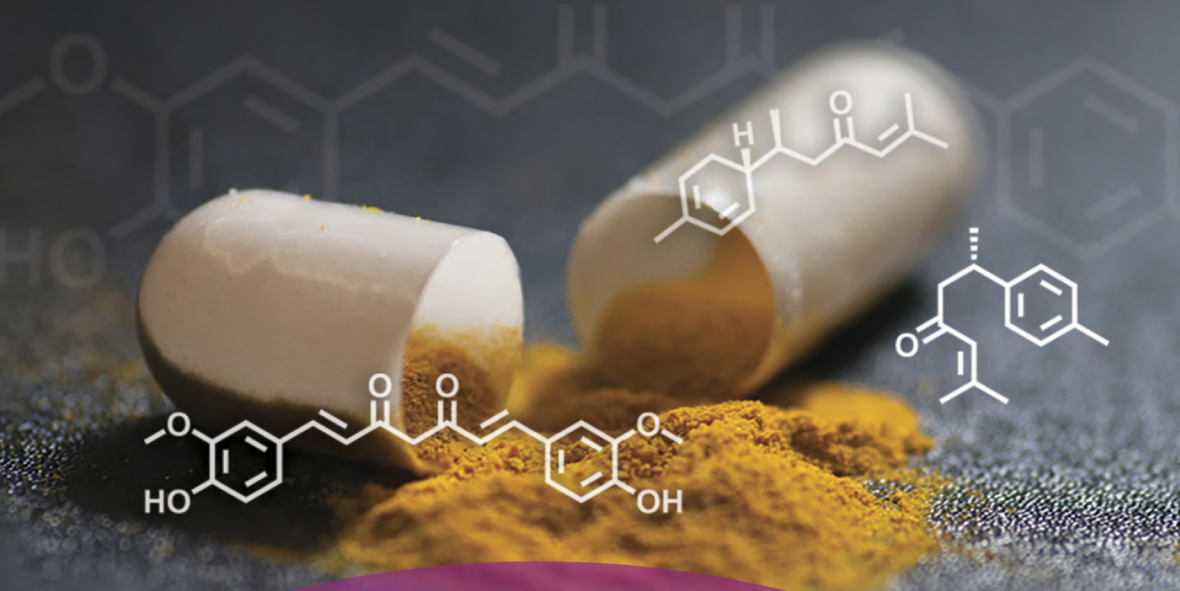


Food Chemistry, Function and Analysis

The Chemistry and Bioactive Components of Turmeric

Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

ROYAL SOCIETY OF CHEMISTRY

The Chemistry and Bioactive Components of Turmeric

Food Chemistry, Function and Analysis

Series editors:
Gary Williamson, *Monash University, Australia*
Alejandro G. Marangoni, *University of Guelph, Canada*
Graham A. Bonwick, *AgriFoodX Limited, UK*
Catherine S. Birch, *AgriFoodX Limited, UK*

Titles in the series:
1: Food Biosensors
2: Sensing Techniques for Food Safety and Quality Control
3: Edible Oil Structuring: Concepts, Methods and Applications
4: Food Irradiation Technologies: Concepts, Applications and Outcomes
5: Non-extractable Polyphenols and Carotenoids: Importance in Human Nutrition and Health
6: Cereal Grain-based Functional Foods: Carbohydrate and Phytochemical Components
7: Steviol Glycosides: Cultivation, Processing, Analysis and Applications in Food
8: Legumes: Nutritional Quality, Processing and Potential Health Benefits
9: Tomato Chemistry, Industrial Processing and Product Development
10: Food Contact Materials Analysis: Mass Spectrometry Techniques
11: Vitamin E: Chemistry and Nutritional Benefits
12: Anthocyanins from Natural Sources: Exploiting Targeted Delivery for Improved Health
13: Carotenoid Esters in Foods: Physical, Chemical and Biological Properties
14: Eggs as Functional Foods and Nutraceuticals for Human Health
15: Rapid Antibody-based Technologies in Food Analysis
16: DNA Techniques to Verify Food Authenticity: Applications in Food Fraud
17: Advanced Gas Chromatography in Food Analysis
18: Handbook of Food Structure Development
19: Mitigating Contamination from Food Processing
20: Biogenic Amines in Food: Analysis, Occurrence and Toxicity
21: Nutrition and Cancer Prevention: From Molecular Mechanisms to Dietary Recommendations
22: Health Claims and Food Labelling
23: Nutraceuticals and Human Health: The Food-to-supplement Paradigm
24: Nutritional Signalling Pathway Activities in Obesity and Diabetes
25: The Chemistry and Bioactive Components of Turmeric

How to obtain future titles on publication:
A standing order plan is available for this series. A standing order will bring delivery of each new volume immediately on publication.

For further information please contact:
Book Sales Department, Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge, CB4 0WF, UK
Telephone: +44 (0)1223 420066, Fax: +44 (0)1223 420247
Email: booksales@rsc.org
Visit our website at www.rsc.org/books

The Chemistry and Bioactive Components of Turmeric

Edited by

Sreeraj Gopi
Aurea Biolabs Private Limited, India
Email: sreerajgopi@yahoo.com

Sabu Thomas
Mahatma Gandhi University, India
Email: sabuchathukulam@yahoo.co.uk; *sabupolymer@yahoo.com*; *sabut@sancharnet.in*

Ajaikumar B. Kunnumakkara
Indian Institute of Technology Guwahati, India
Email: kunnumakkara@iitg.ernet.in

Bharat B. Aggarwal
Inflammation Research Center, USA
Email: bbaggarwal@gmail.com

and

Augustine Amalraj
Aurea Biolabs Private Limited, India
Email: augustin14amal@gmail.com; *amalraj.a@plantlipids.com*

ROYAL SOCIETY OF CHEMISTRY

Food Chemistry, Function and Analysis No. 25

Print ISBN: 978-1-78801-555-4
PDF ISBN: 978-1-78801-593-6
EPUB ISBN: 978-1-83916-059-2
Print ISSN: 2398-0656
Electronic ISSN: 2398-0664

A catalogue record for this book is available from the British Library

The Royal Society of Chemistry is a charity, registered in England and Wales, Number 207890, and a company incorporated in England by Royal Charter (Registered No. RC000524), registered office: Burlington House, Piccadilly, London W1J 0BA, UK, Telephone: +44 (0) 20 7437 8656.

Visit our website at www.rsc.org/books

Printed in the United Kingdom by CPI Group (UK) Ltd, Croydon, CR0 4YY, UK

Preface

"Haridre thu hithe khyathe thabhyam naasthi samam kwachith" (Shodhala Nighantu). This is a famous Sloka in the Shodhala Nighantu describing haridra, which is nothing but turmeric. Shodhala Nighantu was written by Shodhala in the 12th century AD. Nighantus can be considered as the Ayurvedic form of *materia medica* and many of the works were written from the very beginning of the Ayurvedic period. Shodhala Nighantu discusses the properties of 26 groups of medicinal plants and haridra was described as a unique herb with priceless medicinal properties. This clearly indicates that the potency of turmeric has been known since ancient times. Turmeric (*Curcuma longa* Linn), a herb which belongs to the Zingiberaceae family, is a spice of high economic importance due to its wide range of medicinal values and therapeutic potential. Turmeric powder has been used extensively since ancient times as a coloring, flavoring and preserving agent in curries and in cosmetics. Turmeric has traditionally been used for medical purposes for many centuries in different countries, particularly in India for improving digestion, improving intestinal flora, eliminating worms, relieving flatulence, cleansing and strengthening the liver and gallbladder, regulating menstruation, relieving arthritis and swelling and purifying the blood. Turmeric is one of the most popular medicinal herbs, with a wide range of pharmacological properties such as antioxidant, antiprotozoal, antivenom, antimicrobial, antimalarial, anti-inflammatory, antirheumatic, antiproliferative, antidiabetic, anti-Alzheimer's, anti-aging and anti-tumor agents. It has also been used to treat ulcers, parasitic infections, various skin diseases, anti-immune diseases and cure the symptoms of colds and flu. In recent years, several drugs derived from natural products have been developed

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara,
Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

and current drug research is actively investigating the possible therapeutic roles of many Ayurvedic and traditional medicinal remedies and turmeric is one among them. The biological activities of turmeric have been attributed mainly to curcuminoids consisting of curcumin and two related compounds, demethoxycurcumin and bisdemethoxycurcumin. The World Health Organization stated that the acceptable daily intake of curcuminoids as a food additive is in the range of 0–3 mg kg^{-1}. Curcuminoids and turmeric products have been characterized as safe by the Food and Drug Administration in the USA. Curcuminoids have achieved potential therapeutic interest to cure immune-related, metabolic diseases and cancer due to a vast number of biological targets and virtually no side effects, but very limited studies were reported for molecules other than curcuminoids and most of these molecules are equally potent as curcuminoids.

This book provides a comprehensive overview of turmeric, including an Ayurvedic and historical background, geographical variations, chemistry of turmeric, biological activities of turmeric, various drug delivery formulations for curcumin delivery, applications in nutraceutical, functional foods and value-added products, molecular docking and metabolic activities of curcumin, economics and marketing, toxicology aspects of turmeric, *etc.* This book also highlights the chemistry and biological activities of bioactive molecules other than curcuminoids in turmeric. Moreover, the great number of books already published on the subject do not combine details regarding the various compounds in turmeric. However, unlike them, this book takes a leap ahead, presenting a unique, comprehensive discussion relating to all of the therapeutically active compounds of this golden herb.

The book comprises 16 chapters, each written by experts in their respective field and each chapter having an exhaustive bibliography. The editors tried to collect and organize as much information as possible about turmeric in this book and would like to express their gratitude to the various authors of each chapter for their tremendous efforts. This book is directed at scientists, researchers and clinicians working in the areas of experimental and molecular medicine, Ayurveda and herbal medicine and general biomedical sciences. We hope that this book will be precious to all those who are involved in the production, processing, marketing and use of turmeric.

Sreeraj Gopi
Sabu Thomas
Ajaikumar B. Kunnumakkara
Bharat B. Aggarwal
Augustine Amalraj

Contents

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

CHAPTER 1

Turmeric – The Miraculous Herb from Ancient India and its Historical Background

K. S. AKHILA*[a] AND SREERAJ GOPI[b]

[a]Ayushmadam Ayurveda Hospital and Research Center, Annanad Post, Chalakudy–680 309, Kerala, India; [b]R&D Centre, Aurea Biolabs (P) Ltd, Kolenchery, Cochin–682 311, Kerala, India
*E-mail: drakhilaks@gmail.com

1.1 Introduction

The history of Ayurveda starts from 5000 BC onwards when the Vedas, the first written dogma in India, is considered to be written. So, 5000–3000 BC is considered as the Vedic period. Rigveda, Samaveda, Yajurveda and Atharvaveda are the four vedas and their upavedas are Ithihasam, Dhanurvedam, Gandharvavedam and Ayurvedam, respectively. Ayurveda is the upaveda of Atharvaveda.[1] Therefore, it can be assumed that Ayurvedic principles have been in practice since the Vedic period. Hence it is clear that the origin of Ayurveda is about 5000 years ago. In the Vedas, especially Rigveda and Atharvaveda, there are various references about basic anatomy, medicinal herbs, surgery, examination procedures of patients, treatment principles, *etc.* According to Atharva veda, oushadha (medicine) can be divided into four

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

types: atharvani, angirasi, daivi and manushi.[2] The first three categories are related to vitality or will power and the final one, manushi, was the main line of treatment in the Vedic period, in which internal medicines were used. Ayurveda, the science of life, deals with natural methods to treat and prevent diseases and ways to maintain health. For that, Ayurveda utilises the drugs of plant, animal, mineral and metal origin. But most of the Ayurvedic medicinal formulations consist of herbal drugs. The ancient Ayurvedic treatises very well describe the identification, synonyms, properties, actions and varieties of various herbs.

1.2 Turmeric (Haridra) – in the Vedic Period

Turmeric, termed *Curcuma longa* Linn., belongs to the Zingiberaceae family. It is cultivated all over India as a commercial crop, as it has been used as a food ingredient in various Indian cuisines. It is a tall herb, with large leaves. The useful part of turmeric is its rhizome. In Ayurveda, turmeric is called haridra. It has been mentioned in various Ayurveda treatises from the Vedic period onwards. In Agnipurana, it is mentioned that haridra was used for the treatment of diseases such as kamala,[3] prameha,[4] vranaropana[5] and arshas.[6]

1.3 Haridra in Nighantus

According to its morphological specialities and other physicochemical and pharmaceutical properties, many synonyms are given to haridra in various treatises. Nighantus are the main books that discuss those synonyms in detail. Haridra, rajani, nisa, nisakhya, varavarnini, suvarna, varnini, peetha, varangi, peethangi, hemaragini, varnathree, haritha, pinga, syama, kanchani, haldi, haldika, varnavathi, varnavilasini, rangini, vishaghna, siva, deergharaga, gouri, janishta, para, aneshatha, gandhapalashika, gharshani, jwaranthika, kaveri, mehaghni, besava, pindabhara, pinda, paribhra, varnada, mangalya, lambi, bhadra, sobhana, jayanthi, krimighna, yoshipriya, hattavilasini, bharalatha and romashamulika are the common synonyms of haridra that are mentioned in Nighantus. The Dhanwanthari Nighantu[7] (10th century AD) contains 18 synonyms of haridra. The number of synonyms mentioned in various other Nighantus are as follows: Shodalanighantu[8] (12 AD) – 10, Madanapala Nighantu[9] (14 AD) – 10, Kayyadeva Nighantu[10] (15 AD) – 20, Bhavaprakasa Nighantu[11] (16 AD) – 10, Raja Nighantu[12] (17 AD) – 30, Shaligrama Nighantu[13] (19 AD) – 10, Mahoushadhi Nighantu[14] (20 AD) – 14, Priya Nighantu[15] (20 AD) – 4, Nighantu Adarsh[16] (20 AD) – 4. The names haridra, varavarnini, suvarna, peetha, varangi, peethangi, lambi, *etc.* are given according to morphological features. But the names jwaranthika, mehaghni, krimighna, vishaghna indicate pharmaceutical properties.

1.4 Varieties of Haridra

Bhavaprakasa describes four varieties of haridra: haridra, amra haridra, vana haridra and daru haridra.[17] In theses varieties haridra is the one which is used very commonly in Ayurveda formulations, and is identified as *Curcuma longa* Linn., *i.e.* common turmeric. The rhizome of the plant *Curcuma amada* Roxb. is known as amra haridra, which belongs to zingeberaceae family. It is commonly called mango ginger and is cultivated throughout India; due to its rich flavour it is used in making chutneys. Research reveals its anticancer activities against some human cancer cell lines. Also, the rhizome extract shows antioxidant, anti-allergic, antibacterial, antifungal, analgesic and anti-inflammatory activities. *Curcuma aromatica* Salisb. (Zingiberaceae family) is considered as vana haridra. Next to common turmeric it is the most widely used turmeric species that has been mentioned in various Ayurveda medicines. In Ayurveda treatises it is described as a remedy for various skin diseases and respiratory disorders. It has been in traditional use as a medicinal cosmetic to enhance complexion. At the same time, it carries a very promising therapeutic value as it is very effective in sprains, bruises, skin diseases, *etc.* It has also been used as an antidote for snake bites. It is already proven that this particular drug has anti-inflammatory, antibacterial, antifungal, anti-allergic, antioxidant, anticancerous and wound healing properties. Daruharidra is a native shrub that is found commonly in the Himalayas, India. The plant *Berberis aristata* is considered as daruharidra, which belongs to the family berberidaceae. It is commonly called Indian barberry or tree turmeric. According to the ancient Ayurveda treatise bhavaprakasa, it is a very good remedy for various eye, ear and mouth diseases. It has been used for treating various skin disorders and haemorrhoids. Its fruits are a rich source of vitamin C, the roots are used to prepare some types of alcoholic drinks and the whole plant is a very good source of dye. In Ayurveda, it is used for the preparation of a special type of anjana (collyrium) known as rasanjana, which is useful in infective conjunctivitis.

1.5 *Rasa Panchaka* (Five Properties of Drug) of Haridra and its Therapeutic Effects

According to Ayurveda principles each dravya consists of five properties called rasapanchaka. They are: rasa (taste), guna – qualities, virya – potency, vipaka – taste conversion after digestion, prabhava – action that cannot be explained. They are intended to explain the pharmacological activity of the particular drug. Of these, the first four, *i.e.* rasa, guna, virya and vipaka, are perceptible. Haridra is tiktha katu (bitter and pungent) in taste, rooksha (dry) in quality, ushna (hot) in potency and katu (pungent) in post-digestive taste.[18] These properties clearly declare its action on tridoshas (vata, pitha and kapha) and haridra balances all three doshas. It has been

used as a home remedy for thousands of years and its effect on various physical ailments has been proven naturally by repeated usage. It is found to increase skin tone and complexion, heal wounds and scars, manage chronic wounds and skin lesions and is used as an antidote for poisoning. Many studies have been done on the therapeutic effects of turmeric that report anti-inflammatory, antiseptic, antimicrobial, immunomodulatory, antioxidant, hepatoprotective, antidiabetic, anti-asthmatic and anticancerous properties.

1.6 Haridra in Ayurveda Treatises – Charaka Samhitha, Ashtanga Hrudayam, Susrutha Samhitha and Sarngadhara Samhitha

Charaka Samhitha is noted as an excellent Ayurveda treatise on internal medicine. It is believed to have been written between 400 BCE and 200 BCE. It consists of eight sthanas (parts) and 120 chapters in total. The eight sthanas are suthra sthana, nidana sthana, chikithsa sthana, vimana sthana, sareera sthana, indriya sthana, kalpa sthana and sidhi sthana. Ashtanga Hrudaya, which is written by Vagbhata, is considered as the collected information of all eight branches of Ayurveda: kaya, bala, graha, urdhwanga, salya, damshtra, jara and vrisha, in simple language. It is considered to be written around the seventh century AD. It consists of six sthanas and 120 chapters. Suthra, nidana, chikithsa, sareera, kalpa and uthara are sthanas of Ashtanga Hrudaya. It contains all the salient features of Charaka Samhitha, Susrutha Samhitha and Ashtanga Samgraha. Susrutha Samhitha is a book of excellence in the field of surgery. The composition of this book may have begun by Susrutha in the last centuries BCE and it was completed by Drudabala (300–500 CE). It contains six sthanas and 186 chapters in total. The sthanas are: sutra, nidana, chikithsa, kalpa, sareera and uthara. Sarngadhara Samhitha is a well-documented text of pharmaceutical processes and useful medicinal formulations. It is believed to have been written by Sarngadhara around 14th century AD. It contains three sections and 32 chapters in total.

1.7 The Drug Groups of Haridra

All of the main Ayurveda treatises contain dravya ganas (drug groups) where the drugs of similar properties or actions or a particular indication are grouped together under a specific name. These are called ganas. Haridra is included in many such ganas in various treatises. Charaka included haridra in lekhaneeya gana[19] (drugs with an ability to reduce excess tissues are said to have lekhaneeya property and they are shown to have a great effect on weight reduction), kushtaghna gana[20] (beneficial for skin diseases), vishaghna gana[21] (potency to pacify toxic effects),

praja sthapana gana[22] (fertility drugs), vamanadravyas[23] (vamana is one of the panchakarmas in which the increased doshas are expelled out of the body through the mouth), Kashaya skanda dravyas[24] (drugs that have an astringent taste) and sirovirechana gana[25] (sirovirechana or nasya is a panchakarma in which medicine is applied through the nasal cavity to eliminate excess doshas). According to Vagbhata, haridra is a member of tiktha skanda dravyas[26] (drugs having a bitter taste), haridradi ganam[27] (indicated for amatisara, adyavata and sthanya dosha, and for alleviation of medas and kapha, which means it can be very useful in obesity and diabetes), musthadigana[28] (indicated for yoni – sthanya amayaghna, mala pachana, which means it is very useful in uterine vaginal disorders and for purifying breast milk. Also, it is a digestant) and theekshna dhooma dravyas[29] (quickly penetrating medicated fumes). Acharya Susrutha included haridra in haridradi ganam,[30] musthadi ganam,[31] lakshadi ganam[32] (useful for treating ulcers and cutaneous infections), valli panchamullam[33] (roots of five medicinal creepers), samshamana oushadhas[34] (drugs for pacification), kapha samsamana varga[35] (that alleviate kapha, which is one of tridoshas), thiktha varga[36] (drugs that have a bitter taste) and nirooha varga[37] (nirooha is one of the panchakarmas, in which medicine is applied through the anal root to expel the increased doshas).

1.8 The Main Named Formulations of Haridra

The main formulations, where turmeric is a major ingredient, in Charaka Samhitha are: thikthekshuku taila[38] (skin diseases), mahatikthakam ghrtham[39] (skin diseases), mahakhadiram ghritham[40] (skin diseases), kalyanaka ghrtham[41] (mental disorders, infertility), sidharthakadi agadam[42] (psychiatric conditions), maha panchagavyam ghritham[43] (epilepsy, psychiatric problems), patoladyam choornam[44] (ascites, jaundice), moolasavam[45] (indigestion), kadukadyam ghrtham[46] (bleeding disorders), haridradi ghrtham[47] (jaundice), punarnava mandooram[48] (anaemia, spleen disorders), goudarishtam[49] (anaemia), Manasiladi ghratham[50] (respiratory diseases), gudoochyadi ghrtham[51] (respiratory diseases), mrthasanjeevani agada[52] (poison), ganadha hasthi agada[53] (poison, skin diseases), maha gandhahasthi agada[54] (poison), ksharagada[55] (bladder stones, haemorrhoids), khadhira gudika[56] (tooth ache, gingivitis, stomatitis) and khuddaka tailam[57] (burning pain). In Ashtanga Hrudayam, vagbhata details the following formulations of haridra: vasishta rasayana[58] (cough, dyspnea), trikandakayam tailam[59] (diabetes), mahathikthaka ghrtham[60] (skin diseases), nisothamadi kashayam[61] (skin diseases), guggulu marichadi tailam[62] (skin diseases), vajraka ghrtha[63] (skin diseases), gulgulu thikthaka ghrtham[64] (arthritis, haemorrhoids, skin diseases), gruhadhumadi choornam[65] (arthritis), rajanyadi choornam[66] (digestive and respiratory disorders in children), laksha tailam[67] (for body massage to strengthen bones), sidharthakadi ghrtham[68] (mental disorders),

sidharthakadi agadam[69] (psychiatric illness), bhootharava ghrtham[70] (psychiatric problems), mahabhootharavaghrtham[71] (psychiatric problems), hareethakyadi nasyanjanam[72] (psychiatric problems), haridra dwayadi ghrtham[73] (psychiatric illness), kalyanaka ghrtham[74] (mental problems, emaciation), mahakalyanaka ghrtham[75] (mental problems, emaciation), mahath pachagavya ghrtham[76] (fever, epilepsy), hreeberadi nasyam[77] (eye diseases), khadiradi gulika[78] (tooth pain, gingivitis), arimedadi tailam[79] (tooth problems), jathyadi ghrutha[80] (non-healing wounds), madhukadi tailam[81] (fistula), bhadradi tailam[82] (skin disease, skin ulcer), samudradi varthi[83] (pilonidal sinus), manjishtadi trivrtham[84] (facial melanosis), kashmiradi ghrtham[85] (vaginal diseases, infertility), phalasarpis[86] (infertility, uterine disorders), chandrodaya gulika[87] (poison), panchavalkadi agadam[88] (snake poison), vajra gulika[89] (snake poison), vilwadi gulika[90] (poison, indigestion), rajanyadi pratisaranam[91] (scorpion poison), agara dhoomadi lepanam[92] (rat poison), dwinisadi ghrtham[93] (rat poison). Susrutha also describes some very effective formulations that contain haridra. They are aragwadhadhi varthi[94] (wounds), mahathikthakam grtham[95] (skin diseases), vajraka tailam[96] (skin diseases), mahavajraka tailam[97] (skin diseases), sanjeevani agadam[98] (poison), mahagadam[99] (poison), kalyanaka ghrutham[100] (mental problems, infertility), mahakalyanaka ghrutham[101] (fever, general tonic), panchagavya ghrutham[102] (pyrexia), triphaladi ghrutham[103] (fever, respiratory disorders), lakshadi tailam[104] (pyrexia), sidharadhaka ghrtham[105] (psychiatric problems), panchagavya ghrutham[106] (epilepsy), kalyana ghrutham[107] (psychiatric diseases, respiratory diseases) and phala ghrutham[108] (infertility). Sarngadhara Samhitha contains information on the following formulations of haridra: punarnavadi kwatha[109] (oedema), laghu manjishtadi Kashaya[110] (skin diseases), bruhatha manjistadi kashayam[111] (skin diseases, paralysis), pathyadishadangam[112] (headache, eye diseases), sudharshana choornam[113] (fever, pain), panchanibachoornam[114] (skin diseases), chandraprabha guggulu[115] (diabetes, renal stones), paniya kalyana ghrutham[116] (fever, epilepsy, infertility), mahathikthaka ghrutham[117] (skin diseases, arthritis), kasisadyam ghrutham[118] (skin diseases), jathyadi ghrutham[119] (non-healing ulcers, pilonidal sinus), thriphala grutham[120] (eye diseases), gouradyam ghrutham[121] (poison, skin diseases), phala ghrutham[122] (infertility), lagu phala ghrutham[123] (vaginal diseases), lakshadi tailam[124] (pyrexia, cough), ankarakatailam[125] (fever), arka tailam[126] (skin diseases), marichadi tailam[127] (skin diseases), thriphaladi tailam[128] (seborrheic dermatitis), jathyadi tailam[129] (burns, wounds), patadi tailam[130] (sinusitis), chandhanadi tailam[131] (fever, epilepsy), vacha tailam[132] (thyroid diseases), dhathoora tailam[133] (arthritis, paralysis), kumaryasavam[134] (diabetes, irritable bowel disease), devadharvarishtam[135] (diabetes, arthritis), mahavahni rasam[136] (ascites, abdominal distension), kushtadi prathisarana choornam[137] (bleeding gums), dhashanga lepam[138] (poison, skin diseases) and rasanjanavarthi[139] in nakhtandhyatha (night blindness).

1.9 Other Sources where Haridra is Mentioned in Ayurveda Treatises

Even if haridra choorna is indicated for prameha in bruhath trayis (Sharaka Samhitha, Susruta Samhitha and Ashtanga Hrudaya), Ashtanga Hrudaya says haridra is the agryoushadha of prameha.[140] Charaka suggests turmeric powder added with honey and amalaki swarasa,[141] but Vagbhata states that raw haridra along with dhatree rasa (gooseberry juice) will cure prameha.[142] And another important thing, haridra is used as a lepanoushadha for vranasudhi in the context of medo arbuda chikithsa[143] (cancer treatment). Haridra is indicated as a garbhasthapana oushadha[144] (for pregnant women) in Charaka Samhitha. Intake of haridra choorna along with gomutra (cow's urine) is suggested as a good medicine for chronic skin diseases.[145]

Some important choorna prayogas (powder formulations) of haridra available in ancient literature are: for various types of skin disease,[146,147] for external application in parswaruja[148] (pleurodynia), swelling,[149] oedema,[150] for udwarthana[151] (external rubbing) in kushta, mukhadhavana choornam in anorexia,[152] for kasa[153] (cough) for moothrarodhaja udavartha[154] (abdominal pain due to retention of urine), swasa roga[155] (respiratory diseases), pandu[156] (anaemia), rakthapitha[157] (bleeding disorders) and amathisara chikitsa[158] (diarrhoea).

The oil formulations where haridra is a main ingredient are: taila for abyanga after cutting the umbilical cord during delivery,[159] tailam for shirovirechanam[160] (a therapy in which medicine is applied through the nose), for sandhigathavatha[161] (arthritis), of tailam for pana, abhyanga and anuvasana[162] (application through mouth, anal orifice and skin), kaphaja vrudhi chikithsa[163] (hernia), dhava twagadi nasya tailam,[164] vranaropana tailam,[165] taila for pana, abhyanga, gandhoosha, nasya and vasthi,[166] taila for nadivrana[167] (pilonidal sinus), taila for ropana and bhagandara vinashana,[168] taila for bhagandara (fistula).[169]

Kashaya yogas (medicinal decoction) where haridra is included are: for kaphaja prameha,[170] for pithaja prameha,[171] kaphapithajanya kushta,[172] kashaya yoga for tridosha jwara,[173] athisara,[174] pakwathisara,[175] kashaya for pana and abhyanga,[176] sannipatha jwara,[177] amathisara chikitsa,[178] pithathisara chikitsa,[179] kapha jwara,[180] kapha jwara,[181] mooshika visha[182] (rat poison), kashaya for visha damsanm[183] (snake bite), kashaya yoga for prameha chikithsa,[184] kaphadhika vataraktha[185] (rheumatoid arthritis).

Kshara yogas (alkaline preparations), where haridra is an ingredient are: udara,[186] for jadaragni vardhana,[187] chikithsa of udara,[188] *etc.*

Lepana yogas (medicated paste) where haridra is an ingredient are: in kushta chikithsa,[189] in arshas haridra choorna along with sudha ksheera,[190] thwak vishudhikara lepam,[191] vathakapha vathasonitha,[192] dadru,[193] nakha danthadi visha,[194] moosikka visha chikitsa,[195] lutha visha,[196] vrschika visha,[197] savarnakara,[198] pithaja nalivrana,[199] twak visudhi,[200] vrana sodhana,[201] aroomshika,[202] lepana yoga in kukoonaka chikitsa[203] lepana for vrana sodhana,[204]

lepana for pithadhika vataraktha,[205] lepana during asmari chikitsa,[206] lepa for nadi vrana,[207] alepanoushadha for arshas,[208] lepa for kushta chikitsa,[209] lepa for swithra,[210] lepana for kaphaja upadamsa,[211] lepana for nyacha, vyanga chikitsa,[212] lepana in aroomshika chikitsa,[213] lepana after rakthamoksha in visha chikitsa,[214] siro lepam,[215] kushta,[216] kandu,[217] dadru,[218] shopha,[219] vrana shodhana,[220] vasikarana.[221]

Ghrutha yogas (medicated ghee) that contain haridra are: mrthdosha peeditha,[222] of mrthikakrthan sarvan vikaran,[223] mrthikothbhava roga,[224] vathajanya yoni rogam,[225] manovikaras,[226] pitha vidradhi chikithsa,[227] kapha vidradhi chikithsa,[228] karnavardhana ghrtham,[229] pandu,[230] swasakasa,[231] for dhara and pana,[232] for vranapurana,[233] ghrutha for abhyamga on face,[234] for snehapanam[235] and ghrutha for kushta.[236]

Kalka prayogas (herbal paste) of haridra are indicated in pithathisara,[237] in sarkarasmari,[238] kalka prayoga in urusthambha[239] and of kalka prayoga for dourgandha sthanya dosham.[240]

Nasya (nasal therapy), anjana (collyrium) applications that contain haridra are indicated in diseases such as: unmada and apasmara,[241] anjana in the treatment of visha, which affect kaphaja srothas,[242] gulika yoga for nasya and anjana,[243] anjana for grahabadha,[244] anjana for vishoochika,[245] anjana for nakthandhyatha,[246] anjana for divaseshu avasyatha,[247] anjana for timira,[248] nasya for pitha rakha janya peenasa[249] and anjana for abhishyanda.[250]

Haridra in rasayana chikithsa (rejuvenation therapy), is an ingredient in sodhanoushadha, which is indicated prior to rasayana seva,[251] a rasayana yoga of abhayamalakeeya rasayana poda containing haridra, and that yoga is indicated for longevity and health,[252] virechana yoga in rasayana vidhi,[253] jaladadi rasayanam[254] and suntyadi rasayanam[255] contain haridra as a main ingredient.

Haridra in visha chikithsa (treatment of poisons and toxins) it is used for gharshana in visha chikithsa,[256] haridra is good as an agada in the sixth vishavega,[257] kalka yoga anjana, nasya and lepana in visha sopha,[258] kalka yoga for sarpa visha,[259] agada yoga for visha,[260] gruha godha visha chikithsa,[261] agada for nakha, dantha kshatha visham,[262] chathushpada dashtanam agada,[263] amrtham ghrtam sarvavishahari,[264] Vrana shodhana kshalana kashayam and lepam,[265] peya yoga in visha chikitsa,[266] dhoomaprayoga in vrishchika visha[267] and prathisarana for ugra, madhya visha vruschika damsana.[268]

Haridra in vrana chikithsa (treatment of wounds and ulcers) is used in preparatory procedures julooka avacharana,[269] avagharshana choornam,[270] vranashodhana grtham,[271] vranashodhana tailam,[272] vranashopana grtham[273] and vranashopana tailam.[274] For bandhana in kaphaja vrana haridra pathra is used,[275] ropana taila for sadyovrana,[276] ropana taila for sadyovrana,[277] sodhana taila,[278] vrana sodhanoushadha[279] and vrana puranam in kapha vidradhi chikitsa.[280]

Haridra in nethra chikithsa (treatment of eyes) kashaya yoga for nethradhara,[281] varthi for kukoonaka chikitsa,[282] dhara in arma chedana vidhi,[283] manjishtadi dhara,[284] pundrayashtyadi for plutha,[285] dhara for Sudha sukkla

chikithsa,[286] Kashaya dhara for sushkakshipaka,[287] ghrtha for dhara and nasya in pithabhishyanda[288] and aschyothana in praklinna varthna.[289]

Other modes of application of haridra like vasthi (medicated enema), virechana (purgation), gulika (pills), leha (a semi-solid preparation from drugs) and dhooma (inhalation therapy): vasthiyogam for kapharogi,[290] in athisthoulya,[291] of virechanayoga in udara chikithsa,[292] gulika yoga for kushta,[293] a gulika yoga for swithra and kushta,[294] leha, choorna yoga for pithathisara,[295] gulika for gulma,[296] leha for swasa,[297] varthi for dhoomapana,[298] lehyam for aruchi,[299] kalka dhooma prayoga for kandu yoni,[300] vasthi yoga for kapha, pandu,[301] *etc.*

1.10 Conclusion

Nowadays, innumerable research efforts are taking place on the anticancerous, anti-inflammatory, antimicrobial and many other properties of turmeric and it is considered as one of the most precious drugs. However, turmeric (haridra) has been a part of Indian food culture for centuries and it is mentioned as a herb with many medicinal values even in the Vedas, the first written dogma in India. It was used as a home remedy, both for internal external applications. All Ayurveda medicinal treatises mention haridra as a single medicine or as a part of formulations. So, it is clear that the medicinal properties of this precious drug were first recognised by the great science of Ayurveda.

References

1. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 1/6], p. 2.
2. *Atharvaveda bhasha-bhashya*, ed. Dayananda Saraswathi, 1st edn, Dayananda Sansthan, New Delhi, 2007, p. 241.
3. V. Pandey, *Agnipuran, Part-2*, 300.34, Shree Satguru Publications, Delhi, 1st edn, 1997, p. 310.
4. V. Pandey, *Agnipuran, Part-2*, 283.15, Shree Satguru Publications, Delhi, 1st edn, 1997, p. 246.
5. V. Pandey, *Agnipuran, Part-2*, 283.25, Shree Satguru Publications, Delhi, 1st edn, 1997, p. 281.
6. V. Pandey, *Agnipuran, Part-2*, 283.14, Shree Satguru Publications, Delhi, 1st edn, 1997, p. 228.
7. *Dhanwantari Nighantu, Guduchyadi Varga*, edited by B. K. Diwedi, Chaukhambha Krishnadas Academy, Varanasi, 2008, pp. 45–46.
8. Sodhala, *Sodhala Nighantu*, edited by P. V. Sharma, Guduchyadi Varga, Oriental Institute, Baroda, 1st edn, 1978, p. 12.
9. Madanapala, *Madanpal Nighantu, Abhayadi Varga*, edited by J. L. N. Sastry, Choukhamba Orientalia, 2010, p. 187.

10. Kaiyadeva, *Kaiyadeva Nighantu*, edited by P. V. Sharma and G. Sharma, Oushadhivarga, Chaukambha Orientatia, Varanasi, 2006, p. 131.
11. Sri Bhavamisra, *Bhavaprakasa Nighantu*, Commentary by padmshri Prof. K. C. Chunekar, edited by late G. S. Pandey, Haritakyadi Varga, Chaukhambha Bharati Academy, Varanasi, reprint 2015, Shlok No-196-197, pp. 111–112.
12. I. Tripathi, *Raj Nighantu*, Acharya Vishwanath Durvedi, Pippalyadi Varga, Krishna Das Academy, Varanasi, Shlok No-196-198, 2010, p. 174.
13. S. Vaishya, *Shaligrama Nighantu*, Haritakyadi Varga, Khemraj Shrikrishnadas Prakashan, Mumbai, 1st edn, 2002.
14. *Mahausadha Nighantu*, Mahaushadi Varga, edited by P. T. Aryadasa Kumar Singha and I. Tripathi, Chaukhambha Bharati Academy, Varanasi, reprint 2006, Shlok No. 117-118, p. 55.
15. P. V. Sharma, *Priya Nighantu*, Shatapuspadi Varga, Chaukambha Surabharati Prakashana, Varanasi, 2004, Shlok No-170-171, p. 107.
16. *Adarsh Nighantu*, Adrakadi Varga, edited by B. G. Vaidya, Chaukhambha Bharati Academy, Varanasi, reprint 2016, vol. 11, p. 556.
17. P. L. Hedge and A. Harini, *A Text Book of Dravyaguna Vijnana*, Chaukhambha Publications, 1st edn, 2014, vol. 2, p. 335.
18. K. C. Chunekar, *Bhavaprakasha Nighantu, Hareethakyadi Varga*, Chaukhambha Bharathi Academy, Varanasi, 2013, p. 111.
19. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.su 4/9], p. 32.
20. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.su 4/11], p. 33.
21. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.su 4/11], p. 33.
22. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.su 4/18], p. 34.
23. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.vi8/135], p. 283.
24. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.vi8/144], p. 285.
25. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.vi8/151], p. 286.
26. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.su.10/28,29], p. 177.

27. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.su15/35,36], p. 238.
28. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.su15/40], p. 239.
29. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.su21/17,18], p. 297.
30. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 38/27, 28], p. 166.
31. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 38/54, 55], p. 168.
32. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 38/64-65], p. 169.
33. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su38/72], p. 169.
34. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 39/7], p. 171.
35. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 39/9], p. 172.
36. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 42/11], p. 186.
37. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 38/24-28], p. 541.
38. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 7/108-110], p. 455.
39. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi7/144-150], p. 457.
40. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 7/152-156], p. 457.
41. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi9/35-41], p. 471.

42. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi9/69-72], p. 473.
43. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi10/18-24], p. 475.
44. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi13/119-123], p. 497.
45. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi15/156-159], p. 522.
46. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi16/47-49], p. 529.
47. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 16/53], p. 529.
48. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi.16/93-96], p. 530.
49. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi. 16/105], p. 531.
50. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi.17/145, 146], p. 539.
51. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi18/161, 162], p. 546.
52. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi23/54-60], p. 574.
53. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi23/71], p. 575.
54. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi23/79], p. 575.
55. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi23/101], p. 576.
56. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi26/206-214], p. 609.

57. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi29/114], p. 632.
58. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.chi3/133-140].
59. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi12/17,18], p. 679.
60. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.chi19/8-10], p. 711.
61. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.chi19/38,39], p. 714.
62. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [19/71, 72], p. 716.
63. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi19/79-80], p. 717.
64. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.21/58-61], p. 726.
65. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.chi. 22/36, 37], p. 731.
66. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi 2/38-40], p. 783.
67. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u2/54-56], p. 784.
68. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u.5/10-12], p. 794.
69. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u5/13,14], p. 794.
70. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u5/19], pp. 794–795.
71. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 5/20], p. 795.

72. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u.5/36], p. 796.
73. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u.5/45, 46], p. 796.
74. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u.6/26-31], p. 799.
75. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u.6/32-33], p. 799.
76. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u.7/19-23], p. 803.
77. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u13/69], p. 823.
78. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u22/90-94], pp. 856–857.
79. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u22/95], p. 857.
80. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 25/67], p. 869.
81. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 28/35, 36], p. 880.
82. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 30/22-24], p. 885.
83. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 30/39-40], p. 887.
84. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 32/32], p. 892.
85. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 34/28-29], p. 899.
86. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 34/62-66], p. 901.

87. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 35/24-27], p. 904.
88. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 36/63-64], p. 912.
89. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 36/82, 83], p. 913.
90. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 36/84-85], p. 913.
91. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 37/31], p. 916.
92. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 38/18], p. 921.
93. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 38/26], p. 921.
94. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 8/30, 31], p. 440.
95. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 9/8], p. 443.
96. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 9/54-56], p. 44.
97. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 9/57-63], p. 447.
98. Susrutha,*Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.ka 5/73, 74], p. 579.
99. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.ka 5/61, 62], p. 578.
100. Susrutha,*Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u39/229-233], p. 689.
101. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 39/234-239], p. 689.

102. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 39/240-242], p. 690.
103. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 39/245-253], p. 690.
104. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 39/256], p. 690.
105. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 61/31-33], p. 802.
106. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 61/34-37], p. 802.
107. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 62/26], p. 805.
108. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 62/27-29], p. 805.
109. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 120, 121], p. 160.
110. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma kha 136], p. 162.
111. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 137-142], p. 162.
112. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha.143-145], p. 162.
113. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 6/26-36], p. 182.
114. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 6/148-153], p. 194.
115. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 7/40-49], pp. 200–201.
116. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma. kha 9/38-43], p. 217.

117. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma. kha 9/45-50], p. 218.
118. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/51-57], p. 218.
119. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/58,59], p. 219.
120. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha], p. 220.
121. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 72-74], p. 220.
122. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma. kha 9/79-86], p. 221.
123. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/87-90], p. 222.
124. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/93-98], p. 222.
125. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/99-100], p. 223.
126. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/147], p. 227.
127. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/148-152], p. 227.
128. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/153], p. 228.
129. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/168-171], p. 229.
130. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/181], p. 230.
131. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha.9/191-194], p. 232.

132. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/195-197], p. 232.
133. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 9/199-210], p. 232.
134. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 10/18-27], pp. 235–236.
135. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 10/53-59], p. 237.
136. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa.207-212], p. 278.
137. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 10/17, 18], p. 354.
138. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma kha. 11/4-6], p. 356.
139. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 13/85], p. 391.
140. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u], p. 943.
141. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [Ch.chi 6/26], p. 447.
142. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.12/5, 6], p. 678.
143. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 18/41, 42], p. 474.
144. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.sa8/20], p. 343.
145. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 9/45], p. 446.
146. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.su 3/3, 3/8, 3/14], pp. 27–28.

147. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.19/33-36, 19/41], pp. 713–714.
148. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.su 3/25], p. 29.
149. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 12/41, 42], p. 486.
150. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.17/39], p. 707.
151. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.chi.19/65, 66], p. 716.
152. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 57/13], p. 786.
153. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 52/19, 20, 35], pp. 767–769.
154. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 55/24, 25], p. 778.
155. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 51/44], p. 764.
156. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 44/17, su.u 44/22, su.u 44/25, 26], pp. 731–732.
157. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 45/32, 33], p. 736.
158. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 40/40], p. 699.
159. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.sa8/44], p. 348.
160. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi26/184], p. 608.

161. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi29/142], p. 633.
162. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.si 4/18], p. 699.
163. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.13/33, 34], p. 683.
164. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 20/12], p. 844.
165. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 26/55], p. 873.
166. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 37/33-35], p. 533.
167. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 17/41], p. 469.
168. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 8/44-46], p. 441.
169. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 8/48, 49], p. 441.
170. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [cha.chi.6/27], p. 447.
171. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 6/30], p. 447.
172. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi7/100], p. 455.
173. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.], p. 556.
174. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.9/61-63], p. 660.
175. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.chi9/64], p. 660.

176. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u22//104], p. 857.
177. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 39/205, 206], p. 687.
178. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u.40/28], p. 699.
179. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 40/60-62], p. 700.
180. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 39/187], p. 686.
181. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 39/188, 189], p. 686.
182. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.ka 7/38, 39], p. 584.
183. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.ka 8/130-133], p. 593.
184. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 11/19], p. 452.
185. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 5/10], p. 426.
186. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi13/158-161], pp. 498–499.
187. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi15/182], p. 523.
188. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.15/70-73], p. 697.
189. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi.7/87, ch.chi7/167-170], pp. 454–458.
190. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi14/52-57], p. 504.

191. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi25/114], p. 596.
192. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi29/149], p. 633.
193. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.chi19/85], p. 717.
194. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 38/40], p. 922.
195. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 38/17], p. 921.
196. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 83-85], pp. 919–920.
197. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 37/37-38], p. 916.
198. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 32/22], p. 892.
199. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 30/34], p. 886.
200. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 25/60], p. 868.
201. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 25/43], p. 867.
202. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 24/21, 22], p. 862.
203. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u9/25], p. 808.
204. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 2/93, 94], p. 414.
205. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 5/8], p. 425.

206. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi7/35], p. 437.
207. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi8/42], p. 441.
208. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 6/12], p. 432.
209. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 9/10], p. 443.
210. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 9/27, 28], p. 445.
211. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 19/33-35], p. 476.
212. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 20/35, 36], p. 480.
213. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 20/27, 28], p. 479.
214. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.ka 7/33], p. 583.
215. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 39/296-299], p. 694.
216. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha11/50, 51], p. 360.
217. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 11/55], p. 360.
218. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 11/56], p. 360.
219. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 11/82, 83], p. 364.
220. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 11/88], p. 364.

221. Sarngadhara, *Sarngadharasamhitha with Dipika and Gudarthadipika commentaries*, Chaukhambha Publications, Varanasi, reprint 2013, [sa. ma.kha 11/120], p. 368.
222. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi.16/119-121].
223. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi16/36, 37], p. 703.
224. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 2/76], pp. 531–532.
225. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 30/53], p. 637.
226. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.ka 4/16, 17], p. 660.
227. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.13/4, 5], p. 681.
228. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.13.6/7], p. 681.
229. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [18/56-58], p. 840.
230. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 44/14, 15], p. 730.
231. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 51/21, 22], p. 762.
232. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 17/10-13], p. 467.
233. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 17/22], p. 468.
234. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 25/38-42], p. 496.
235. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 10/15], p. 451.

236. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 9/48, 49], p. 446.
237. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 19/52-56], p. 552.
238. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 26/63], p. 601.
239. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 27/31], p. 614.
240. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 30/273, 274], p. 645.
241. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 9/64, 65], p. 471.
242. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 23/69], p. 575.
243. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 5/15-17], p. 794.
244. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 60/44], p. 789.
245. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 56/18], p. 783.
246. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 17/17], p. 626.
247. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 17/27], p. 627.
248. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 18/101], p. 640.
249. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 24/29], p. 653.
250. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 9/14], p. 611.

251. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 1/25], p. 377.
252. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 1/76], p. 380.
253. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 39/11], p. 924.
254. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 39/46-47], p. 926.
255. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 39/105], p. 930.
256. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 23/42], p. 573.
257. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi23/50], p. 574.
258. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 23/190], p. 579.
259. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 23/198], p. 580.
260. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi23/212], p. 580.
261. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi23/216], p. 580.
262. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi23/220], p. 581.
263. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi23/231], p. 581.
264. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi 23/246], p. 581.
265. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.chi25/85], p. 595.

266. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 35/21, 22], p. 904.
267. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.ka 8/72, 73, 74], p. 591.
268. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.ka 8/67], p. 590.
269. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 13/19], p. 57.
270. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 14/35], p. 65.
271. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 37/16], p. 162.
272. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 37/19], p. 162.
273. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 37/25], p. 162.
274. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 37/26], p. 162.
275. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 1/113-119], p. 406.
276. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 2/75], p. 413.
277. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.su 2/82-84], p. 414.
278. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 2/89, 90, 91], p. 411.
279. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 8/43], p. 441.
280. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 16/24-26], p. 465.

281. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u.9/21], p. 808.
282. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u.9/32], p. 808.
283. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 11/21,22], p. 813.
284. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 16/13], p. 831.
285. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 16/15], p. 831.
286. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [a.h.u 11/38], p. 814.
287. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 9/21, 23, 24].
288. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 10/3, 4, 5], pp. 612–613.
289. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 12/49], p. 619.
290. A. Charaka, *Charaka samhitha revised by Dipika commentary of Chakrapanidatta*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Prakashan, Varanasi, reprint 2013, [ch.si 10/22, 23], p. 725.
291. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.su 14/25-28], p. 226.
292. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash-chi.15/10, 11, 12], p. 693.
293. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.chi. 19/44], p. 714.
294. Vagbhata, *Astanga hrudaya with the commentaries of Arunadatta and Hemadri*, edited by Pt. Hari Sadasiva Sastri Paradakara, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2012, [ash.chi. 19/64], pp. 715–716.
295. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 40/63-65], p. 700.

296. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 42/49-51], pp. 720–721.
297. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 51/41], p. 764.
298. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 51/50], p. 764.
299. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 57/9-11, 8/67], pp. 590, 785.
300. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.u 38/27], p. 670.
301. Susrutha, *Susrutha samhitha with Nibandha samgraha commentary*, edited by Vaidya Jadavji Trikamji Acharya, Chaukhambha Sanskrit Sansthan, Varanasi, reprint 2013, [su.chi 38/64-66], p. 544.

CHAPTER 2

Chemistry of Turmeric

AKHILA NAIR[a] AND SREERAJ GOPI*[a]

[a]R&D Centre, Aurea Biolabs (P) Ltd, Kolenchery, Cochin–682 311, Kerala, India
*E-mail: sreerajgopi@yahoo.com

2.1 Introduction

Turmeric is a medicinal spice that belongs to the Zingiberaceae family. This earthy orange-yellow rhizome is a perennial herb that is erect with sheath leaves and thick fleshy rhizomes. Turmeric is scientifically termed *Curcuma longa* L. In 1753, Linnaeus substantiated the genus *Curcuma* and in 1977, Burtt reinstated the synonym as *C. longa* L. and C. *domestica.*[1] This kitchen spice has been known from time immemorial and closely associated with Asian culture for its multi-dynamic characteristics, especially its culinary and medicinal properties. The wide range of medicinal properties of turmeric include its use as an antioxidant, anti-inflammatory, anti-tumor, anti-malarial, anti-microbial, anti-protozoal, anti-aging, anti-venom, anti-angiogenic agent *etc.*[2] Also, due to the diverse pharmacological activities of turmeric, various names have been created such as golden spice, spice of life, herb of the sun and so on.

The literature states that turmeric, when orally administered, enters the blood circulation and is present in the form of glucuronide and glucuronide–sulfate conjugate forms. It is documented that these conjugates are one of the reasons behind its diverse physiological activities.[3] Indeed, the

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

structure of each compound of turmeric, especially the functional groups and the reactions occurring amidst them, are elements responsible for their multifarious biological activities. It has also been observed that various turmeric extracts possess different compounds exhibiting different levels of anti-inflammatory activities. In the same way, phenolic compounds present in turmeric are accountable for their antioxidant properties. The exploration of anti-inflammatory and antioxidant properties of this plant have opened a new pathway that elevates the value of this spice. These discoveries prove that turmeric exhibits diverse pharmacological activities due to the existence of multitudinous compounds, each holding distinct characteristics. Hence, more studies in regard to the chemistry, especially the physicochemical properties, extraction processes, biosynthesis, structure activity relationship, metal chelating activities, spectroscopic properties and other chemical reactivities during addition and degradation reactions of turmeric are essential to explore its in-depth potency.[4] Also, knowledge of the chemistry of turmeric could help to unwind the bona fide mode of action of known pharmacological activities possessed by respective turmeric compounds.[5] Unearthing their chemistry could help one to design numerous turmeric based drug formulations that could be used in a safe and effective manner. Moreover, with the help of this science, reduction in the risk of many malignant diseases could transpire, to which these formulations are the only solution.[6] Numerous clinical trials, whether completed or ongoing, have evidenced the fact that a thorough knowledge of the medicinal chemistry of turmeric is the key to the lock.[7] In this context, this chapter is an initiative penned to reveal the plethora of compounds contained in this golden herb, including structural properties, analysis, biosynthesis, biological activities and stability studies.

2.2 Conventional Stratification on Turmeric

Turmeric, the 'golden spice', is renowned for its color, flavor and odor, which symbolizes the presence of valuable and pharmacologically active compounds. Commercially, turmeric is also available as dried whole rhizomes, ground or powdered rhizomes apart from oil or oleoresin. Other constituents of turmeric are dietary fibers, mineral matter, proteins, fats, moisture and carbohydrates. These elements can be broadly categorized as volatile and non-volatile components. Distinct compositions of individual constituents are 3–7% of volatile oil, non-volatile compounds (curcuminoids about 1–6%, dietary fiber about 2–7%, mineral matter around 3–7%, protein approximately 6–8%, moisture ranging from about 6–13%, fat about 5–10% and carbohydrate around 60–70%)[8] (see Table 2.1). This spice also contains, in general, water-soluble vitamins such as niacin, riboflavin, thiamine and ascorbic acids as well as minerals.[9] The illimitable reaches of this phenomenal spice have been extensively studied in terms of numerous phytochemicals, which are their secondary metabolites. These phytochemicals comprise monoterpenes, triterpenes, sesquiterpenes, flavonoids, steroids, fatty acids,

Table 2.1 General classification of turmeric.

General classification of turmeric		Composition (w/w%)
Non-volatile components	Volatile oils	3–7
	Curcuminoids	1–6
	Fiber	2–7
	Moisture	6–13
	Minerals	3–7
	Proteins	6–8
	Carbohydrate	60–70
	Fats	5–10

saccharides, alkaloids and phenolic compounds. These phytochemicals are proven through clinical and preclinical studies to exhibit various therapeutic effects such as neuroprotective, hepatoprotective, anti-inflammatory, antioxidant, anticancer, anti-diabetic, antimicrobial, lipid decreasing, anti-HIV, anti-fungal, antifibrinogenic, nematocidal, radioprotective, anti-parasitic, antispasmodic, wound-healing, antimutagenic, immunomodulating and anti-bacterial activities, as well as being used to treat Alzheimer's disease.[2] Hence, these diverse pharmacological properties of turmeric necessitate an understanding of their extraction processes, which yield turmeric oil, oleoresin and other valuable phytochemicals including proteins, dietary fibers and carbohydrates that becomes the basis of their further classification into volatile and non-volatile compounds.

2.3 Turmeric Oleoresin

Turmeric is hydrophobic in nature; therefore, it is treated with solvent for the extraction of oleoresin. Anderson *et al.* discovered a technique for isolating curcumin from turmeric by using dichloromethane, refluxing for an hour, suction filtering and then titrating the residue with hexane.[10] After this breakthrough, Baghi reported another method for the extraction of curcumin using ethanol and acetone.[11] Rigorous studies over the years have brought about a gradual and elaborated procedure that consists of a chronicle of extraction methods that are mainly focused on enrichment of the active recovery. Rhizomes are subjected to harvesting, after debris and dirt have been removed and washed. They are further subjected to slicing through a pilot plant slicer or dried in a dryer at 65 °C and reduced in a mill. Usually, the sliced portion is taken for further processing, either cold solvent extraction or hot storage extraction. In cold solvent extraction the weighed rhizomes are blended with ethanol and stirred on a magnetic stirrer for six hours. Then, the extract is filtered. This process is repeated twice or thrice and concentrated in a rotary evaporator set in a water bath at 50 °C. The oleoresin obtained is then analyzed for yield. Another method is hot solvent extraction in which the dried rhizomes are weighed and blended

with ethanol and transferred to a round-bottomed flask with additional ethanol. A condenser is fixed and the setup is refluxed for six hours in a water bath at a temperature of 80 °C. Thereafter, the extract is subjected to vacuum filtration and the residue is re-extracted. Both the extracts are combined and concentrated in a rotatory evaporator. The oleoresin thus obtained is analyzed for yield.[12] Moreover, the industrial-scale extraction method involves percolation, using ethyl acetate as a solvent followed by concentrating it to obtain turmeric oleoresin, oil and eventually curcuminoids of the highest purity. Industrial-scale developments also utilize different solvents like ethyl alcohol, acetone, isopropyl alcohol *etc.* for the extraction of curcuminoids.

The current extraction method involves molecular distillation and supercritical CO_2 extraction technologies to obtain the extract 'turmeric oleoresin' from mainly ground turmeric spice along with turmeric oil. The solvents used for the extraction of oleoresin are ethyl acetate, acetone, ethylene dichloride or ethanol. This oleoresin is orange-red in color and considered to be a value-added product that is in high demand in all countries. For the extraction process, an optimum pressure of about 22 MPa is required. Recently, a successful modification was carried out in the process of obtaining oleoresin and curcuminoids by Nagvekar *et al.*, who worked to produce a greater yield of turmeric oleoresin as well as curcuminoids by a pretreatment with Stargen®002 enzyme (a granular starch hydrolyzing enzyme) and utilization of 30% ethanol as modifier with supercritical fluid of 99.9% purity of carbon dioxide, and thereafter optimization of the pressure, temperature and time. In this procedure, before loading the sample in the column, they evenly mixed the modifier with the sample, which was closed at one end and a polypropylene frit of 45 μ (where the sample was placed) was placed near to this end and the vessel was firmly packed for the proper and uniform distribution of carbon dioxide throughout the sample matrix. This setup was connected to an oven by vessel connectors keeping the frit side above it; the temperature, pressure and time were varied and measured for optimization purposes during the extraction procedure. The extracted samples were stored at −20 °C. It was observed that at 350 bar the maximum yield of oleoresin was achieved at about 35.37 ± 3.43 mg g^{-1}. Thus, with the help of a suitable modifier that is 30% ethanol, using Stragen®002 as a mode of enzymatic pretreatment as well as optimizing the pressure temperature and time to about 350 bar/65 °C/150 min variant, a better yield of oleoresin and curcuminoids was obtained.[13] Further, Kurmudle *et al.*, studied the yield in the process of enzyme pretreatment for the extraction of oleoresin, which utilized acetone as a solvent. The enzymes used were glucoamylase, amylase and a mixture of xylanase and amylase. The various variables checked were pH, concentration, incubation time and extraction time. There was a significant increase in the yield of oleoresin to about 13.7% and 22.5% for amylase and glucoamylase enzyme, respectively. Although utilization of supercritical carbon-dioxide is the most common extraction method (Soxhlet apparatus)

for oleoresin,[14] there are other techniques such as three-phase partitioning and the leaching method used for extraction. The three-phase partitioning technique utilizes ammonium sulfate and *t*-butanol, which are added simultaneously into the aqueous slurry of turmeric as stated by Kurmudle *et al.*, who optimized the three-phase partitioning technique to increase the yield of oleoresin. For this, they pretreated the slurry with glucoamylase with or without amylase and optimized the oleoresin extraction by varying the ratio of *t*-butanol and the concentration of ammonium sulfate. It was observed that the treatment with enzymes reduced the extraction time and improved the yield of oleoresin.[15]

Another method is leaching using acetone, which is utilized to separate one or more constituents from a solid mixture by transferring them into the cellular matrix of the solid and diffusion of components out of the solid until equilibrium. Haldar *et al.* calculated the time required for equilibrium through extraction kinetics, which could be utilized to reduce the extraction time. In their study, a temperature range from 35–50 °C and solid to solvent ratio was taken between 1/5 and 1/30 (g g^{-1}). A non-linear regression model was applied to find the yield of oleoresin and the effects of various parameters such as temperature, solid–solvent ratio, maximum extraction percent dry basis (k) and initial extraction rate (a). The outcome of the study denoted that the maximum yield of oleoresin was 6.49% decibel at 50 °C having a solid to solvent ratio 1/30 g g^{-1}.[16] Furthermore, they optimized the effects of parameters such as temperature, particle size and solvent to solid ratio in Soxhlet apparatus for the extraction of oleoresin yield. Their studies demonstrated that a temperature of 28.94 °C, particle size of 0.24 mm and solid–solvent ratio 20.95 were considered optimum for producing an oleoresin yield of 9.57%.

The extracted oleoresin is subjected to oxidative degradation and the pigments having hydroxyl groups are readily converted into ketones that are highly unstable. They are further decomposed to a short carbon skeleton of colorless compounds. Nampoothiri *et al.* studied the antioxidant and antidiabetic study of spent turmeric oleoresin (STO). They studied the antioxidant properties by superoxide radical scavenging activity, DPPH activity, metal chelating activity, ABTS and antidiabetic activity by α-glucosidase and α-amylase. The STO exhibited a moderate chelating property and IC_{50} value of 61.5, 33, 58.1 μg mL^{-1} for the superoxide free radical, ABTS and DPPH, which proved STO to be a potent antidiabetic and antioxidant agent.[17] The oleoresin further underwent crystallization and purification to obtain curcuminoid powder (highest purity) and oil. Therefore, the major part of turmeric oleoresin comprises curcuminoids about 30–40% and volatile oil covers the rest of the portion around 15–20%.[18] Nagarajan *et al.* demonstrated that the spent turmeric oleoresin remaining after partial isolation of curcuminoids, if enriched with curcuminoids, is a potent antioxidant. Through radical scavenging activity it was observed that curcuminoid-enriched spent turmeric oleoresin had a high radical

scavenging activity of 84% at 50 μg mL^{-1} and antioxidant activity of 74% at 25 μg mL^{-1}. The total phenolic content was also doubled to 267.27 + 5.75 mg GAE g^{-1}. Thus proving that the antioxidant properties of spent turmeric oleoresin should not be neglected.[19] Other volatile and non-volatile components of turmeric along with their structural composition and analysis are further described.

2.4 Volatile Oil and/or Non-curcuminoid Component of Turmeric

Of the total 3–7% composition of volatile oil present in turmeric, dried rhizomes comprise about 5–6% and leaves about 1–2%.[18] The composition of essential oil could vary depending upon the different agricultural practices that are carried out as well as the utilization of fertilizers.[19] The volatile oil is obtained by following a series of processes such as drying, coarse grinding, steam distillation, hydrodistillation or supercritical fluid method.[8] Previously, hydrodistillation was carried out using Clevenger's apparatus by mixing dried rhizomes with water and heating for 6–10 hours. The evolved condensed vapors were separated through an auto/oil water separator. The upper layers containing oil were thereafter separated.[20] Recently, the supercritical fluid method has been employed using carbon dioxide that produces oil as well as oleoresin.[18] This oil is yellowish in color with a medicinal aroma.[8] It has been stated that the steam-distilled volatile oil of turmeric contains sesquiterpene ketones. However, the oil obtained along with the oleoresins has also been considered commercially and has been subjected to further purification to obtain the desired compounds. They are lipophilic in nature, which promotes the transportation of other turmeric compounds mainly curcuminoids (the non-volatile compound). Therefore, they promise to improve the bioavailability of curcumin when administered orally. They are also loaded with numerous pharmacological properties exhibiting anti-mutagenic, neuroprotective, anti-hyperlipidaemic, atherosclerotic, disease modifying, anti-arthritic, anti-ischemic, antifibrosis, hepatoprotective, anti-dermatophytic, antibacterial, antifungal, anti-oxidant, anti-aflatoxigenic, anti-apoptotic, anti-platelet, anti-hyperglycemic, anti-depressant and immunostimulant activities.[21] Hence, the Food and Drug Administration have approved turmeric oil as a safe food additive. The compounds are also generally recognized as safe (GRAS). Generally, a dose of 1–2 drops is recommended and is utilized in aromatherapy. Studies on the physicochemical properties, especially structural elucidation and analysis, have a major role in the discovery of the abundant therapeutic activities. Research illustrates that turmeric oil is lipophilic in nature, which facilitates brain accessibility and helps in stroke management.[22] Hence, the composition, structural studies and method of analysis are subjects of interest.

2.5 Constituents and Structural Studies of Turmeric oil and/or Non-curcuminoids

In the volatile portion of turmeric, the leaves contain monoterpenes and the rhizomes are dominated by sesquiterpenes. To date, 68 monoterpenes have been discovered primarily from the leaves of turmeric. Their basic molecular formula is C_{10} $H_{16.}$ They have two isoprene groups and can be in the form of cyclic or acyclic units. Some important monoterpenes are α-phellandrene, carvacrol, limonene, cineole, myrcene, terpinolene, *p*-cymene and *p*-cymen-8-ol.

Another important group is the sesquiterpenes; they are the most dominant group of turmeric rhizomes as they cover around 40% of essential oils. Sesquiterpenes with the basic molecular formula C_{15} $H_{20}O_2$ are the key factors responsible for the peculiar aroma and taste that are the characteristics of turmeric.[23] So far, about 109 sesquiterpenes have been discovered. The compounds identified as sesquiterpenes are 54 bisabolenes, seven guaianes, six germacranes, four selinanes, three santalanes, three elemanes, two caryophyllenes, aristolene, acorane, carabane, bergamotanes, himachalane, cedrane and sesquisabinane.[24] Their ratio and existence depend majorly on the geographic locations of the cultivation of turmeric as well as the changes in different species. The most abundantly found bisabolanes are turmerones, which occupy around 50–80% in Brazilian species and around 27% in Asian species.[24] These turmerones are further divided as α, β turmerones and Ar-turmerones. Ar-turmerones are especially studied for their various biological activities. In addition, elemenes are cataloged under bisabolanes and are classified as α, β and δ elemenes. Among them, β elemenes are more popular for their diverse medicinal activities. Other bisabolanes known for therapeutic activities are bisacurone, curlone, β-sesquiphelandrene and dihydrozingerone. Similarly, germacrone categorized under germacranes has achieved recognition as a pharmacologically active compound. Similarly, cyclocurcumin is formed by the pyrone ring cyclization, and is the intermolecular Michael addition product of the enol oxygen group to the enone group. Cyclocurcumin is also cataloged under compounds other than curcuminoids that possess medicinal properties. Another categorization is phenylpropenes and to date six phenylpropenes have been discovered. Among these phenylpropenes, known for their biological activities, are Calebin-A, vanillin and ferulic acid. Kim and Kim originally isolated and thereafter identified Calebin-A at about 0.0001%.[25] This compound has a ferulic ester bond that is devoid of a 1, 3-diketonic structure similar to the curcuminoids.[26] Similarly, ferulic acid with the IUPAC name (*E*)-3(4-Hydroxy-3-methoxy-phenyl) prop-2-enoic acid has a phenolic structure. These compounds are also considered valuable for their therapeutic effects and the literature denotes them as having potential anticancer, anti-diabetic, cardiovascular, Alzheimer's and skin properties.[27] The major compounds of turmeric oil are listed in Table 2.2. Mostly, the compounds of turmeric oil have a proven history of a series

Table 2.2 Specified classification of certain major components of turmeric including IUPAC.

S. No.	Curcuminoids and its derivative	Other compounds than curcuminoids
1	**Curcumin**, diferuloylmethane [1,7-bis (4-hydroxy-3-methoxy-phenyl)-hepta-1,6-diene-3,5-dione]	**Ar-turmerone**, 6*S*-2-methyl-6-(4-methylphenyl)hept-2-en-4-one **α-turmerone**, 2-Methyl-6-(4-methylcyclohexa-2,4-dien-1-yl) hept-2-en-4-one **β-turmerone**, (6*S*)-2-methyl-6-[(1*R*)-4-methylidenecyclohex-2-en-1-yl]hept-2-en-4-one
2	**Demethoxycurcumin**, [4-hydroxycinnamoyl-(4-hydroxy-3-methoxycinnamoyl) methane]	**α-elemene**, (6*S*)-6-ethenyl-6-methyl-1-propan-2-yl-3-propan-2-ylidenecyclohexene **β-elemene**, (1*S*,2*S*,4*R*)-1-ethenyl-1-methyl-2,4-bis(prop-1-en-2-yl) cyclohexane **δ-elemene**, (3*R*,4*R*)-4-ethenyl-4-methyl-1-propan-2-yl-3-prop-1-en-2-ylcyclohexene
3	**Bisdemethoxycurcumin**, [bis-(4-hydroxy cinnamoyl) methane]	**Bisacurone**, (6*S*)-6-[(1*R*,4*S*,5*S*)-4,5-dihydroxy-4-methylcyclohex-2-en-1-yl]-2-methyl hept-2-en-4-one
4	1-(4-hydroxy-3-methoxy-phenyl)-7-phenyl-heptan-3-one	**Furanodiene**, (5*E*,9*E*)-3,6,10-trimethyl-4,7,8,11-tetrahydrocyclodeca[*b*] furan
5	(*E*)-1-(4-hydroxy-3-methoxyphenyl)-7-phenylhept-1-en-3-one	**Germacrone**, (3*E*,7*E*)-3,7-Dimehyl-10-propan-2-ylidenecyclodeca-3,7-dien-1-one
6	(*E*)-(*E*)-4-(4-hydroxy-3-methoxyphenyl)-2-oxobut-3-en-1-yl 3-(4-hydroxy-3-methoxyphenyl) acrylate	**β-sesquiphellandrane**, 3-(6-methylhept-5-en-2-yl)-6-methylidene cyclohexene
7	(1*E*,4*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,4,6-trien-3-one	**Curlone**, 2-methyl-6-(4-methylidenecyclohex-2-en-1-yl) hept-2-en-4-one
8.	(*E*)-7-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl) hept-1-ene-3,5-dione	**Curdione**, (6*Z*)-6,10-dimethyl-3-propan-2-ylcyclodec-6-ene-1,4-dione
9.	(*E*)-1,7-bis(4-hydroxyphenyl) hept-1-ene-3,5-dione	**Calebin-A**, (3*E*)-4-(4-hydroxy-3-methoxyphenyl)-2-oxobut-3-en-1-yl(2*E*)-3-(4 hydroxy-3-methoxyphenyl) prop-2-enoate)
10.	(1*E*,4*E*,6*E*)-1,7-bis(4-hydroxyphenyl) hepta-1,4,6-trien-3-one	**Cyclocurcumin**, (*E*)-2-4-hydroxy-3-methoxyphenyl-6-(4-hydrox-3-methoxystyryl)2*H*-pyra-4(3*H*)-one

(*continued*)

Table 2.2 (*continued*)

S. No.	Curcuminoids and its derivative	Other compounds than curcuminoids
11.	(1*E*,4*E*)-1,5-bis(4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one	**Curcumol**, (1*S*,2*S*,5*S*,9*S*)-9-isopropyl-2-methyl-6-methylene-11-oxatricyclo(6.2.1.$0^{1,5}$) undecan-8-ol
12.	**Dehydrogingerone**, 4-(4-Hydroxy-3-methoxyphenyl)-3-buten-2-one	**1,8-Cineole**, 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane
13.	**Tetrahydrocurcumin**, (*Z*)-5-hydroxy-1,7-bis (4-hydroxy-3-methoxyphenyl) hept-4-en-3-one	**Myrcene**, 7-methyl-3-methylideneocta-1,6-diene
14.	**Hexahydrocurcumin**, 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl) heptan-3-one	**Carvacol**, 2-methyl-5-propan-2-ylphenol
15.	**Octahydrocurcumin**, 1,7-bis(4-hydroxy-3-methoxyphenyl) heptane-3,5-diol	**(*Z*),(*E*)-γ-Alantone**
16.	**Hexahydocurcuminol**, 1,7-bis(4-hydroxy-3-methoxyphenyl) heptane-3,5-diol	**2-Carene**, 2,7,7-trimethylbicyclo[4.1.0]hept-3-ene
17.	(2*S*,3*S*,4*S*,5*R*,6*S*)-3,4,5-trihydroxy-6-(4-((1*E*,3*Z*,6*E*)-3-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-5-oxohepta-1,3,6-trien-1-yl)-2-methoxyphenoxy) tetrahydro-2*H*-pyran-2-carboxylic acid	**Methyleugenol**, 1,2-dimethoxy-4-prop-2-enylbenzene
18.	**Curcumin di-glucuronide**, (2*S*,2′*S*,3*S*,3′*S*,4*S*,4′*S*,5*R*,5′*R*,6*S*,6′*R*)-6,6′-((((1*E*,3Z,6*E*)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylic acid	**Geraniol**, (2*E*)-3,7-dimethylocta-2,6-dien-1-ol
19	**Ferulic acid**, (*E*)-3-(4-hydroxy-3-methoxyphenyl) acrylic acid	**Zingiberene**, (5*R*)-2-methyl-5-[(2*S*)-6-methylhept-5-en-2-yl] cyclohexa-1,3-diene
20.	**Dihydroferulic acid**, 3-(4-hydroxy-3-methoxyphenyl) propanoic acid	**β-bisabolene**, 1-methyl-4-(6-methylheptan-2-yl)cyclohexane
21.		**α-phellandrene**, 2-methyl-5-propan-2-ylcyclohexa-1,3-diene

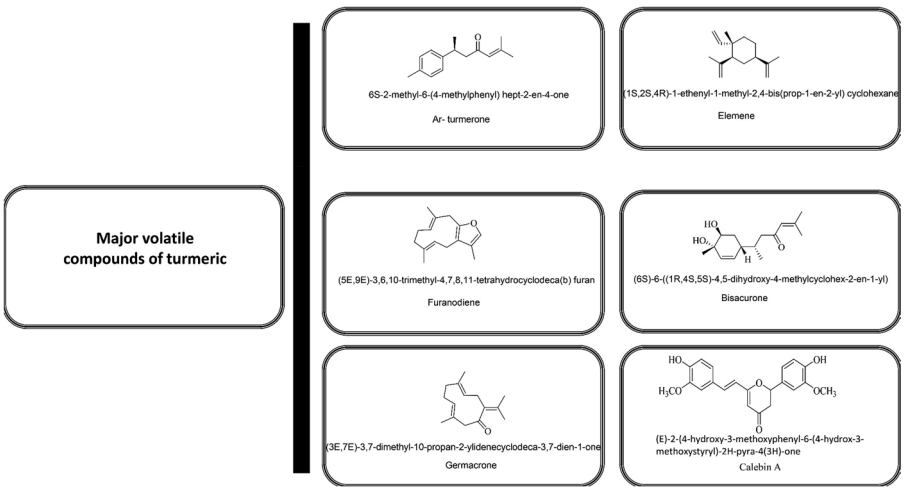


Figure 2.1 Volatile compounds of turmeric.

of biological activities that are equipotent to non-volatile compounds, especially curcuminoids, but these compounds still must be explored for their therapeutic activities through more evidenced-based studies.[28] The volatile compounds of turmeric are depicted in Figure 2.1.

2.6 Analysis of Turmeric Essential Oil and/or Non-curcuminoids

The fractions in turmeric oil are separated by silica gel chromatography and preparative thin layer chromatography and fractional distillation.[28,29] These constituents of turmeric oil content have been analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).[8] The structural elucidation was carried out by infrared spectroscopy (IR), nuclear magnetic resonance (NMR) and mass spectrometry (MS). Furthermore, absolute stereo structures of some carabrane type sesquiterpenes were analyzed by the nuclear Overhauser effect (NOE) and circular dichroic (CD) spectroscopic analysis. This structural elucidation proved to be beneficial as the pharmacological activities of these compounds came into the limelight.[30]

2.7 Biological Activities of Volatile and/or Non-curcuminoid Compounds of Turmeric

Turmeric, called the golden spice, is known to exhibit enormous therapeutic benefits due to the compounds present in it. The oils extracted from this plant have undergone serious investigation for their distinct activities. There

are more than 5600 studies in the literature that focus on the different medicinal properties of the volatile compounds of turmeric.[31] Verma *et al.* reviewed the pharmacological outline of turmeric oil and stated that this volatile component carries a plethora of activities that can be utilized for anti-mutagenic, neuroprotective, anti-inflammatory, antioxidant, antinociception, antihyperlipidaemic, anti-atheroclerosis, anti-arthritic, anti-iscehmic, antiplatelets, antifibrosis, cytoprotective, anti-apoptogenic, anti-dermatophytic, chemopreventive, antidiabetic, anti-epiletic, antidepressant, anti-aflatoxigenic, immunostimulant, antioxidant, anticancer purposes.[22] In this series, Aggarwal *et al.* streamlined the anti-inflammatory and anticancer potential of major compounds other than curcuminoids such as turmerone, elemene, cyclocurcumin, curdione, Calebin-A, bisacurone, germacurone, furanodiene.[32] Zang *et al.* recently reported the antioxidant, anti-microbial, anti-inflammatory and anticancer activities of these oils, especially ar-turmerone, β-turmerone, α-zingiberene, ar-curcumene and sesquiphellandrane. These identified components were in the ratio of 0.92–42.85%; ar-turmerone 5.13–42.54%, β-turmerone 0.25–25.05% and α-zingiberone 1.21–15.70%. Through DPPH, ABTS and radical scavenging activities, the IC_{50} values of each were found to be 4.37–11.59 μg mL^{-1}, 4.21–13.25 μg mL^{-1} and 10.72–11.42 μg mL^{-1}, respectively. The cytotoxity against B16 cells and LNCaP was evaluated to be IC_{50} 13.96–135.97 μg mL^{-1} and 19.63–127.81 μg mL^{-1}. The anti-inflammatory effects demonstrated downregulation of the expression of inflammatory cytokines, tumor necrosis factor alpha (TNFα) and cyclooxygenase 2 (COX-2); thus expressing the rationale behind the potency of vivid biological activities of volatile oil.[33] In addition, the potency of Calebin-A was demonstrated as a promising candidate in osteoporosis by suppressing RANKL signaling, possessing anticancer and neuroprotective activities.[25,34] Moreover, another compound having structural similarity with curcumin is cyclocurcumin, which possesses antioxidant, anti-cancer and neuroprotective activities.[35] Thus, these studies prove that components other than curcumin exhibit promising therapeutic effects and can be utilized as promising entities separately as well as in synergism with other compounds for curing various diseases. The biological activities of the compounds in turmeric are presented in Table 2.3.

2.8 Non-volatile Components of Turmeric

Diphenyl alkanoids and phenyl propene (cinnamic acid derivatives) are non-volatile compounds of turmeric, proteins, dietary fibers and carbohydrates. Further, diphenyl alkanoids are subdivided into diphenylheptanoids and diphenylpentanoids. Curcuminoids are diphenylheptanoids, which cover about 1–6% of turmeric.[32] Turmeric oleoresin also contains curcuminoids, but in low purity. Crystallization followed by purification will result in curcuminoids with the highest purity. Other than curcuminoids there are natural,

Table 2.3 Biological properties of the major components of turmeric.

S. No.	Major compounds of turmeric	Biological activities	Reference
Non-volatile compounds			2
1	Curcumin	Anti-inflammatory, neuroprotective, premenstrual syndrome, depressive disorder, Alzheimer's disease, anti-tumor, radioprotective, cardioprotective, antiviral, antifungal, anti-angiogenic, hepatoprotective, antimutagenic, arsenic and chromium toxicity, anti-arthritic, anti-acanthamoebic, anti-acidogenic, anti-malarial, cardiovascular, pulmonary, neoplastic	
Natural derivatives of curcumin			37
2	Demethoxycurcumin	Neuroprotective, anticancer, antifungal, Alzheimer's disease, antioxidant,	
3	Bisdemethoxycurcumin	Neuroprotective, Alzheimer's disease, anticancer, antioxidant	
4	Dihydrogenerone	Antioxidant, anticancer, anti-inflammatory	
Hydrogenated derivatives of curcumin			39
5	Tetrahydrocurcumin	Antioxidant, anticancer, neuroprotective, hepatoprotective	
6	Hexahydrocurcumin	Antioxidant	
7	Octahydrocurcumin	Antioxidant, hepatoprotective	
8	Hexahydocurcuminol		
Curcumin metabolites			40
9	Curcumin glucuronides	Chemopreventive, non-toxic	
10	Curcumin sulfonamides	Chemopreventive	
Volatile compounds			18
11	Turmeric oil	Anti-mutagenic, neuroprotective, anti-inflammatory, antioxidant, antinociception, antihyperlipidemic, anti-atherosclerosis, anti-arthritic, anti-ischemic, antiplatelets, antifibrosis, cytoprotective, anti-apoptogenic, anti-dermatophytic, chemopreventive, antidiabetic, anti-epileptic, antidepressant, anti-aflatoxigenic, immunostimulant	
12	Ferulic acid	Antioxidant, anticancer	
13	Calebin-A	Anticancer, neuronal cell protective, osteoporosis	
14	Cyclocurcumin	Antioxidant, antivasoconstrictive, anticancer, anti-arthritic, neurodegenerative diseases, nematocidal	
Other compounds			8
15	Dietary fibers	Anti-microbial activity, wound dressings, composites and textiles	
16	Proteins	Antioxidant, antihyperglycemic, nutrition	
17	Minerals	Nutrition	
18	Carbohydrates	Nutrition	
19	Fats	Nutrition	

semi-synthetic, synthetic, hydrogenated derivatives of curcumin (Table 2.2). In addition, curcumin possesses certain metabolites such as glucuronide and sulphonamide. The metabolites exhibit diverse pharmacological activities that are clinically proven and well documented.[36] These pharmacological activities are well correlated with the individual structure and analysis. Hence, knowledge of chemistry of this medicinal plant proves beneficial in understanding the underlying mechanism of action of any particular property.

2.8.1 Constituents and Structural Studies of Non-volatile Components of Turmeric

Phenolic and methoxy substitutions on the phenyl ring of diphenyl heptane are the cause of the formation of compounds named curcumin, demethoxycurcumin and bis-demethoxycurcumin.[36] (Figure 2.2). The major compound of turmeric, curcumin in ratio 71–72% with chemical name 1,7-bis-(4-hydr oxy-3-methoxyphenyl)-hepta-1, 6-diene-3, 5-dione and chemical formula $C_{21}H_{20}O_6$ has a molecular weight 368.37 and pKa value 8.54. It contains an aryl-C7-aryl skeleton that was discovered in 1910. Solubility studies state that this compound is insoluble in water at acidic and neutral pH; however, it is soluble in ethanol, methanol, acetone and dimethylsulfoxide. The yellow

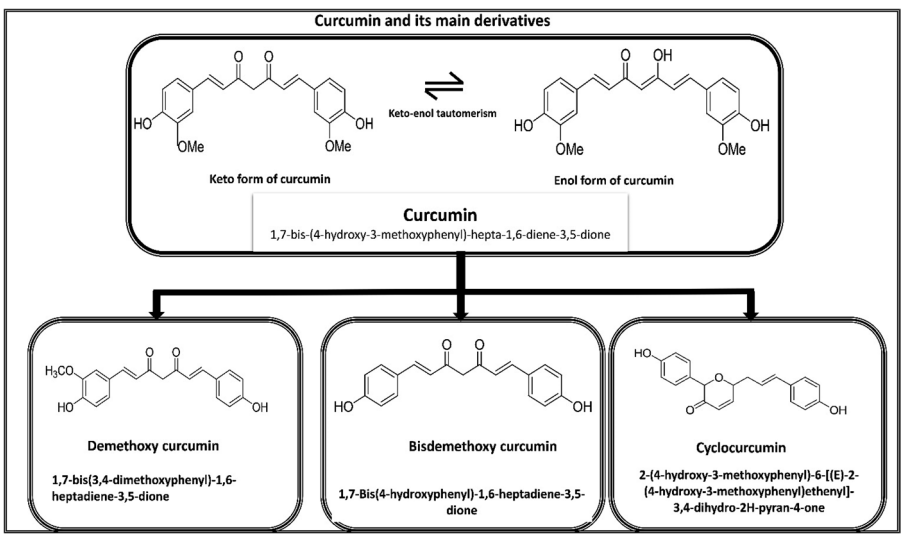


Figure 2.2 Curcumin and its main derivatives.

color of curcumin is sustained from pH 2.5 to 7 and changes to a reddish color in alkaline pH. The molecular configuration exists in bis-keto and an enolate tautomer forms. The enol form has three ionizable protons, namely two phenolic groups and one corresponding to the enolic group. The enolic form dominates in an alkaline medium and in conditions such as acidic or neutral; the keto form dominates crowning curcumin as the potential donor of hydrogen atoms. An NMR study of curcumin suggested that in solutions it exists in keto–enol tautomers.[37] Furthermore, the other curcuminoids in turmeric are dimethoxy curcumin and bisdemethoxycurcumin. Demethoxycurcumin was found in the ratio 19–20%, having the chemical formula 1, 7-bis (3, 4-dimethoxyphenyl)-1, 6-heptadiene-3, 5-dione and molecular formula as well as molecular weight $C_{23}H_{24}O_6$ and 396.4, respectively. Bisdemethoxycurcumin in the ratio 9–10%, another curcuminoid in turmeric, has the chemical formula 1,7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione with the molecular formula $C_{19}H_{16}O_4$ and molecular weight 308.33. Furthermore, there are six naturally occurring analogs of curcumin that were isolated by a bioassay-teamed fractionation. This assay was also utilized to determine the ability of these naturally occurring curcumin derivatives to protect PC12 cells from β-amyloid. The ED_{50} was about 0.5–10 μg mL^{-1} and the ED_{50} value of Congo red was found to be about 37–39 μg mL^{-1} [38] (Figure 2.3). Moreover, the hydrogenated or reduced derivatives such as tetrahydrocurcumin (THC), hexahydrocurcumin (HHC) and octahydrocurcumin (OHC)

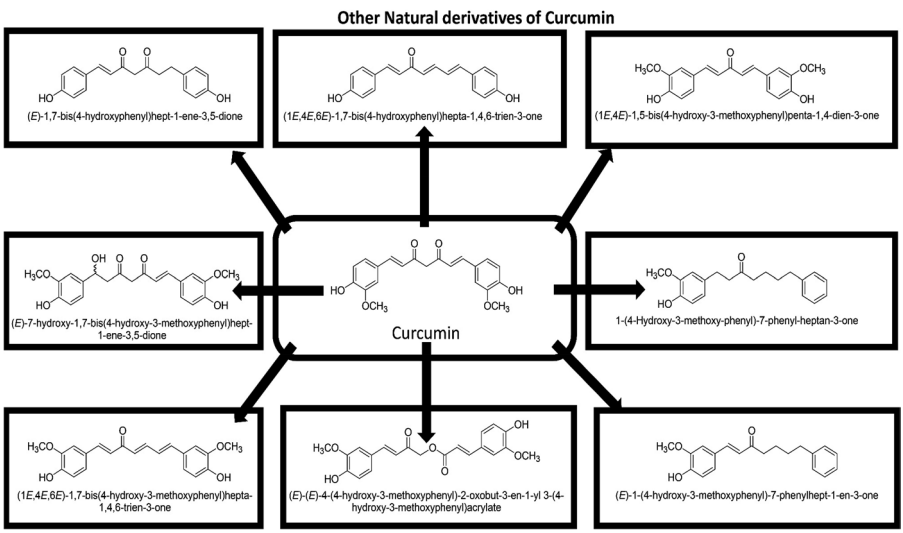


Figure 2.3 Other natural derivatives of curcumin.

were found to have potent DPPH activity (Figure 2.4). Tetrahydrocurcumin is hypothesized to absorb easily in the gastrointestinal tract and is most stable in 0.1 N phosphate buffer at various pH. Limtrakul *et al.* reported that conjugation groups of curcumin, which are responsible for the yellow color, are reduced to colorless dihydrocurcumin and tetrahydrocurcumin by an endogenous reductase system and furthermore they were converted to monoglucoronoside conjugates such as curcumin-glucuronide, THC-glucuronoside, dihyhyrocurcumin-glucuronoside with the help of Uridine 5′-diphospho glucuronosyl transferase.[39] These water soluble metabolites are easily subjected to enzymatic degradation as curcumin and their analogs contain two phenolic groups that most likely undergo conjugation with glucuronide and a sulfate group. Generally, curcumin monosulfate, curcumin monoglucuronide and curcumin sulfate-glucuronide are found in the human metabolism after oral consumption of curcumin[40] (Figure 2.5).

2.8.2 Analysis of Non-volatile Turmeric Compounds

Spectrophotometrical studies denote that the maximum absorption (λ max) of curcumin in methanol is 430 nm having a Beer's law range from 0.5 μg mL^{-1} to 5 μg mL^{-1}. The absorption maximum in acetone is 415 to 420 nm; also, it has been observed that 1% solution of curcumin has 1650 absorbance units. The absorption spectrum of curcumin contains certain structures in toluene that disappear with the addition of more polar solvents such as

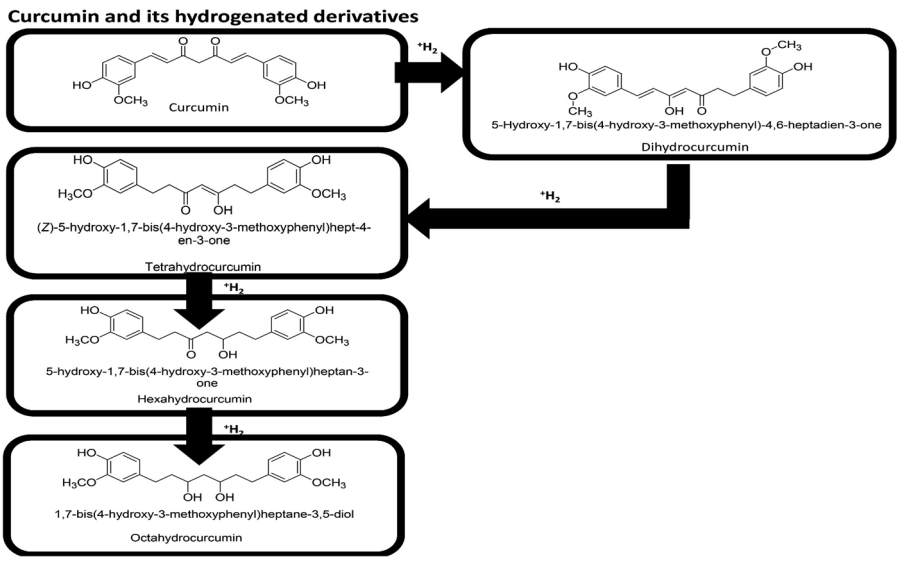


Figure 2.4 Curcumin and its hydrogenated derivatives.

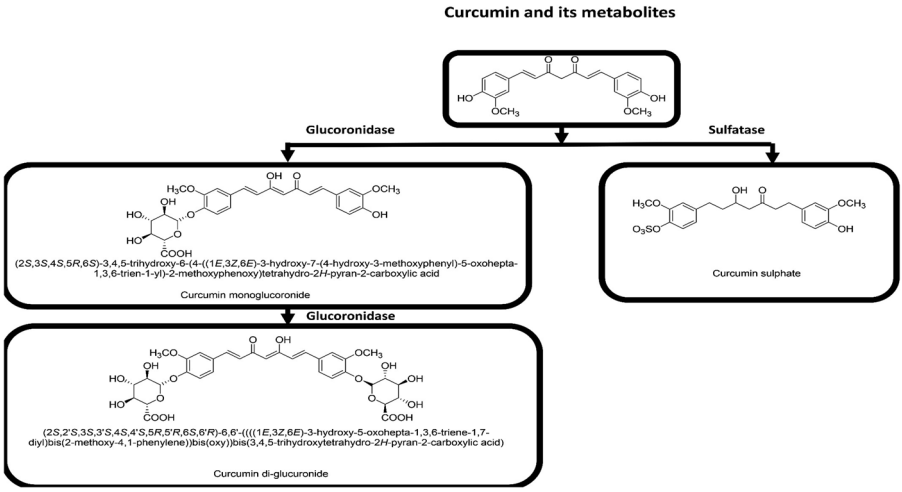


Figure 2.5 Curcumin and its metabolites.

acetonitrile and ethanol. The fluorescence of curcumin denotes certain structures in toluene at absorption maximum (λ max) 460, 488 nm and signifies a broad spectrum in ethanol with λ max 549, acetonitrile with λ max 524 and micellar solution with λ max 557. The fluorescence quantum yield of curcumin in acetonitrile is higher at ϕ, 0.104 and low in sodium dodecyl sulfate ϕ. 0.011.50 μm of curcumin tend to produce singlet oxygen upon irradiation in acetonitrile or toluene at an absorption maximum of more than 400 nm. Also, in acetonitrile solvent, curcumin tends to quench singlet oxygen (kq = 7 $\times 10^6$ M s^{-1}). It is also observed that curcumin produced ten times more singlet oxygen atoms than in aqueous micellar solution of Triton-X and produced no singlet oxygen phosphorescence in sodium dodecyl sulfate. It is also capable of generating superoxide in ethanol and toluene. Mishra *et al.* demonstrated the reaction of curcumin with a superoxide-crown ether complex. Through optical rotation it was observed that a blue color is formed intermediately at an absorption maximum 560 nm on reaction with the superoxide but decayed eventually with the development of the absorption band corresponding to the parent curcumin.[41] Moreover, the regeneration of curcumin was found to be 100% at low superoxide without any chemical changes and the regeneration of curcumin was decreased to 60% at high superoxide concentration because of the inhibition of superoxide activity. Furthermore, Mishra *et al.* also explained that the mechanism of action of curcumin on superoxide was due to the reaction of 1 mole of curcumin with 6 mole of anionic radical forming perhydroxyl radical that subsequently reduces to oxygen and hydrogen peroxide. Research also unveiled striking photophysics and photodynamic

properties of curcumin through spectroscopical studies. The excited state photophysics of curcumin explained that it gets excited at a sub-nanosecond timescale and transfers hydrogen atoms.[42] Mukergee *et al.* studied fluorescence anisotropy and linear dichroism of curcumin by demonstrating that the transition moments for the long wavelength absorption and emission band is along the molecular axis and that intermolecular hydrogen atom transfer is directly dependent on intermolecular conformational changes.[43] Adhikary *et al.* studied excited-state intramolecular hydrogen atom transfer (ESIHT) and solvation through ultrafast fluorescence up-conversion spectroscopy. They suggested that curcumin undergoes deuteration in ethylene glycol and methanol exhibiting an isotropic effect at a timescale 100 ps whereas it remains unaffected in a shorter timescale of 12–20 ps in these solvents due to the solvation process of excited-state curcumin.[44] These studies concluded that the processes of ESIHT and the ultrafast excited state such as solvation help to increase the demand of curcumin in photodynamic therapy. Photochemical studies gained focus due to the low solubility of curcumin in aqueous medium. These studies researched new solvents that could help lift the relatively low solubility of curcumin. Concerning this quest, acid-base effects on the ultraviolet-visible spectrum of curcumin were monitored in different solvents such as ethanol, methanol, acetic acid and trifluoroethanol. It was observed that the increase in the acid concentration was directly proportional to the fluorescence decay rate of curcumin.[45]

Furthermore, the radical scavenging mechanism was studied for curcumin and half-curcumin different solvents such as acetonitrile, chloroform, benzene, ethanol, methanol and Triton-X-100 (5%) aqueous at different pH by a stopped-flow spectrophotometer. It was observed that the radical scavenging activity is strongly affected by the solvent and pH conditions. The core reason for this activity is the enol structure of curcumin having intermolecular hydrogen bonding and phenol proton acid base dissociation equilibrium in half-curcumin and curcumin.[46]

2.9 Biological Activities of Non-volatile Compounds of Turmeric

Continuous research efforts on the potential biological benefits of curcuminoids are being reported. These studies provide ample evidence that curcuminoids exhibit antioxidant, anti-inflammatory, anti-acidogenic, anticancer, neuroprotective, cardioprotective, radioprotective, antifungal, antiviral, angiogenic properties.[2] Furthermore, innumerable studies demonstrate the different mechanistic pathways of these compounds, which are able to regulate their growth factors: fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor (TGF), β1 vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), hepatocyte growth factor (HGF); transcription factors: nuclear factor-kappa B (NF-κB), activator protein (AP-1), signal transducer and

activator of transcription (STAT), early growth response-1 (Egr-1), peroxisome proliferator-activator receptor-gamma (PPAR-γ), estrogen receptor element (ERE), nuclear respiratory factor (NRF2), phosphoprotein p53, β-catenin, CREB-binding protein (CBP), hypoxia inducible factor1 (HIF-1); inflammatory cytokines, protein kinase: c-Jun-N-terminal (JNK), 5-adenocine monophosphate activated protein kinase (AMPK), protein kinase B or Akt, cell signaling proteins (5-lipoxygenase), cyclooxygenase 2 (COX-2), tubulin and cell proliferation factors (c-myc, cyclin D1), to bring about their abilities and suppress, proliferate and inhibit tumor metastasis, inducing apoptosis. In addition, there are numerous ways in which their solubility, bioavailability and stability can be improved, which hinder establishing their potency as biologically active components. The structure of curcumin 1, 7-bis (4-hydroxy-3-methoxyphenyl) hepta-1, 6-diene-3, 5-dione contains two ferulic acid residues that are linked by a methylene bridge. Their antioxidant activity is due to the methylene and O-methoxy phenol group. Both the presence of tautomeric and aromatic structures are key responsible factors for the hydrophobicity as well as the hydrogen bond formation, inclusive of the flexibility of the linker group causing non-covalent interactions. β-diketone and α unsaturated bonds are responsible for covalent interaction with the protein thiol. Furthermore, the β-keto group helps in reducing the toxicity of metals along with their pro-oxidative activity by forming complexes with transition metals and chelation of these metals function through the O-methoxy phenolic group of curcumin. It is also noted that chelation can occur through the diketo group but the influence of the O-methoxy group of curcumin on the diketo group affects the chelation of metals. Thus, chemical modification of curcumin is beneficial for their enhanced biological activities. Studies prove that the natural analogs of curcumin and their reduced derivatives have ample potency being antioxidant, anticancer hepatoprotective and neuroprotective.[47,48] Therefore, recent focus on structural modification synthesis with functional substituents, formation of metal complexes and encapsulation with various encapsulates such as nano or micro particle formation have helped to overcome the shortcomings of low solubility and bioavailability[49] (Table 2.3).

2.10 Biosynthesis of Curcumin

Earlier studies have quoted through radiotracer investigations that there are two major biosynthetic mechanisms known for turmeric (Figure 2.6).[18] Firstly, retrieval of compounds from the condensation of intermediates and other molecules derived from the fatty acid chain and acetate pathways in the phenyl propanoid pathway. In this context, the structural elucidation highlighted that two cinnamoyl units play a major role in the biosynthesis of curcumin. Secondly, an alternative pathway consisting of a cinnamate starter having five malonate or acetate units that was followed by cyclization,

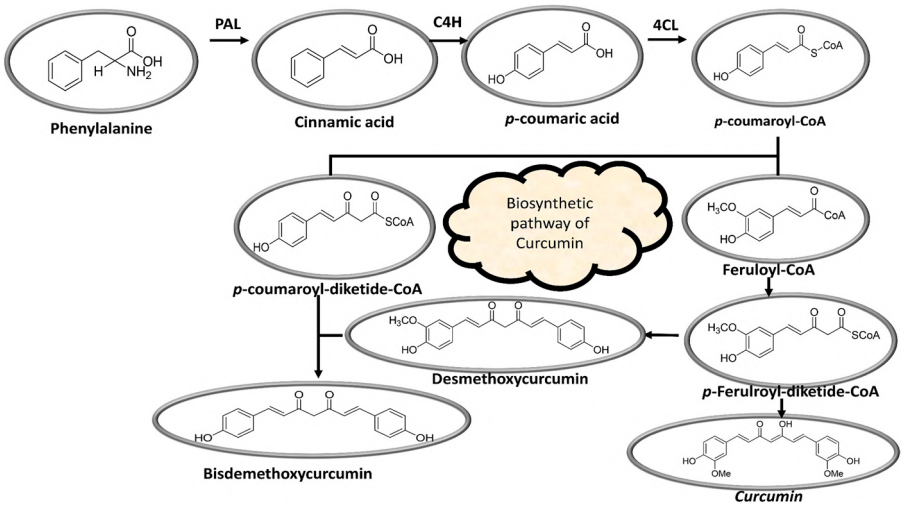


Figure 2.6 Biosynthesis of curcumin and its derivatives.

hydroxylation and methylation. In addition, the authors demonstrated a higher degree of phenylalanine incorporation into curcumin with the help of tracer investigation using 1–14C-phenylalanine with tritated cinnamic acids such as caffeic, coumarin and ferulic acid. Moreover, phenylalanine ammonia lyase (PAL) located in the lumen of the membrane is responsible for the initialization of the biosynthetic sequence in the germination phase, majorly acting in the leaves of the herb. Further investigation led to the discovery that one or more polyketide synthase or enzymes such as hydroxycinnamoyl-CoA obtained from the phenylpropanoid or malonyl-CoA pathways are curcuminoid synthase. These synthases are responsible for forming curcuminoids in turmeric. Later, it was reported that along with curcumin synthase, diketide-CoA synthase also plays a major role in the biosynthesis of curcuminoids. Feuloyl-CoA and malonyl-CoA are condensed to form feruloyldiketide-CoA under the catalytic activity of diketide-CoA synthase. The formation of curcuminoids from feruloyl-CoA and cinnamoyldiketide-*N*-acetylcysteamine was catalyzed by curcumin synthase. Also, high efficiency curcumin resulted when both the type II polyketide synthase, namely curcumin synthase and diketide-CoA synthase, are co-incubated in the presence of malonyl-CoA and feruloyl-CoA. From earlier research it was clear that polyketide synthase plays an important role in curcumin biosynthesis, so Deepa *et al.* recently studied the cDNA of this enzyme. The results of this study reflected that out of 63 transcriptome of Curcuma, CIPKS11 exhibits a higher expression level with an open reading frame of 1176 bp (base pair) and around 391 amino acid polypeptide encoding capacity carrying a molecular mass of 42.9 kDa.

This expression showcased amino acid differences in cyclization pockets, binding pocket and geometry shapers that surround the active site. Even through molecular docking studies, it was demonstrated that CLPKS11 has a high substrate affinity.[50] Thus, the intrinsic level of these expressions can be utilized for curcumin screening as well as curcumin biosynthesis. Similarly, the biosynthetic pathways of some compounds of turmeric are known, yet there are some other compounds in this medicinal plant whose biosynthesis is still to be elucidated.[18]

2.11 Stability Studies of Curcumin

To date, studies have highlighted that the stability of the compounds of turmeric, whether volatile or non-volatile, vary depending upon the extraction and storage conditions. The studies showed that the non-volatile compounds, especially curcumin, have poor gastrointestinal absorption and are subjected to pre-systematic transformation.[24] They are unstable in light, alkaline medium and high temperatures that limit further application. Poor solubility also results in instability and better understanding of this parameter could resolve stability problems. The reasons for poor dissolution of curcumin at higher temperatures are perhaps the exposure of polar groups due to the breaking of intermolecular hydrogen bonding. However, when cooled to a temperature of 25 °C, the stability increases due to intermolecular aggregates.[51] With the help of nanoindention, mechanical properties such as hardness and elasticity of curcumin polymorphs can be identified, which results in a better understanding of the stability of curcumin in the solid state. It was observed that the solubility of curcumin is highly dependent on its hardness and softer curcumin polymorphs are more soluble.[52] Furthermore, minimum light conditions also prove favorable for curcumin as it becomes more stable. pH also has a great impact on the stability of curcumin. The half-life of curcumin is mainly affected at different pH conditions, as it is found to have increased half-life in acidic pH in contrast to basic pH. Also, curcumin readily decomposes into vanilion, feruloymethane, ferulic acid and *trans*-6-(4-hydroxy-3-methoxyphenyl)-2, 4-dioxo-5-hexenal at 7 pH. Moreover, it is reported that 90% of curcumin likely degrades at a phosphate buffer of 7.2 pH at 30 minutes.[42] This is caused due to the changes in the molecular structures of curcumin at different pH states.[53] It is also observed that the stability of curcumin in aqueous solution is enhanced with the addition of an antioxidant. When sparged with methanol and air, the photo-oxidation stability of curcumin is greater among the curcuminoids.[54] Hence, knowledge of the physicochemical characteristics of curcumin is important as it would favor target-oriented activities. Moreover, more attempts to improve the stability of curcumin by incorporating it in different delivery systems such as emulsions, liposomes or solid lipid nanoparticles could be facilitated.

2.12 Conclusion

Time and again, turmeric has proved to be an idiosyncratic moiety having miscellaneous biological properties, which are utilized as antioxidant, anti-inflammatory, anticarcinogenic, anti-coagulant, antibacterial, anti-venom, antimutagenic, antifertility, anti-fungal, antiviral, antidiabetic, antiprotozoal, anti-ulcer, anti-tumor agents and so on. Turmeric holds a strong position in the economic market for its cosmeceutical, nutraceutical and medicinal properties. Research has proved through evidenced-based studies that it is safe and effective at high concentration exhibiting low toxicity. This kitchen spice has a flip side that has gained acclamation as a spice, food additive, flavoring and coloring agent. This chapter includes a brief introduction of the biological active compounds in this herb and their phytochemical classification. In fact, the revelations of their unique potential to the world can only be possible if each compound of turmeric, whether diminutive or not, is studied for structural elucidation, physicochemical parameters, analysis, biological activities, biosynthesis and stability. In addition, a basic understanding of the chemistry of turmeric is essential to understand the root cause of any therapeutic benefit that is exhibited. This knowledge could be also utilized for any further developments that can be made to amplify its pharmacological activities.

References

1. S. Agarwal, R. Mishra, A. K. Gupta and A. Gupta, in *Synthesis of Medicinal Agents from Plants*, ed. A. Tiwari and S. Tiwari, Elsevier B.V., 2018, pp. 105–125.
2. A. Amalraj, A. Pius, S. Gopi and S. Gopi, *J. Tradit. Complementary Med.*, 2017, **7**, 205.
3. K. I. Priyadarsini, *Molecules*, 2014, **19**, 20091.
4. G. K. Jayaprakasha, L. Jagan, M. Rao and K. K. Sakariah, *Trends Food Sci. Technol.*, 2005, **16**, 533.
5. H. Yang, Z. Du, W. Wang, M. Song, K. Z. Sanidad, E. Sukamtoh, J. Zheng, L. Tian, H. Xiao, Z. Liu and G. Zhang, *J. Agric. Food Chem.*, 2017, **65**, 4509.
6. C. Schneider, O. N. Gordon, R. L. Edwards and P. B. Luis, *J. Agric. Food Chem.*, 2015, **63**, 7606.
7. K. M. Nelson, J. L. Dahlin, J. Bisson, J. Graham, G. F. Pauli and M. A. Walters, *J. Med. Chem.*, 2017, **60**, 1620.
8. M. Attokaran, *Natural Food Flavors and Colorants*, Blackwell Publishing Ltd., Institute of Food Technologists, 2nd edn, 2011, vol. 98, pp. 391–398.
9. F. C. Meng, Y. Q. Zhou, D. Ren, R. Wang, C. Wang, L. G. Lin, X. Q. Zhang, W. C. Ye and Q. W. Zhang, in *Natural and Artificial Flavoring Agents and Food Dyes,* ed. A. M. Grumezescu and A. M. Holban, *Handbook of Food Bioengineering*, Academic press, Elsevier Inc, 2018, pp. 299–350.
10. A. M. Anderson, M. S. Mitchell and R. S. Mohan, Isolation of curcumin from turmeric, *J. Chem. Educ.*, 2000, **77**, 359.

11. A. Bagchi, *IOSR J. Environ. Sci., Toxicol. Food Technol.*, 2012, **1**, 1.
12. C. E. Green, S. L. Hibbert, Y. A. Bailey Shaw, L. A. D. Williams, S. Mitchell and E. Garraway, *J. Agric. Food Chem.*, 2008, **56**, 3664.
13. N. Nagavekar and R. S. Singhal, *Ind. Crops Prod.*, 2019, **134**, 134.
14. N. Kurmudle, L. D. Kagliwal, S. B. Bankar and R. S. Singhaln, *Food Biosci.*, 2013, **3**, 36.
15. N. N. Kurmudle, S. B. Bankar, I. B. Bajaj, M. V. Bule and R. S. Singhal, *Process Biochem.*, 2011, **46**, 423.
16. S. Haldar, G. C. Majumdar and H. N. Mishra, *J. Food Eng.*, 2015, **146**, 116.
17. S. V. Nampoothiri, P. C. Lekshmi, V. V. Venugopalan and A. N. Menon, *Asian Pac. J. Trop. Dis.*, 2012, S169.
18. K. P. P. Nair, *The Agronomy and Economy of Turmeric and Ginger, the Invaluable Medicinal Spice Crops*, Elsevier Inc., USA, 2013, pp. 179–204.
19. S. Nagarajan, I. R. Kubra and L. J. Rao, *J. Food Sci.*, 2010, **75**, H158.
20. G. Padmanaban and V. A. Nagaraj, in *Studies in Natural Products Chemistry*, ed. A. U. Rahman, Elsevier B.V., 2018, vol. 57, p. 179.
21. V. Singh, M. Pal, S. Gupta, S. K. Tiwari, L. Malkunje and S. Das, *Int. J. Maxillofac. Surg.*, 2013, **4**, 198.
22. S. Verma and V. Kumar, *Lek. Sirovine*, 2015, **35**, 3.
23. J. J. Chen, C. S. Tsai, T. L. Hwang, P. C. Shieh, J. F. Chen and P. J. Sung, *Food Chem.*, 2010, **119**, 974.
24. S. Li, W. Yuan, G. Deng, P. Wang, P. Yang and B. B. Aggarwal, *Pharm. Crops*, 2011, **2**, 28.
25. D. S. H. L. Kim and J. Y. Kim, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2541.
26. W. S. Liou, C. Lin, P. S. Lee, N. Kalyanam, C. T. Ho and M. H. Pan, *J. Funct. Foods*, 2016, **26**, 781.
27. M. Bacanlıa, S. Aydına, G. Tanerb, H. G. Göktas, T. Sahin, A. A. Basarane and N. Basaran, *Environ. Toxicol. Pharmacol.*, 2014, **38**, 774.
28. C. Mancuso and R. Santangelo, *Food Chem. Toxicol.*, 2014, **65**, 185.
29. A. Nair, A. Amalraj, J. Jacob, A. B. Kunnumakkara and S. Gopi, *Biomol*, 2019, **9**, 13.
30. T. Masuda, T. Maekawa, K. Hidaka, H. Bando, Y. Takeda and H. Yamaguchi, *J. Agric. Food Chem.*, 2001, **49**, 2539.
31. R. Lobo, K. S. Prabhua, A. Shirwaikara and A. Shirwaikar, *J. Pharm. Pharmacol.*, 2009, **61**, 13.
32. B. B. Aggarwal, W. Yuan, S. Li and S. C. Gupta, *Mol. Nutr. Food Res.*, 2013, **57**, 1529.
33. L. Zhang, Z. Yang, F. Chen, P. Su, D. Chen, W. Pan, Y. Fang, C. Dong, X. Zheng and Z. Du, *Ind. Crops Prod.*, 2017, **109**, 60.
34. A. K. Tyagi, S. Prasad, M. Majeed and B. B. Aggarwal, *Arch. Biochem. Biophys.*, 2016, **593**, 80.
35. Y. Li, M. Toscano, G. Mazzone and N. Russo, *New J. Chem.*, 2018, **42**, 12698.
36. J. N. Jacob, in *Studies in Natural Products Chemistry*, ed. A. U. Rahman, Elsevier B.V., 2016, vol. 48, pp. 101–135.
37. F. Payton, P. Sandusky and W. L. Alworth, *J. Nat. Prod.*, 2007, **70**, 143.

38. D. K. Agrawal and P. K. Mishra, *Med. Res. Rev.*, 2010, **30**, 818.
39. P. Limtrakul, W. Chearwae, S. Shukla, C. Phisalphong and S. V. Ambudkar, *Mol. Cell. Biochem.*, 2007, **296**, 85.
40. B. J. Douglass and D. L. Clouatre, *J. Am. Coll. Nutr.*, 2015, **34**, 347.
41. B. Mishra, K. I. Priyadarshini, M. K. Bhide, R. M. Kadam and H. Mohan, *Free Radical Res.*, 2004, **38**, 355.
42. S. Prasad, S. C. Gupta, A. K. Tyagi and B. B. Aggarwal, *Biotechnol. Adv.*, 2014, **32**, 1053.
43. A. Mukerjee, T. J. Sørensen, A. P. Ranjan, S. Raut, I. Gryczynski, J. K. Vishwanatha and Z. Gryczynski, *J. Phys. Chem. B*, 2010, **114**, 12679.
44. R. Adhikary, P. Mukherjee, T. W. Kee and J. W. Petrich, *J. Phys. Chem. B*, 2009, **113**, 5255.
45. Y. Erez, I. Presiado, R. Gepshtein and D. Huppert, *J. Phys. Chem. A*, 2012, **116**, 2039.
46. B. B. Aggarwal, I. D. Bhatt, H. Ichikawa, K. S. Ahn, G. Sethi, S. K. Sandur, C. Natarajan, N. Seeram and S. Shishodia, in *Turmeric- The Genus Curcuma*, ed. P. N. Ravindran, K. N. Babu and K. Sivaraman, CRC Press, 2006, pp. 297–368.
47. A. Asai and T. Miyazawa, *Life Sci.*, 2000, **67**, 2785.
48. P. Somparan, C. Phisalaphong, S. Nakornchal, S. Unchern and N. P. Morales, *Biol. Pharm. Bull.*, 2007, **30**, 74.
49. F. C. Rodrigues, N. V. A. Kumar and G. Thakur, *Eur. J. Med. Chem.*, 2019, **177**, 76.
50. K. Deepa, T. E. Sheeja, O. B. Rosana, V. Srinivasan, K. S. Krishnamurthy and B. Sasikumar, *Ind. Crops Prod.*, 2017, **97**, 229.
51. R. Jagannathan, P. M. Abraham and P. Poddar, *J. Phys. Chem. B*, 2012, **116**, 14533.
52. M. K. Mishra, P. Sanphui, U. Ramamurty and G. R. Desiraju, *Cryst. Growth Des.*, 2014, **14**, 3054.
53. M. Kharat, Z. Du, G. Zhang and D. J. McClements, *J. Agric. Food Chem.*, 2017, **65**, 1525.
54. L. C. Price and R. W. Buescher, *J. Food Biochem.*, 1996, **20**, 125.

CHAPTER 3

Geographical Variations of Turmeric and Curcumin

JOBY JACOB*[a], SHINTU JUDE[a] AND SREERAJ GOPI*[a]

[a]R&D Centre, Aurea Biolabs (P) Ltd, Kolenchery, Cochin, Kerala, India
*E-mail: joby.jacob@plantlipids.com, sreerajgopi@yahoo.com

3.1 Introduction

Turmeric is an important member of the *Zingiberacea* family and started its geo-history in India. The medicinal properties, economic importance of its value-added products and the potential towards improving human wellbeing has made turmeric a golden crop and it has been cultivated in many places around the world.

Although the origin is marked as being Indian, turmeric shows variations across areas and almost more than 90 scientifically accepted species of turmeric have been identified to date.[1] They are majorly cultivated in countries like India, Pakistan, Bangladesh, China, Taiwan, Thailand, Sri Lanka, East Indies, Burma, Indonesia, Costa Rica, Haiti, Jamaica, Peru, Brazil and Northern Australia.[2] Figure 3.1 shows a clear picture of the major turmeric producing countries along with their share in total production. From the long list of cultivating countries, it is understood that turmeric can grow in varying soil types, altitudes and weather. But for the productivity, growth and quality parameters, everything will vary depending on many factors such as agro-climatic parameters, cultivation practices, supply of nutrition, quality

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara,
Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

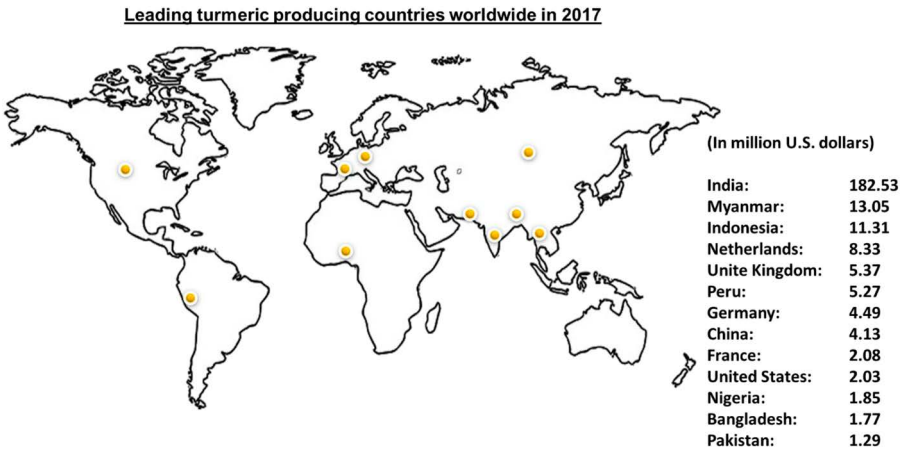


Figure 3.1 Worldwide production of turmeric in US dollars (millions) in 2017.

of planting material, plant genotype *etc.* Among the different climatic conditions, an optimum warm and humid climate is suitable for turmeric cultivation. It can grow well in tropical and subtropical climates of 20–30 °C, with sufficient rainfall and good irrigation.

Knowledge of the bioactive components such as curcuminoids present in turmeric add value to the coloring spice of history. The content of phytoconstituents – plant secondary metabolites – plays a key role in the properties of turmeric. The overall quality of turmeric and its products depends on these components. The quantity and quality of the product may vary depending on various ago-climatic factors and geographical peculiarities. Studies have proved that, with the variation in agro-climatic zones, the composition of the constituents of turmeric vary by a wide range.[3] The influence of habitat and agro-climatic conditions on the production and product optimization of natural products is one of the major challenges in product standardization and commercialization. The impact of geographical and environmental parameters such as climate, water, soil, altitude, rainfall, temperature *etc.* are very important factors of interest while dealing with turmeric production and quality.[3,4] Knowledge of these effects can positively alter the cultivating parameters, in order to meet the needs for higher quantity and quality of large-scale productions.

The basic touchstone of the natural product industry is agriculture. So, the industrial aspects will affect the modes, requirements and considerations of the growers. It will result in a search for better yield and quality. In the case of turmeric, the value of the material depends on the appearance, content of minerals and phytochemicals *etc.* Many studies have been conducted to relate the yield with cultivars, genotypes, locations, environments, *etc.*[4] Presentation of evidential data and systematic comparisons will help to sort out the best options for high quality products.

Altogether, the economic and qualitative traits of turmeric comprise curcumin content, oleoresin content, content and components of rhizome oil and leaf oil.[5]

3.2 Studies on the Variations of Curcumin Content

The color and many of the activities for which turmeric is widely cultivated are the properties of curcuminoids. So, the content of curcuminoids is an important quality parameter and hence control of it during cultivation is a commonly investigated area. Variations in curcuminoids in a range of 3.7–5.9% with different accessions were recorded in a farm study conducted in India.[6] Within another group of 120 turmeric accessions, there was a variation in the content of curcuminoids of 2.8–10.9%.[7] Curcumin, the dominant curcuminoid, showed a deviation of 0.61–1.45% in North Indian turmeric samples.[8] At the same time, samples from different parts of India proved to have a varying curcuminoid content ranging from 0.1212% to 2.3536%.[9]

Studies of Thailand turmeric reported curcumin in a range of 1.28–6.6% and total curcuminoids in the range of 0.46–10.23%.[10,11] The content of curcuminoids was assessed for turmeric samples from Indonesia, and was found to vary from 0.53% to 5.33%.[12] A study conducted in Sri Lanka focused on the maturity effects on curcumin content of turmeric. Curcumin content was increased up to a particular point and declined with further maturity, showing a range of 4.85–8.08% in the dry rhizomes.[13] All these studies point towards the significant diversity of turmeric varieties in terms of curcumin content, which can vary by a large range, on scales from less than 0.15% to more than 11%.

3.3 Studies on the Variation in Essential Oil Content

Curcumin is celebrated more than anything else in turmeric. However, the features, properties and biological activities of turmeric oil have found their own way to make a mark. In the era of natural products, turmeric essential oil is a very important ingredient for many applications. It is found to be a bioavailability enhancer and is a potential anti-inflammatory, antioxidant and anticancer agent. Turmeric oil is a potential choice in perfumery as well as in aromatherapy.[14] There are many studies that have attempted the profiling of the chemical constituents and pharmacological activities of turmeric essential oil.[15] The content and components of turmeric volatile oil were found to vary with the maturity of the rhizome. The monoterpene content decreases and the sesquiterpene content increases with maturity in the turmeric fingers.[13] The total turmeric oil content was found to vary in a range of 3.32–8.19% among different accessions from Uttar Pradesh, India.[6] In the samples collected from all over India, the variation was 1.5–9%. These variations were accounted for by the varietal differences, origin, method of curing turmeric, its age of harvesting and the conditions of distillation.[7]

3.4 Genetic Variations and Environmental Factors on the Yield and Quality of Turmeric

As in other organisms, genes also play a vital role in the behavior and properties of plants. Genetically, turmeric is a cross-pollinated, triploid species. Studies have proved that the chromosome numbers of turmeric can vary from $2n = 63$ to $2n = 86$.[16] Research studies dealing with characterization and improvement of turmeric encounter hurdles from environmental and genetic factors and their interactions.

3.4.1 Factors with the Curcumin *Synthase* (CURS) Gene

Curcumin synthase (*CURS*) consists of three enzyme isoforms present in the rhizomes and leaves of the turmeric plant and part of curcumin synthesis pathway. Data on the variations in the differential expression of the *CURS* gene, across different agro-climatic zones and its correlations with curcumin content, deliver useful genetic knowledge of turmeric. In a particular cultivar, the *CURS* expression does not cause any change to the curcumin content at different experimental zones, while keeping a positive correlation with the curcumin content in different harvesting phases. These functions enable the possibilities of site-specific cultivation, in order to obtain high curcumin content.[17] Even the genetic features were experimentally confirmed with field experiments.

3.4.2 Genetic Diversity in Different Agro-climatic Regions

Sufficient knowledge of genetic diversity and its effects helps to combine favorable characteristics and standardize the breeding programs, as per the requirements and thus to meet the demand for higher production and yield of phytochemicals. Considering this context, many studies have been conducted to establish the genetic factors of turmeric. While comparing the chemical and morphological features of 84 different turmeric accessions from various geographical regions of India, there was notable genetic variations in plant vigor, plant height, leaf mass, content and components of leaf essential oil, rhizomes number and yield, oil and curcumin content of rhizome, components of rhizome essential oil, *etc.* The study revealed many interesting correlation pairs such as rhizome mass and curcumin yield, leaf oil yield and rhizome curcumin yield, leaf mass and rhizome mass, *etc.* Along with the genetic peculiarities of different accessions, the data provide the parameters for chemotype identification and quality.[18] The interrelations between yield parameters and active components were also studied for the selection of a potential cultivar.[19]

The environment to which the genotype is exposed is an important factor in determining the plant's character. Variations in yield and phytochemical content with geographical properties were studied extensively. One genotype

producing high yield and phytochemical content in a particular agro-climatic region may not provide the same in another geographical location. Interpretations of these interactions are consolidated as genotype environment interactions (GEI). Stable genotypes with few GEI are preferred for improvement programs as the environmental and genetic interactions are very predictable for them.[20] Wide variation was observed in the yield and quality of properties among different turmeric germplasm. Considering these facts, effective germplasm selection can be made based on the phenotypic variances.[9] A field experiment on ten different turmeric genotypes brought to light the better yielding varieties suitable for the low input rain-fed agricultural system of Odisha, India. Furthermore, the significant effects of interactions between varieties and nutrient treatments in the same agro zone towards the yield and curcumin content was explored in the same work.[21] Direct impact of environmental factors on many of the biochemical, morphological, yield and quality traits were described by Vinodhini *et al.*, which evidenced the masking effect of geographical factors over genetic factors.[22]

3.4.3 Effect of Agro-climatic Zones in a Single Cultivar

Variations in agro-climatic conditions such as rainfall, humidity, temperature, soil conditions, nutrients availability *etc.* results in varied production of plant secondary metabolites. In the case of turmeric, the effects are notable for curcumin and oil content. A recent study provides sufficient evidential experimental data for the effect of different 'nature factors' on the quantity and quality of curcumin and essential oil contents. A high yielding turmeric cultivar 'Surama' was cultivated in nine different agro-climatic zones, and the influence of different soil and environmental parameters was studied. The study unveiled the controlling elements that determine curcumin content, and rhizome and leaf essential oils. Altitude, nitrogen content, soil pH and potassium content play a major role in determining curcumin content, while temperature and phosphorus content influences leaf essential oil. The levels of nitrogen phosphorous and potassium affect rhizome essential oil.[23]

It is proved that many of the improved, released varieties of plants exhibit greater productivity in environments that are similar to their original place of origin. Bansal *et al.* characterized the yield and essential oil content of rhizomes and leaves for *Curcuma longa* L. cv Roma, cultivated in the subtropical agro-climate of the Indo-Gangetic Plains of Lucknow, India.[24] The same cultivar was examined for the effect of soil, environment and other agro-climatic properties in growth and yield parameters in ten different agro-climatic zones. The study's results relate the factors behind growth and high yield of phytochemicals. On comparing the results, the rhizome yield and curcumin content were recorded in South Eastern Ghat and Eastern Ghat high land. Eastern Ghat high land is the releasing research station of the variety, and for good yield, a similar climatic and environmental condition is preferred.

At the same time, the North Eastern Coastal plain is recommended for better leaf essential oil yield and South Eastern Ghat as well as the North Eastern Coastal plain for rhizome essential oil.[25]

3.4.4 Effect of Agro-climatic Zones on Different Cultivars

The primary concern of agricultural studies is yield/productivity and many of the turmeric cultivars have been subjected to studies on growth and yield parameters. All of these studies resulted in a huge set of comparative data regarding growth attributes such as number of tillers, petiole length, number of leaves, leaf area, plant height, primary finger length, number of leaves per clump *etc.*, and yield attributes such as fresh rhizome yield, curing percentage, period of maturity, number of rhizomes *etc.*[26–33]

Later, studies on the variation in phytochemical compounds with different cultivars came into the picture, along with concerns regarding yield. A two-year study on different cultivars delivered data on their physical and biochemical parameters. Within ten cultivars, the yield/plant was varied from 191.46 to 303 g; curcumin varying in range of 3.7–5.9% and the essential oil varying from 3.32% to 8.19%.[6] Studies on genetic variability and association among different genotypes identified the traits that have positive correlations with yield and curcumin content.[9] Also, examinations on the qualitative traits of turmeric from different locations evaluated the potential genotypes for each desired trait and identified Surama as a high curcumin content yielding variety and Lakadong as a high essential oil yielding variety.[5] M. Anandaraj *et al.*, reported the variation of curcumin content as well as fresh and dry yields of turmeric from place to place, after examining the performance of eleven different genotypes across different environments all over India. Some of the varieties performed well for specific traits in specific locations. The study put forward three potential varieties of turmeric – Mega turmeric 1, IISR Prathiba and IISR Kedaram – as stable genetic sources having high yield and curcumin content across environments.[20] The results of phytochemical parameters and yields analyzed from 120 turmeric accessions from all over India are helpful evidential data on the variation of curcumin in contents of curcumin, essential oil and oleoresin and curing percentage, according to the accessions. While the cultivar 'Konni' gave the maximum oleoresin content (19.2%), 'Edapalayam' possessed the highest curcumin content (10.9%).[7] Figure 3.2 depicts the influence of geographical and climatic effects on turmeric cultivation.

3.5 Agricultural Study Patterns

Agricultural studies comprise many patterns. As per the requirements, facilities and style of study of the mode of execution will be different. It could be greenhouse studies or field trials. In the case of turmeric, every possible trial has been conducted worldwide.

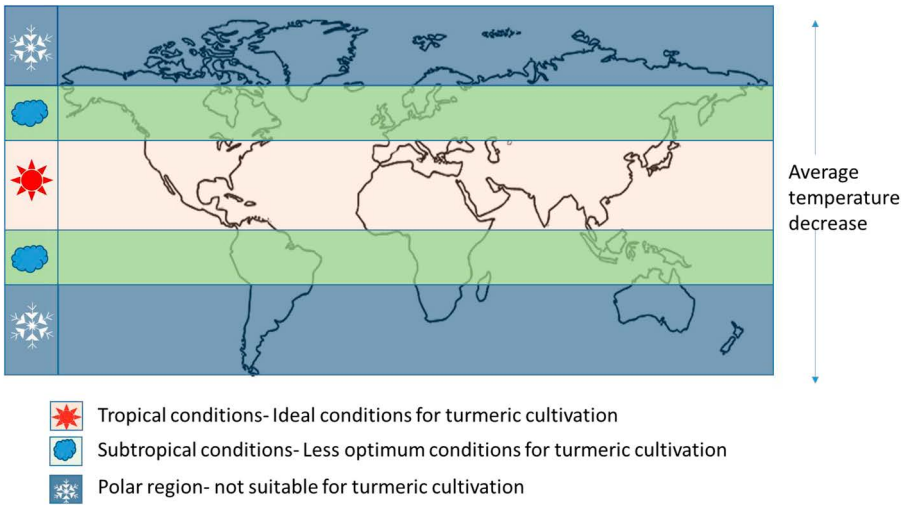


Figure 3.2 Impact of climatic zone changes in turmeric cultivation.

3.5.1 Experiments by Greenhouse Studies

Greenhouse studies serve as miniature cross sections of field studies. 'Greenhouses' are structures with walls and roofs made of transparent materials where the experimental conditions can be controlled and results can be obtained in a short time span. The influences of fertilizer treatments, mineral concentrations and plant density on the growth of turmeric plants were studied in a greenhouse. Within six months, the optimum conditions for multiple factors affecting the concentrations of curcuminoids were identified.[34]

3.5.2 Field Trials

Field trials require much effort and time; however, they produce real as well as reliable data. Turmeric may be the most experimentally cultivated crop around the globe in order to collect data. An on-farm experiment, conducted on different cultivars in the North-Western Himalayan region suggests the use of high yielding turmeric cultivars (HYTCs) for productivity, curcumin yield and profitability. This two-year study provided sufficient data on the research methodologies to be adapted for the remote hill agro-ecologies.[35] Even in the same environmental, geographical and manuring conditions, different cultivars resulted in different yields and rhizomes with varied content of minerals, curcumin and essential oil.[6] In such an environment, the phenotypic co-efficient of variance (PCV) estimates show higher values than genotypic co-efficient of variance (GCV).[9,20]Based on the level of curcumin content and yield, the performance of eleven different genotypes across different environments was examined. The study put forward three potential varieties of turmeric – P44, P36, P40 – as stable genetic sources having high

yield and curcumin content.[36] A field study on the performance of different genotypes and the effect of nutrient management practices on the phytochemical content as well as yield was worthy, as the study reported significant interactions between the parameters. Furthermore, the results have been used to suggest better practices and genotypes to be adapted for the Kandhamal district of Odisha.[21]

3.6 Molecular Markers' Studies

Genotypic diversity recording is important in the geo-history and identification of plants. Characterization of plants using morphological data and qualitative traits often fails, as these properties are vulnerable to environmental factors. So, plant identification should be based on a stable platform. For plants like turmeric (cultivated worldwide, researched enormously) the small variations may not be identified, and there is a chance to group them mistakenly. Genetic improvement of plants can be achieved by germplasm selection, open pollinated progeny selection, hybridization and mutation breeding. For an effective genetic improvement, proper identification of the parent plant is the prime requirement. In this scenario, instead of morphological evaluations, molecular and genotype based characterization and grouping are important. Molecular and cytological markers fulfill this purpose.

Molecular marker techniques make use of molecular markers such as DNA, proteins, *etc.* for the characterization of organisms. Molecular marker techniques are accepted as having more potential than morphological or biochemical markers, as they are independent of the environmental factors and can be used for the efficient management of genetic resources, henceforth making the agro-climatic zone based genetic characterization possible.[37] For example, the possibility of a combination of random amplification polymorphism DNA (RAPD) analysis and curcumin analysis is successfully worked out for the profiling of different turmeric accessions. Evaluation of different accessions of turmeric could find clones for large-scale production.[38] Many studies have been conducted for genotype discrimination and identification. RAPD analysis, together with 4C nuclear DNA content, serve well for the assessment of genetic variation between 17 promising Indian cultivars with the same chromosome number. The intraspecific polymorphism ranged from 35.6–98.6%.[39] Genotype grouping of turmeric from India by using RAPD markers was seconded by their curcumin content analysis. These results were parallel to the intraspecific variation between two Indian turmeric accessions with the same number of chromosomes, which was found to be greater than 9%. The latter study put forward the possibility for intraspecific variation occurring as a result of adaptive targeted selection during long-term cultivation.[40] RAPD markers were successfully used for assessing the genetic variability of many closely related genotypes from Pakistan. Cluster analysis of the produced data could place the genotypes into different

groups, which exactly matched the geographical and morphological groupings.[41] But, the RAPD analysis of samples from the Philippines showed high genetic diversity without any location specification.[42] Broadening knowledge of the germplasm base is helpful in identifying the potential mothers for breeding programs.

In a study using microsatellite markers, a relatively low genetic variability was present among 39 Brazilian turmeric accessions, suggesting the possibility of the presence of a few genotypes in the area.[43] However, another genetic characterization study on *Curcuma longa* germplasm collections from Brazil, processed with simple sequence repeats (SSR) produced polymorphic patterns.[44] The genetic diversity of turmeric accessions was tested in ten different agro-climatic regions of India, by using DNA based molecular marker techniques: RAPD and inter simple sequence repeats (ISSR). The study has demonstrated the relationship between the genetic properties and geographical location. The accession from the Kandarpur delta region clearly forms an individual cluster due to a high content of silt and increased fertility of the soil.[45] Highly reproducible SSR markers were employed to assess the genetic relationships between turmeric genotypes. Genetic grouping of turmeric accessions from India by using SSR markers indicated a loose pattern, independent of geographical origin.[46] In all these studies, smaller genetic diversity is observed between the accessions from hill zones, while higher genetic diversity is experienced within plains and plateaus. It is explained that hilly areas are almost undisturbed and possess ecological adaptations, while the plain land region faces high disturbance of agricultural and research practices. The genetic variability of turmeric accessions was also attributed to the adaptability of crops in different agro-climatic conditions.[45]

Sequence characterized amplified region (SCAR) markers were used for the identification and determination of Curcuma species.[47] Turmeric samples, even on a large scale, could be analyzed for adulteration by using this specific, reproducible and sensitive method with SCAR markers.[48]

3.7 Different Techniques and Technologies Regarding Curcumin Content

Alongside traditional methodologies, new technologies are also used to evaluate, optimize and predict the geometrical variations of turmeric production and its physicochemical parameters. A. Akbar *et al.* developed an artificial neural network (ANN) prediction model from the data created out of different turmeric samples from different agro-climatic regions of Odisha. It was successfully used for the prediction of oil yield for turmeric at a particular site.[49] In some cases, a combination of Raman spectroscopy and multivariate partial least square regression (PLS-R) found potential for the prediction of curcumin content in the turmeric rhizomes.[50] ^{1}H NMR spectroscopy, coupled with High performance thin layer chromatography (HPTLC), provides an effective platform for correlating the production conditions of turmeric to

the metabolomics composition of products therefrom.[51] In another study, a TLC-densitometry was demonstrated as a simple, but powerful tool for the quantitative analysis of curcuminoids.[12] A combination of TLC, UV and FTIR methods was used successfully for the characterization of curcumin.[52]

3.8 Effect of Maturity in Curcumin Content

The productivity and assay of curcuminoids and essential oil components were studied for their variation with plant maturity. It was found that parameters such as rate of plant growth and biosynthesis, gene expression for curcuminoids *etc.* control the curcumin content, and the changes in these factors over time restrict the phytochemical content reaching its maximum at a certain stage of plant growth. On comparison with turmeric cultivated in tropical countries, those from Iran show an almost similar pattern for essential oil composition. A linear significant relation between the biomass and curcumin concentration was indicated by statistical analysis of turmeric of Thailand origin, cultivated and studied in Iran. But their increase rates were not parallel to each other. The yield and phytochemical content increased over the maturation of the plant. Studies on the effects of maturity on the yield and composition of turmeric rhizomes shine light on the appropriate time for harvesting. An Iranian phenological study on Thailand turmeric reported a maximum of 2.7% curcumin.[53] Along with the harvesting time and location of growth, processing of the rhizome also makes a mark on the quantity and quality of turmeric oleoresin from turmeric and its content of curcuminoids as well as antioxidant activity. The study put forward suggestions on the suitable harvesting period, in order to obtain high curcumin content (of 15.65–22.96%) and high oleoresin yield (of 14.21–14.87%) for turmeric cultivated in different areas of Jamaica.[54]

3.9 Studies on Growth, Yield and Quality Parameters with Agro-climatic Zones in India

In India, turmeric is not just a spice. It is a centerpiece of culture, religious rituals and medicine. Therefore, turmeric production has a recorded history of more than 4000 years. Now, this sacred spice is one of the highest earning commercial crops cultivated in India. The requirement for Indian turmeric is much more, and turmeric from India exceeds that from other countries in terms of both quantity and quality. There are many studies conducted all over the nation, focused on meeting these needs. The major turmeric producing states and their contribution towards the total production is given in Table 3.1.

India, having a wide variety of agro-climatic zones ranging from tropical to polar climates, is representative of the variety of climate zones in the rest of the world. So, the agricultural studies carried out in different parts of India can be adopted for cultivating practices in different countries, matching

Table 3.1 Different varieties of turmeric studied in India at different climatic conditions.

State at which the study conducted	Agro-climatic nature	Variety	Curcumin content (%)	Yield (t ha^{-1})		Reference
				Dry	Fresh	
Meghalaya	Mid-hill region	Lakadong	6.8–7.5	—	—	59
Tamil Nadu	Dry zone	BSR 1		11.45	—	67
		BSR 2		12.05	—	
		Salem	4.75	—	—	
West Bengal	Gangetic alluvial plains	Suguna	4.53	—	30.8	61
		Rajendra Sonia	4.16	—	32.04	
Uttar Pradesh	Tarai	Krishna		—	40.56	62
Karnataka	Dry zone	Salem		—	33.67	63
		PTS 24	7.2	—		
Maharashtra	Konkan	Salem	4.4	—	44.39	64
		CA-70-1	4.8	—	16.32	
	Marathwada	Prathibha		—	33.53	65
		Waigoan	5.74	—	—	
Kerala	Wayanad	Alleppey	6.2	—	—	68
Andhra Pradesh	Rainfed coastal areas	Roma	6.3	—	37.61	70

geographical peculiarities. As discussed earlier, planting methods, manuring, maturing, date of harvesting *etc.*, may affect the growth parameters, yield and phytochemical content of turmeric. According to climate changes, the yield and curcumin content of turmeric varies widely. The varietal stability of different turmeric genotypes was assessed across different environments in terms of curcumin content and yield. The study suggests that 'Mega' turmeric, IISR Prathibha and IISR Kedaram are stable genotypes, possessing stable yield and quality traits under different environmental conditions all over India, with predictable performance levels and less environmental interaction.[55] A delay in harvesting produced better results for yield and curcumin content from turmeric cultivated in Ludhiana (Punjab).[56] Although the methods of planting did not affect the results much, harvesting time played a similar role (the soil of the experimental field was loamy sand, normal with respect to pH (8.0) and electrical conductivity(EC) (0.2 dSm-1 at 25 °C), low in organic carbon (0.23%) and available nitrogen (210 kg ha^{-1}), medium in available phosphorus (17 kg ha^{-1}) and potash (207 kg ha^{-1})), which was in agreement with the studies of Kaur *et al.* A delay in the harvesting from November to mid-March resulted in an increase of yield as well as oil and curcumin content.[57] Chandra *et al.*, reported the high yielding and highest curcumin containing varieties of turmeric under the mid-hill conditions of Meghalaya. This three-year study evaluated the performance of 25 different genotypes and produced an important finding on the negative correlation between dry rhizome recovery and fresh rhizome yield per clump.

The work suggests PCT13, PCT 11, GL Puram and PCT15 to be the most yielding varieties under the mid-hill conditions of Meghalaya, and Lakadong to be a high curcumin containing variety.[58]

Different turmeric genotypes were evaluated for their performance in terms of growth, yield and quality parameters, while cultivating in the Gangetic alluvial plains of West Bengal.[59] A similar field experiment was conducted at Bareilly and identified 'Krishna' to be the highest yielding variety in the Tarai regions of Uttar Pradesh.[60] 'Salem' turmeric produced the highest yield and 'PTS-24' produced high curcumin content in the southern, dry zone of Karnataka.[61] In Sikkim, being the organic hub of India, only organic matters are permitted for input during cultivation. Hence, the crops produced in Sikkim are considered to be globally important. So, many turmeric varieties have also been assessed for their adaption to the agro-climatic peculiarities of the land. Studies conducted at the Konkan region of Maharashtra recommends Salem turmeric as the best yielding turmeric variety, with good growth traits, and CA-70-1 to contain the most curcumin.[62] Twelve different varieties of turmeric were cultivated in another field trial under Marathwada conditions at Parbhani, Maharashtra, where each of the traits have exhibited superiority in different parameters. The study included varieties with high qualities as recorded in previous studies conducted at different places. Surprisingly, the genotypes chose yet another parameter to show off. For example, 'Suvarna', which was recorded as a high curcumin yielding variety in the previous study, was superseded by 'Waigoan' (5.74%), and was awarded for high recovery, minimum moisture content and maximum sprout numbers per plant. Prathibha exhibited maximum yield (33.53 t ha^{-1}).[63] In Odisha, the Roma variety was subjected to the influence of different environmental factors on the growth and yield of phytochemicals. It was found that organic carbon and potassium from the soil positively affect the curcumin content up to a particular level, after which they exert a negative impact. Also, variations in the soil nutrients, altitude and environmental factors provide varied results for phytochemical components, rhizome yield, essential oil *etc.*[64] A later study proved a better performance of the Lakdong variety under the same climatic zone and nutrient treatments. It proposed 'lime @10% LR (lime requirement) + 50% organic + 50% inorganic' as the best among the five nutrient treatment compositions.[21]

Field experiments were conducted in Tamil Nadu, in order to find the appropriate field inputs to achieve better growth, yield and quality. Closer spacing and proper nitrogen plot treatment increased the production of turmeric and early planted crops resulted in better yield as well as curcumin content. The normal weather conditions of the field at Bhavanisagar are a mean annual rainfall of 659 mm received in 43 rainy days. The mean maximum and minimum temperatures are 33 and 22 °C, respectively. The soil of the experimental fields was well drained sandy loam, low in available nitrogen and phosphorous, and high in available potassium with near neutral pH. The crop was raised with irrigation from the Lower Bhavani Reservoir. These conditions favored the product yielding of the 'BSR 2' variety.[65] Salem

Table 3.2 Major state-wise cultivation area and production of turmeric in India.

State	Area (hectare)	Production (tons)
Telangana	51 000	294 000
Maharashtra	14 050	224 680
Andhra Pradesh	19 180	79 730
Karnataka	14 990	76 490
Gujarat	3710	73 150
Tamil Nadu	16 190	57 150
Orissa	27 860	54 500
West Bengal	18 000	45 500
Mizoram	7740	29 820
Hariyana	1500	22 000
Assam	17 110	19 170

turmeric produced comparatively good results for curcumin content in a study conducted at Tamil Nadu.[66] The highest yield and curcumin content were recorded for Alleppey turmeric in a study that dealt with turmeric varieties from Kerala.[67] These details are tabulated in Table 3.2. It is clear from the results that even the same variety of turmeric gave different curcumin contents and yields under varying environments. The strong impact of geographical variation in turmeric regarding the curcumin content exceeded a little, while comparing the effect of other factors like type of soil, amount of rainfall, method of cultivation *etc.* Curcumin content in turmeric samples collected from nine states of India, representing different agro-climatic zones, revealed the contour line pattern of curcumin content in relation to geographical variations.[68] Among the twelve varieties of turmeric studied in agency areas of North Coastal Andhra Pradesh, Roma exhibited superiority in all the yield, growth and quality traits. Here, under the general cultivation practices, Roma had a longer maturation time.[69]

In a study, a quality assessment of 184 turmeric accessions from all over India was carried out by considering curing percentage, oleoresin, essential oil and curcumin content as the parameters. Among the subjected accessions, 'Edapalayam' from the West coast of India exhibited the maximum curcumin content and 'Konni' produced maximum oleoresin.[7] Here, we can relate the production information from Table 3.1 to the agro-climatic peculiarities. From Figure 3.3, it is clear that the major turmeric producing states belong to related climatic regions.

3.10 Agricultural Practices and Processing Methods

Even with the same environmental conditions, turmeric can produce different results with different agricultural practices. However, for a sustained production of higher quality rhizomes for this long duration crop, a good supply of nutrients and fertilizing matter along with supporting weather is required. Use of organic nutrients is suggested for improving soil health by soil fertility

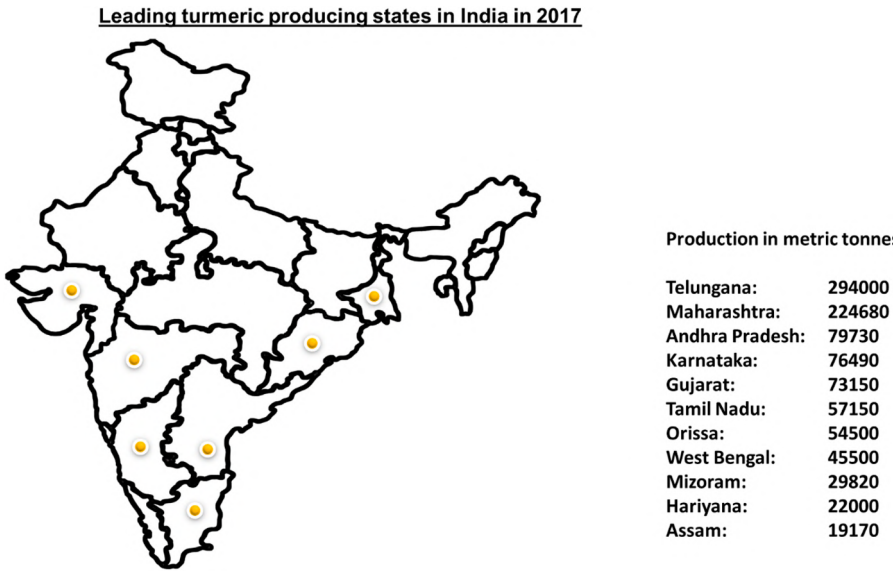


Figure 3.3 Leading turmeric producing states in India in metric tonnes in 2017.

enhancement, soil productivity retention and soil biomass increase.[70] The effects of different manuring and fertilizing techniques on the turmeric yield and quality were studied by field trials across different agro-climatic conditions. Field experiments with organic manures resulted in better rhizome yields with improved quality characteristics and enhanced soil fertility.[71] Among the many manures, poultry manure was found to deliver good responses in morphological, quality and yield parameters under an average annual rainfall of 1350 mm, in a 6.80–460 °C temperature range with an average humidity of 74%. Use of biofertilizer also increased the yields.[72] Not only the yield, but the growth and physicochemical parameters were found to be positively altered by the usage of organic manures.[73] The proper selection of type as well as amount of manures, fertilizers and minerals is important for the required quality and yield of turmeric.[70]

There are studies relating growth, yield and quality of turmeric with depth of planting, time and pattern, seed size, planting soil *etc.* Difference in soil can cause variations in root biomass, yield, color, curcumin and mineral content.[74] The temperature and planting date affect the emerging pattern, yield parameters and growth factors.[75] Yield and weed competition were markedly different while planting turmeric at different depths.[76] Furthermore, the planting pattern and distance between plants make a difference to weed interference and yield.[77] Also, the size and properties of the mother rhizome play a role in the growth and yield of turmeric.[78] The effects of planting material, planting method and plant density on the yield studied further in a different environment was in agreement with earlier studies.[79] Modifications

in micro-topography and mulching suppress weed growth and nutrient loss, which will result in improved rhizome yield and productivity.

The modulation of growth and metabolite synthesis in turmeric was also promoted by microorganisms. Studies have revealed that microbial organisms endophytes deliver positive effects for promoting plant growth, resistance capacity, metabolite synthesis and antioxidant activities.[80–82] Also, inoculation of bacterial stains promoted rhizome yield, weight, plant height morphological yields and curcumin content.[83,84] All of these significant effects are exerted by many microbes including bacteria and fungi, and are proved in many studies.[85–87]

3.11 Conclusions

Turmeric, even with a recorded history of millennia, still finds scope for further studies and research. There will not be any competitor for turmeric in the field of herbal medicine. The increased requirement and usage push turmeric production to yield better productivity and quality. Research in this field often encounters problems arising from environmental factors. So, agricultural research is supposed to consider the agro-climatic peculiarities, where the crop is meant to be grown. Here we have dealt with the efforts and achievements made to improve the production and phytochemical content of turmeric under different genetic and geographic conditions. From this information it is clear that irrespective of genetic properties, climate as well as other environmental features determine the yield, curcumin content and oil qualities in the turmeric varieties. Also, variation in each factor contributes differently towards the properties. Altogether, studies on the effects can reveal either suitable a variety for a particular agro-climate zone, or a suitable climatic zone for a particular variety.

References

1. N. Akarchariya, S. Sirilun, J. J. Ulsrigival and S. Chansakaowa, *Asian Pac. J. Trop. Biomed.*, 2017, **7**, 881.
2. N. Dosoky and W. Setzer, *Nutrients*, 2018, **10**, 1196.
3. J. I. L. Morison and D. W. Lawlor, *Plant, Cell Environ.*, 1999, **22**, 659.
4. S. Li, W. Yuan, G. Deng, P. Wang, P. Yang and B. B. Aggarwal, *Pharm. Crops*, 2011, **2**, 28.
5. S. Singh, R. K. Joshi and S. Nayak, *Ind. Crops Prod.*, 2013, **43**, 165.
6. S. Kumari, P. Singh and R. N. Kewat, *Int. J. Sci. Res. Publ.*, 2014, **4**(8), August 2014 Edition.
7. M. J. Ratnambal, *Plant Foods Hum. Nutr.*, 1986, **36**, 243.
8. K. Tanaka, Y. Kuba, T. Sasaki, F. Hiwatashi and K. Komatsu, *J. Agric. Food Chem.*, 2008, **56**, 8787.
9. S. Venugopal, A. Pariari, C. S. Karthik and P. Dineshkumar, *Bull. Environ., Pharmacol. Life Sci.*, 2017, **6**, 382.
10. P. Chavalittumrong and T. Dechatiwongse, *Thai J. Pharm. Sci.*, 1988, **13**, 317.

11. R. Thaikert and Y. Paisooksantivatana, *Kasetsart J.*, 2009, **43**, 507.
12. D. S. C. Wahyuni, A. N. Artanti and Y. Rinanto, *Conf. Ser.: Mater. Sci. Eng.*, 2018, **349**, 012015.
13. N. F. Cooray, E. R. Jansz, J. Ranatunga and S. Wimalasena, *J. Natl. Sci. Found. Sri Lanka*, 1988, **16**, 39.
14. A. Amalraj, A. Pius, S. Gopi and S. Gopi, *J. Tradit. Complementary Med.*, 2017, **7**, 205.
15. I. A. Oyemitan, C. A. Elusiyan, A. O. Onifade, M. A. Akanmu, A. O. Oyedeji and A. G. McDonald, *Toxicol. Rep.*, 2017, **4**, 391.
16. R. R. Nair and B. Sasikumar, *Cytologia*, 2009, **74**, 153.
17. I. S. Sandeep, S. Das, N. Nasim, A. Mishra, L. Acharya, R. K. Joshi, S. Nayak and S. Mohanty, *Plant Physiol. Biochem.*, 2017, **118**, 348.
18. S. Kumar, R. P. Bansal, S. N. Garg, R. Goel, M. M. Gupta, J. R. Bahl and V. Singh, *Proc. Indian Natl. Sci. Acad.*, 2014, **80**, 143.
19. S. Singh, S. Sahoo, S. Dash and S. Nayak, *Ind. Crops Prod.*, 2014, **62**, 373.
20. R. Rajyalakshmi and K. Umajyothi, *J. Spices Aromat. Crops*, 2014, **23**, 258.
21. A. Mishra, A. Mishra, B. S. Rath, S. K. Mohanty and B. Behera, *J. Spices Aromat. Crops*, 2017, **26**, 114.
22. V. Vinodhini, B. Senthamizh Selvi, S. Balakrishnan and R. Muthuragavan, *Electron. J. Plant Breed.*, 2018, **9**, 1060.
23. I. S. Sandeep, A. Kuanar, A. Akbar, B. Kar, S. Das, A. Mishra, P. Sial, P. K. Naik, S. Nayak and S. Mohanty, *Ind. Crops Prod.*, 2016, **85**, 229.
24. R. P. Bansal, J. R. Bahl, S. N. Garg, A. A. Naqvi and S. Kumar, *Pharm. Biol.*, 2002, **40**, 384.
25. I. S. Sandeep, N. Sanghamitra and M. Sujata, *Indian J. Exp. Biol.*, 2015, **53**, 406.
26. S. K. Jagadish, *Selection of Rhizobacteria Antagonistic to Ralstonia Solanacearum E.F. Smith Causing Bacterial Wilt in Tomato and Their Biocontrol Mechanisms*, University Of Agricultural Sciences GKVK, Bangalore, 2000.
27. N. S. Anusuya, M.Sc. thesis, *Evaluation of Different Genotypes of Turmeric and Response of Turmeric cv. Salem to Organic and Inorganic Fertilisers*, University Of Agricultural Sciences GKVK, 2004.
28. J. C. Jana and B. Bhattacharya, *Environ. Ecol.*, 2001, **19**, 463.
29. K. S. Kumar and D. S. Yadav, *Indian J. Hill Farming*, 2001, **14**, 147.
30. S. P. Singh and R. Prasad, *Int. J. Plant Sci.*, 2006, **1**, 22.
31. N. A. Deshmukh, S. U. Gondane, P. S. Ingole and S. R. Patil, *J. Soil. Crops*, 2009, **19**, 88.
32. T. K. Hrideek, K. M. Kuruvilla, G. P. B. Indianumol, P. P. Menon, K. J. Madhusoodanan and J. Thomas, *J. Plant. Crops*, 2006, **34**, 178.
33. M. Anandaraj, D. Prasath, K. Kandiannan, T. J. Zachariah, V. Srinivasan, A. K. Jha, B. K. Singh, A. K. Singh, V. P. Pandey, S. P. Singh, N. Shoba, J. C. Jana, K. Ravindra Kumar and K. U. Maheswari, *Ind. Crops Prod.*, 2014, **53**, 358.
34. S. S. Roy and J. K. Hore, *J. Crop Weed*, 2012, **8**, 90.
35. A. K. Choudhary and S. Rahi, *Ind. Crops Prod.*, 2018, **124**, 495.
36. S. Singh, S. Sahoo, S. Dash and S. Nayak, *Ind. Crops Prod.*, 2014, **62**, 373.

37. P. Kumar, V. K. Gupta, A. K. Misra, D. R. Modi and B. K. Pandey, *Plant Omics*, 2009, **2**, 141.
38. N. Arya, O. Prakash, S. Kumar, Vivekanand and A. K. Pant, *Asian Pac. J. Trop. Dis.*, 2016, **6**, 70.
39. S. Nayak, P. K. Naik, L. K. Acharya and A. K. Pattnaik, *Cytologia*, 2006, **71**, 49.
40. J. Leong-Škorničková, O. Šída, V. Jarolímová, M. Sabu, T. Fér, P. Trávníček and J. Suda, *Ann. Bot.*, 2007, **100**, 505.
41. H. U. Jan, M. A. Rabbani and Z. K. Shinwari, *J. Med. Plants Res.*, 2011, **5**, 823.
42. E. A. Corcolon, A. C. Laurena and M. L. Dionisio-Sese, *Procedia Chem.*, 2015, **14**, 157.
43. M. S. Sigrist, J. B. Pinheiro, J. A. A. Filho and M. I. Zucchi, *Genet. Mol. Res.*, 2011, **10**, 419.
44. M. S. Sigrist, J. B. Pinheiro, J. A. A. Filho and M. I. Zucchi, *Crop Breed. Appl. Biotechnol.*, 2011, **11**, 70.
45. S. Singh, M. K. Panda and S. Nayak, *Ind. Crops Prod.*, 2012, **37**, 284.
46. S. Senan, D. Kizhakayil, T. E. Sheeja, S. Bhas, A. Bhat and P. Va, *Acta Bot. Croat.*, 2013, **72**, 407.
47. T. J. Singh, R. K. Patel, S. N. Patel and P. A. Patel, *Int. J. Curr. Microbiol. Appl. Sci.*, 2018, **7**, 552.
48. K. Dhanya, S. Syamkumar, S. Siju and B. Sasikumar, *Food Res. Int.*, 2011, **44**, 2889.
49. A. Akbar, A. Kuanar, J. Patnaik, A. Mishra and S. Nayak, *Comput. Electr. Agric.*, 2018, **148**, 160.
50. I. M. A. G. Wirasuta, C. I. T. R. Dewi, N. P. L. Laksmiani, I. G. A. M. Srinadi and D. P. Putra, *Indones. J. Pharm. Sci. Technol.*, 2018, **5**, 88.
51. A. Booker, D. Frommenwiler, D. Johnston, C. Umealajekwu, E. Reich and M. Heinrich, *J. Ethnopharmacol.*, 2014, **152**, 292.
52. H. Pawar, M. Karde, N. Mundle, P. Jadhav and K. Mehra, *Med. Chem.*, 2014, **4**, 588.
53. G. Asghari, A. Mostajeran and M. Shebli, *Res. Pharm. Sci.*, 2009, **4**, 55.
54. C. E. Green, S. L. Hibbert, Y. A. Bailey-Shaw, L. A. Williams, S. Mitchell and E. Garraway, *J. Agric. Food Chem.*, 2008, **56**, 3664.
55. M. Anandaraj, D. Prasath, K. Kandiannan, T. J. Zachariah, V. Srinivasan, A. K. Jha, B. K. Singh, A. K. Singh, V. P. Pandey, S. P. Singh, N. Shoba, J. C. Jana, K. R. Kumari and K. U. Maheswari, *Ind. Crops Prod.*, 2014, **53**, 358.
56. B. Kumar and B. S. Gill, *J. Spices Aromat. Crops*, 2009, **18**, 22.
57. S. Kaur, M.Sc thesis, Punjab Agricultural University, Ludhiana, 2001.
58. R. Chandra, S. Govind and A. R. Desai, *J. Appl. Hortic.*, 1999, **1**, 142.
59. S. Venugopal, A. Pariari, C. S. Karthik and P. Dineshkumar, *Bull. Environ., Pharmacol. Life Sci.*, 2017, **6**, 382.
60. A. S. Chaudhary, S. K. Sachan and R. L. Singh, *Indian J. Crops Sci.*, 2006, **1**, 189.
61. J. Venkatesha and S. Siddalingayya, *Acta Hortic.*, 2016, **1125**, 339.
62. P. V. Sinkar, P. M. Haldankar, R. G. Khandekar, S. A. Ranpise, G. D. Joshi and B. B. Mahale, *J. Spices Aromat. Crops*, 2005, **14**, 28.

63. U. B. Jadhav, M.Sc. thesis, Effect of Foliar Application of Nutrients on Yield, Quality and Uptake of Soybean (Glycine max (L) Merrill), Vasantrao Naik Marathwada Agricultural University, 2018.
64. I. S. Sandeep, S. Nayak and S. Mohanty, *Indian J. Exp. Biol.*, 2015, **53**, 406.
65. K. Kandiannan and K. K. Chandaragir, *Indian J. Agric. Sci.*, 2006, **76**, 432.
66. S. Muthuswamy and H. A. Shah, *Indian Cocoa Arecanut Spices J.*, 1982, **5**, 77.
67. A. Muralidharan and M. N. Ramankutty, *Agric. Res. J. Kerala*, 1976, **14**, 191.
68. A. Geethanjali, P. Lalitha and M. J. Firdhouse, *Int. J. Pharm. Chem. Res.*, 2016, **2**, 55.
69. R. K. Kurimella, *Progress. Res. An Int. J.*, 2015, **10**, 2417.
70. S. Datta, J. C. Jana, P. T. Bhaisare and K. H. Nimbalkar, *J. Appl. Nat. Sci.*, 2017, **9**, 1981.
71. S. K. Sanwal, K. Laxminarayana, R. K. Yadav, N. Rai, D. S. Yadav and M. Bhuyan, *Indian J. Hortic.*, 2007, **64**, 444.
72. S. R. Pratap, P. K. Jain, A. Tiwari, A. K. Verma and S. K. Dwivedi, *Int. J. Agric. Sci.*, 2015, **7**, 824.
73. S. S. Roy and J. K. Hore, *J. Crops Weeds*, 2012, **8**, 90.
74. A. Hossain and Y. Ishimine, *Plant Prod. Sci.*, 2005, **8**, 482.
75. Y. Ishimine, M. A. Hossain, K. Motomura, H. Akamine and T. Hirayama, *Jpn. J. Trop. Agric.*, 2004, **48**, 10.
76. Y. Ishimine, M. A. Hossain, Y. Ishimine and S. Murayama, *Plant Prod. Sci.*, 2003, **6**, 83.
77. A. Hossain, Y. Ishimine, K. Motomura and H. Akamine, *Plant Prod. Sci.*, 2005, **8**, 95.
78. A. Hossain, Y. Ishimine, H. Akamine and K. Motomura, *Plant Prod. Sci.*, 2005, **8**, 86.
79. B. Kumar and B. S. Gill, *J. Spices Aromat. Crops*, 2010, **19**, 42.
80. C. Anisha, J. Mathew and E. K. Radhakrishnan, *Int. J. Biol., Pharm. Allied Sci.*, 2013, **2**, 593.
81. S. Lee, M. Flores-Encarnación, M. Contreras-Zentella, L. Garcia-Flores, J. E. Escamilla and C. Kennedy, *J. Bacteriol.*, 2004, **186**, 5384.
82. Bustanussalam, F. Rachman, E. Septiana, S. J. Lekatompessy, T. Widowati, H. I. Sukiman and P. Simanjuntak, *Pak. J. Biol. Sci.*, 2015, **18**, 42–45.
83. N. Suryadevara and P. Ponmurugan, *Int. J. Biol. Technol.*, 2012, **3**, 39.
84. A. Kumar, R. S. Vandana, M. Singh, P. P. Singh, S. K. Singh, P. K. Singh and K. D. Pandey, *Biocatal. Agric. Biotechnol.*, 2016, **8**, 1.
85. A. Karnwal and M. Guleria, *Annals of "Valahia" University of Târgovişte*, 2011, p. 34.
86. S. C. Dutta and B. Neog, *Sci. Hortic.*, 2016, **204**, 179.
87. R. El-Hawaz, N. Tharayil, W. Bridges and J. Adelberg, *Ind. Crops Prod.*, 2016, **83**, 186.

CHAPTER 4

Turmeric – Active Ingredients Other than Curcuminoids

AUGUSTINE AMALRAJ*[a], NIMISHA PULIKKAL SUKUMARAN[a] AND SREERAJ GOPI*[a]

[a]R&D Centre, Aurea Biolabs (P) Ltd, Kolenchery, Cochin–682 311, Kerala, India
*E-mail: augustin14amal@gmail.com, sreerajgopi@yahoo.com

4.1 Introduction

Natural products have been used in traditional medicines for thousands of years, and have shown assurance as a source of components for the development of new drugs. Turmeric (*Curcuma longa* Linn) is a member of the Zingiberaceae family and is cultivated in tropical and subtropical regions around the world. It originates from India, Southeast Asia and Indonesia. The powder form of turmeric is extensively utilized as a coloring and flavoring agent in curries and mustards. Traditionally, turmeric has been used for medical purposes in Asian countries such as India and China for the treatment of jaundice and other liver ailments for many centuries. Moreover, it is one of the most popular medicinal herbs, with a wide range of pharmacological activities due to its antioxidant, anti-protozoal, anti-venom, anti-microbial, anti-malarial, anti-inflammatory, anti-proliferative, anti-angiogenic, anti-tumor and anti-aging properties. Furthermore, it has also been used to treat ulcers, parasitic infections, various skin diseases, anti-immune diseases and to cure the symptoms of colds and flu. The pharmacological activity of this rhizome

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

has been recognized as mainly from curcuminoids consisting of curcumin (CUR) and two related compounds demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). Curcuminoids are commonly used as coloring agents as well as food additives due to their bright orange-yellow color. The World Health Organization (WHO) stated that the acceptable daily intake of curcuminoids as a food additive is in the range of 0–3 mg kg^{-1}. Curcuminoids and turmeric products have been characterized as safe by the Food and Drug Administration (FDA) in the USA. Curcuminoids have achieved potential therapeutic importance to cure immune related, metabolic diseases and cancer due to a vast number of biological targets and almost no side effects.[1,†]

There are more than 6000 published works on curcumin (diferuloylmethane), which is the most extensively studied component of this spice. Curcumin makes up between 2 and 5% of dried turmeric root. Curcumin is believed to be responsible for most of the activities of turmeric, recent research has shown that other curcuminoids in turmeric are as effective as or even more effective than curcumin containing turmeric. Many components of turmeric, particularly phenolic compounds, terpenes including sesquiterpenes, monoterpenes, diterpenes, triterpenes and flavonoids *etc.* are reported to possess potent biological activities.[2]

4.2 Chemical Composition of Turmeric

Turmeric (*Curcuma longa* L.) is the most chemically investigated species. Extracts of turmeric are prepared by using solvents like ethanol, methanol, water, or ethyl acetate. Turmeric extracts are both water-soluble and water-insoluble. The water-insoluble fraction consists of turmeric oil and polyphenols, which are primarily diarylheptanoids, also called curcuminoids. The latter consists of CUR (80%), DMC (18%) and BDMC (2%). However ethanol (70%) is most favored for the extraction of curcuminoids from turmeric,[2] followed by hexane extraction after hydrodistillation, which is the most commonly chosen process for separating essential oils from turmeric. CUR, DMC and BDMC account for over 30% of the ethanol extract of turmeric.[2,3] One more important constituent of turmeric is turmeric oil, which is responsible for the aromatic taste and smell of the spice. Dried turmeric usually contains 1.5 to 5% essential oils, which are dominated by sesquiterpenes.[4]

To date, approximately 235 compounds, primarily phenolic compounds and terpenes, have been identified in this spice. Previous phytochemical investigations on turmeric led to the isolation and identification of many types of secondary metabolites including monoterpenes, sesquiterpenes, diterpenes, triterpenes, curcuminoids, and the products conjugating curcuminoids with monoterpenes or sesquiterpenes, phenolic compounds, flavonoids, saccharides, steroids, fatty acids and alkaloids. In the turmeric oil, around 109 sesquiterpenes have been identified in turmeric oil, including 54 bisabolanes, seven guaianes, six germacrones, four selinanes, three

[†]Adapted from ref. 1 with permission from Elsevier. Copyright 2017.

santalanes, two elemenes, two caryophyllanes, as well as the following: aristolene, acorane, carbrane, cedrane, bergamotane, himachalene and sesquisabinane. Moreover sesquiterpenes, 68 monoterpenes, five diterpenes, three triterpenes[5,6] and four steroids have been identified in turmeric.[2] In this chapter the major active components of turmeric, curcumin and those other than curcumin are discussed. Of these compounds, 22 are diarylheptanoids, 26 terpecurcumin and polypentanoids; and also include other phenolic compounds (Figure 4.1), 67 sesquiterpenes (Figure 4.2), 68 monoterpenes, four diterpenes, two triterpenes (Figure 4.3) and six flavonoids (Figure 4.4). Those phytochemicals are responsible for the anticancer, anti-oxidative, anti-inflammatory, antimicrobial, antidiabetic, lipid-decreasing, hepatoprotective and neuroprotective activities of turmeric.

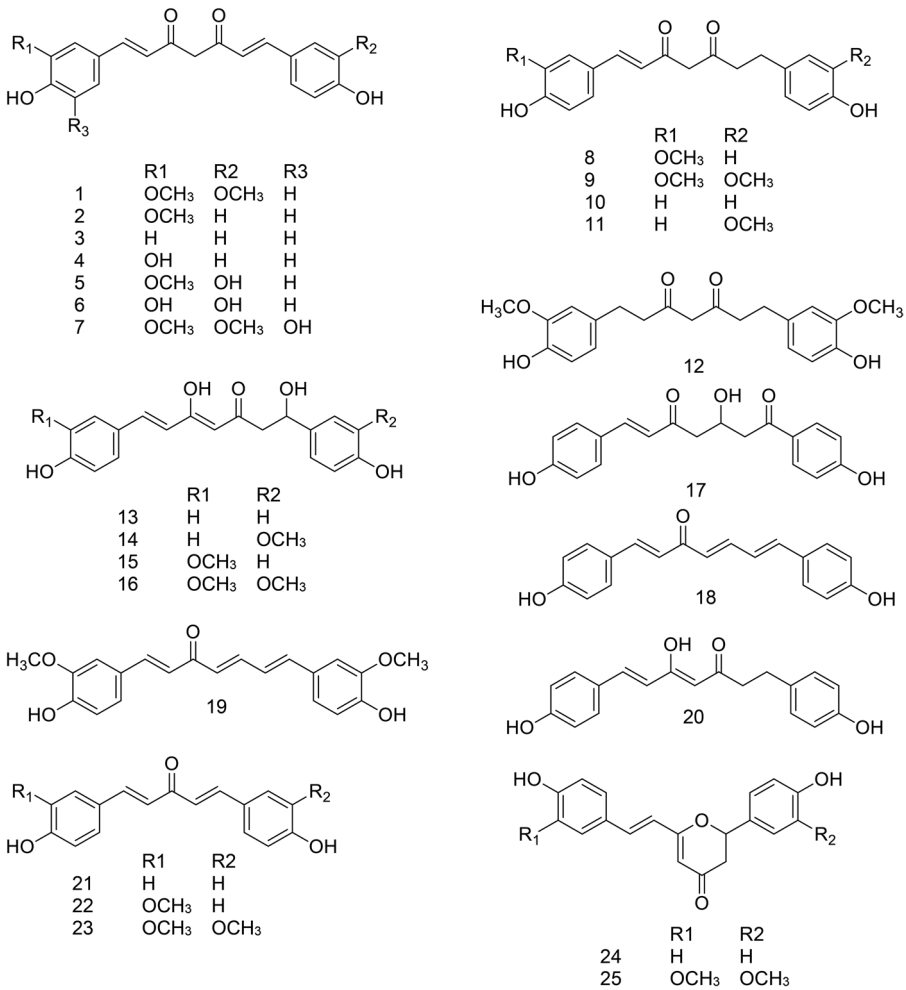


Figure 4.1 Chemical structures of phenolic compounds (curcuminoids and their related components) in turmeric.

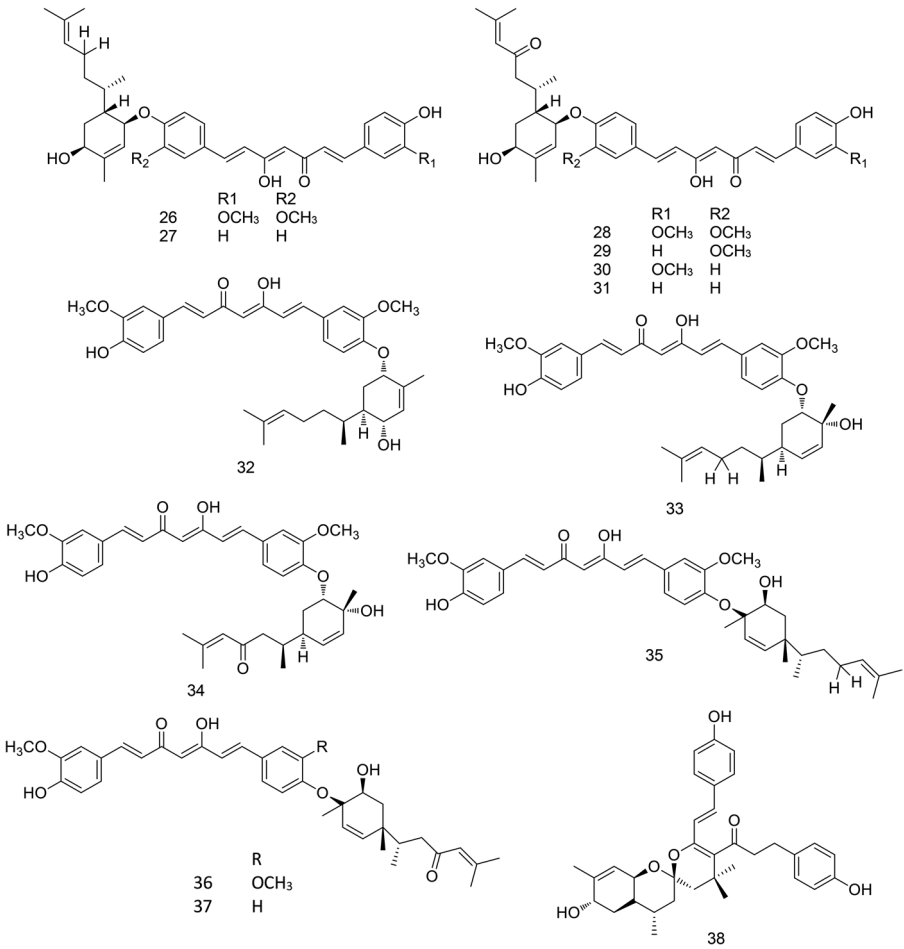


Figure 4.1 *(continued)*

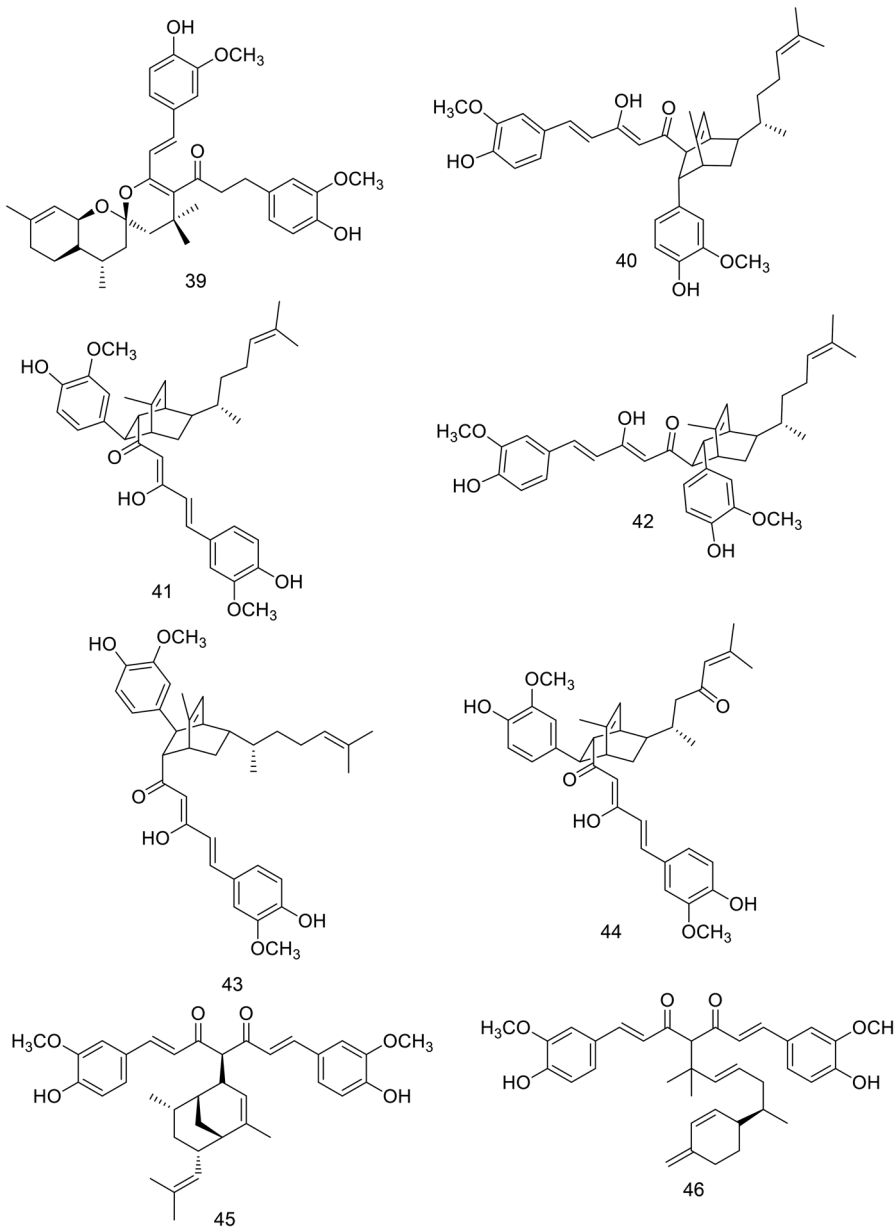


Figure 4.1 *(continued)*

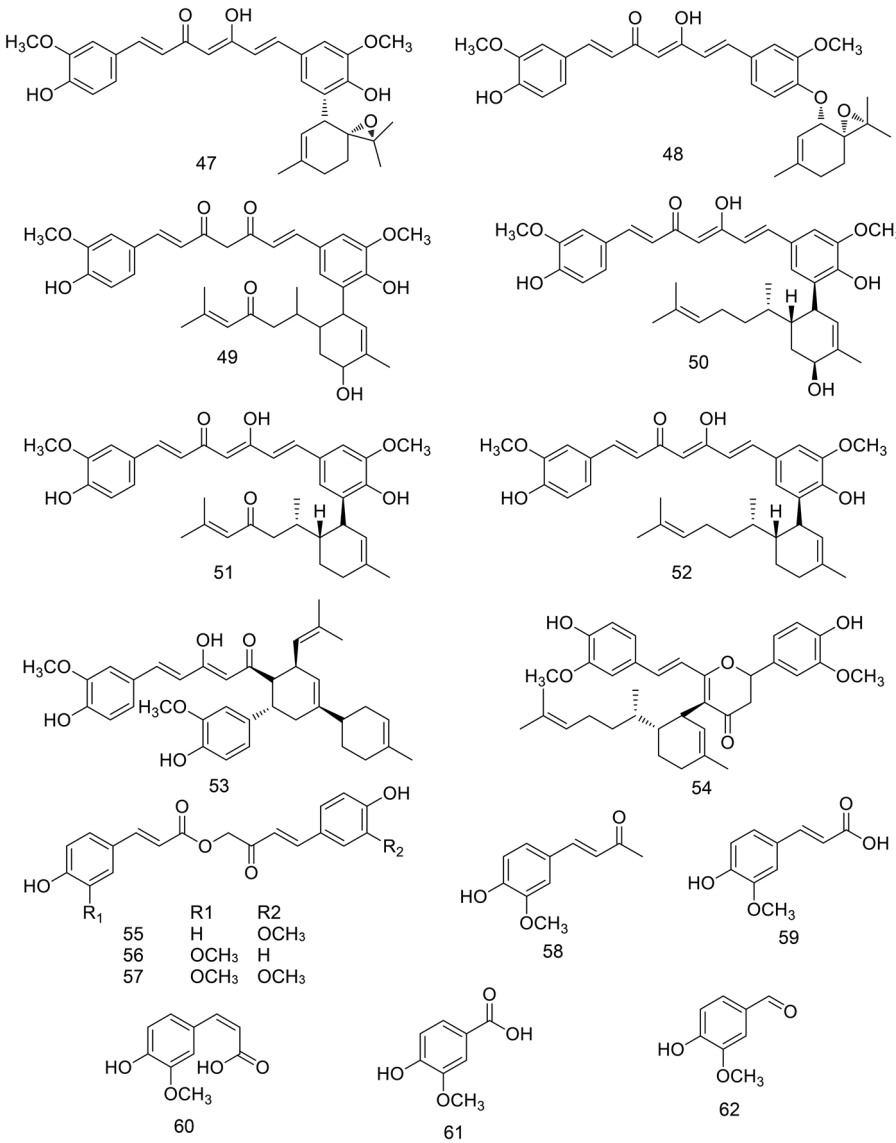


Figure 4.1 *(continued)*

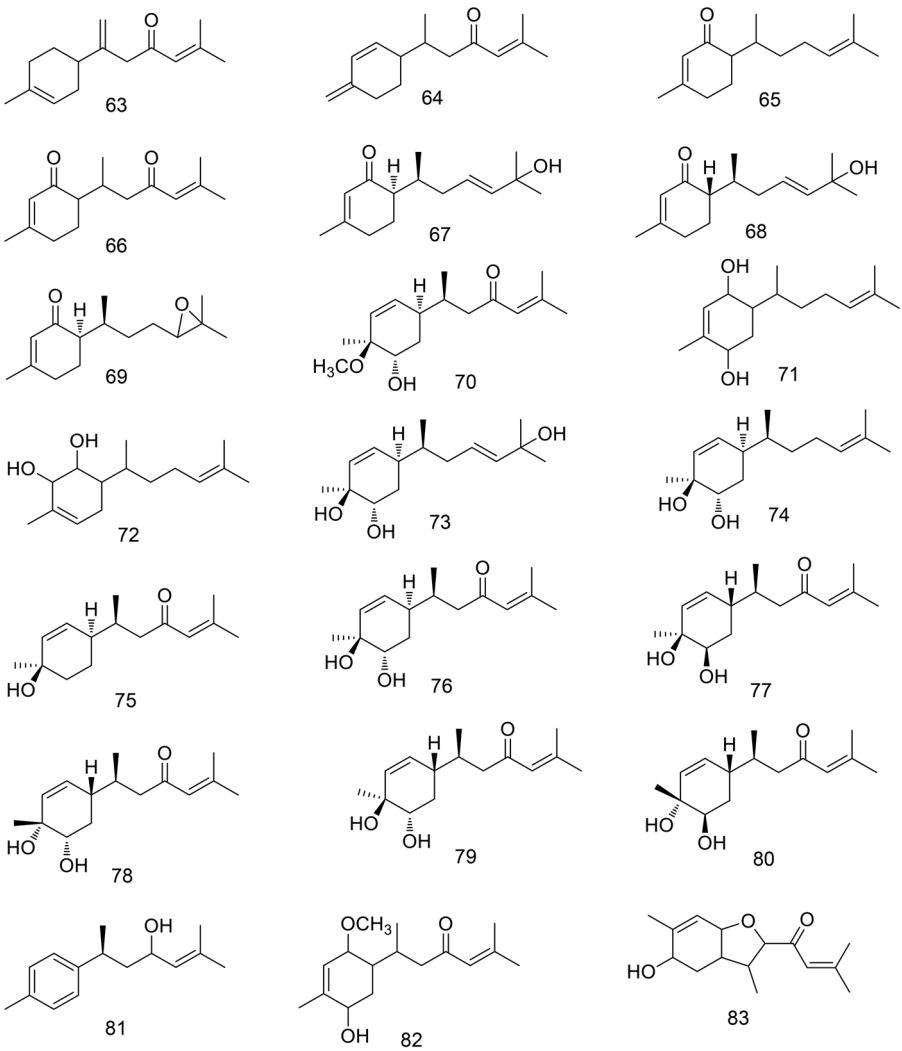


Figure 4.2 Chemical structures of sesquiterpenes in turmeric.

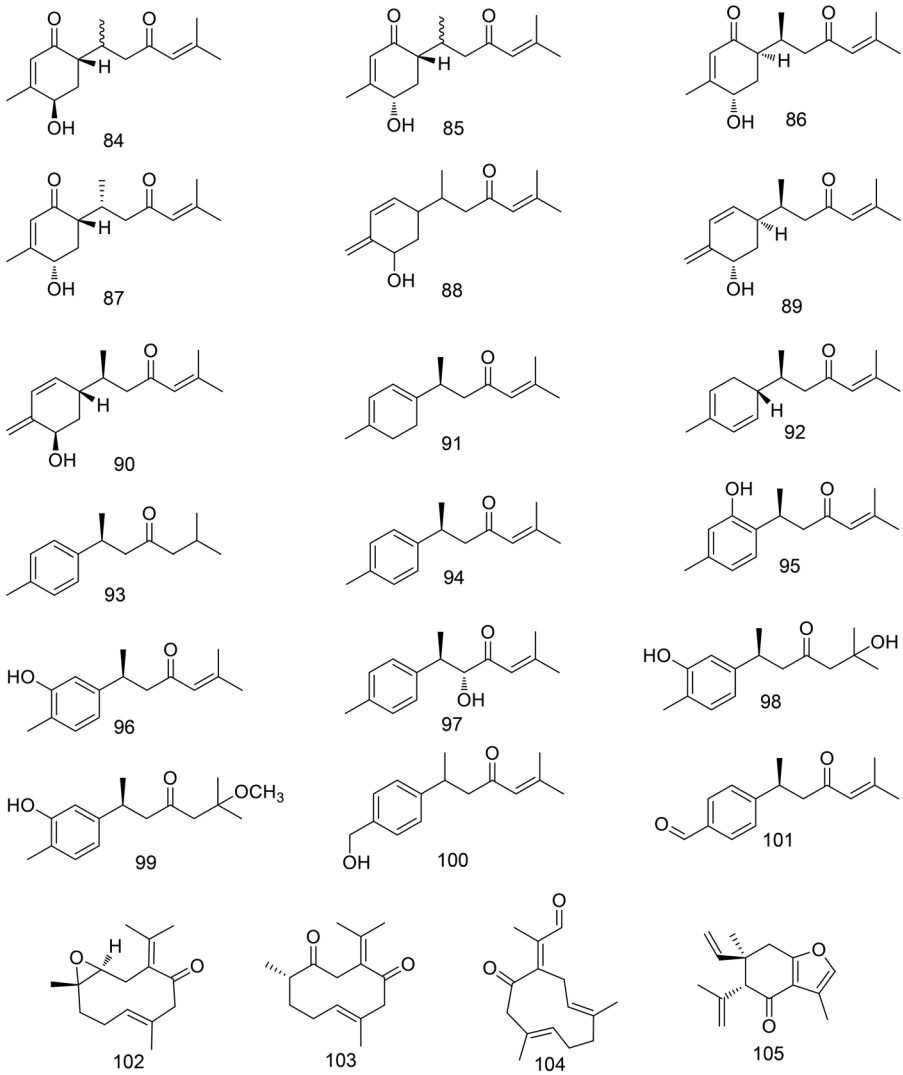


Figure 4.2 *(continued)*

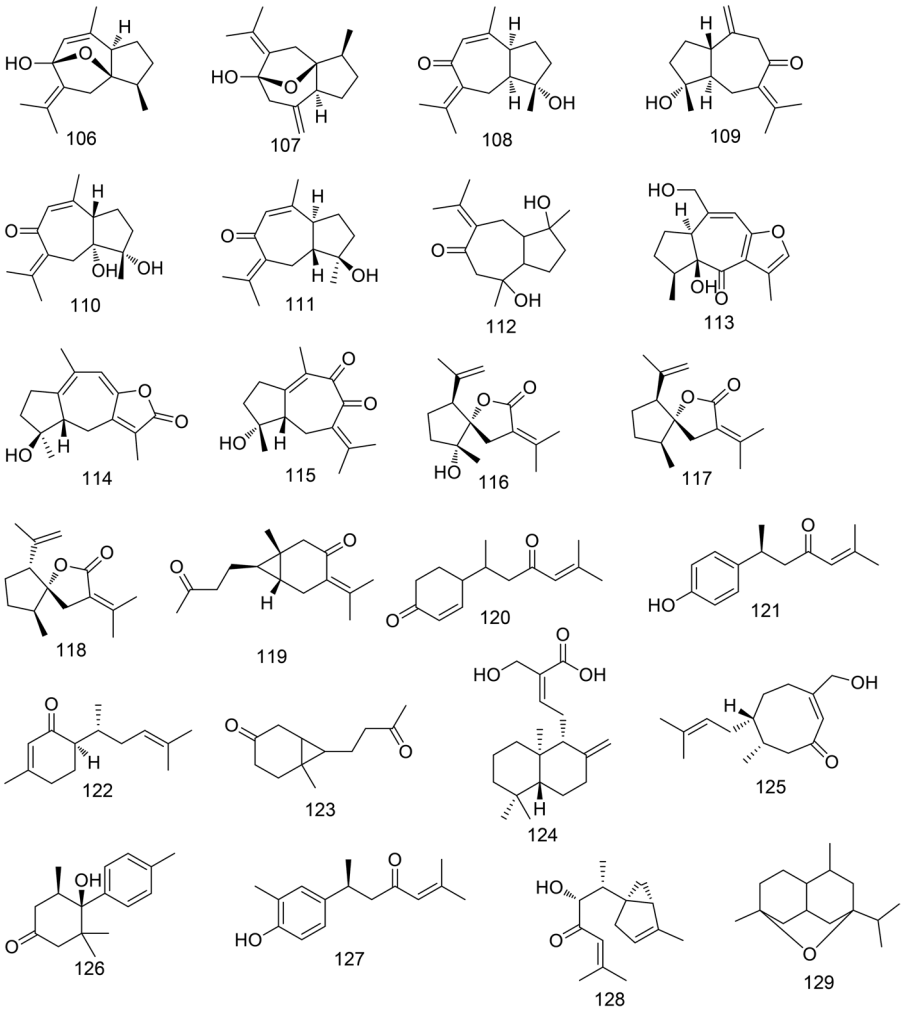


Figure 4.2 *(continued)*

4.2.1 Curcuminoids and Their Related Components

Curcuminoids are also the main components from turmeric. The orange–yellow color of turmeric is produced by the highly unsaturated conjugated chemical structures of the curcuminoids. Curcuminoids are a type of constituent in turmeric, which is chemically associated with its principal ingredient CUR. All curcuminoids share the same characteristic skeleton of two aromatic rings connected by an aliphatic chain, usually a heptane chain. Some curcuminoids have a 1,3-diketone group, which has at least two keto–enol tautomers. The enol form of CUR predominated in its keto–enol tautomeric mixtures in the solid phase and in solution.

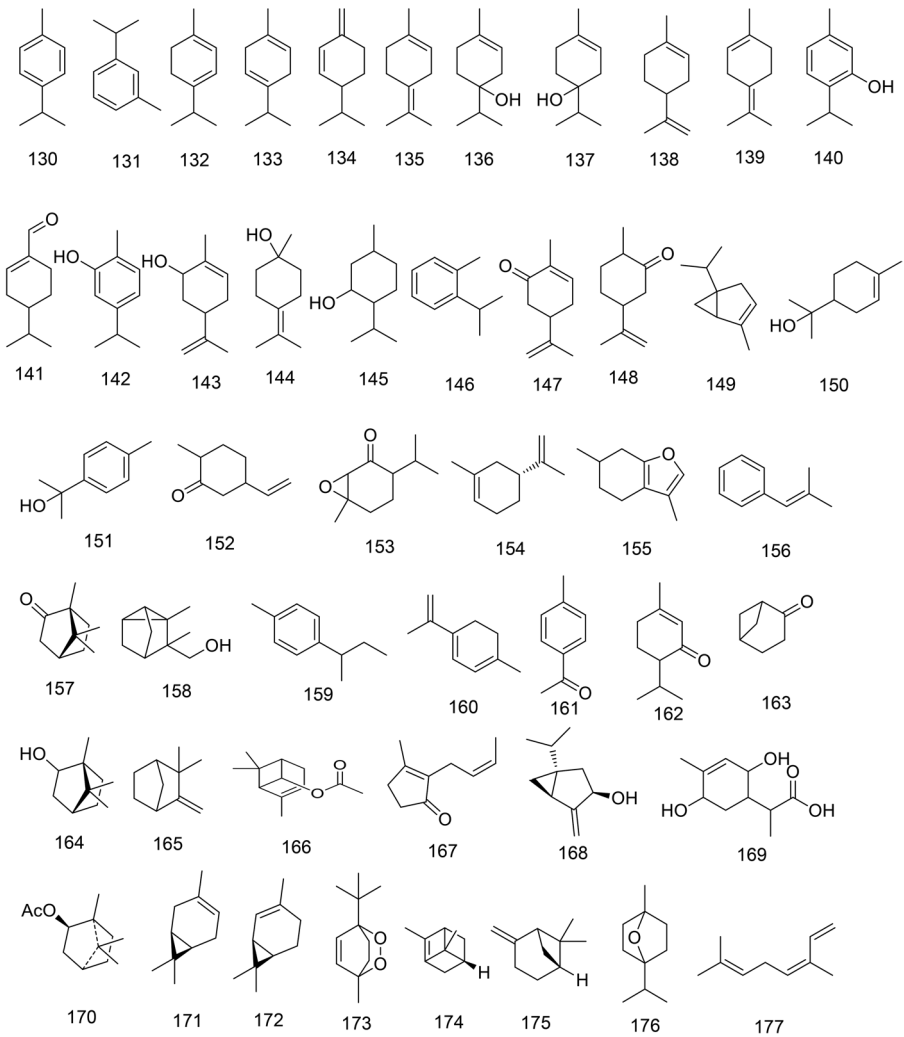


Figure 4.3 Chemical structures of terpenes in turmeric.

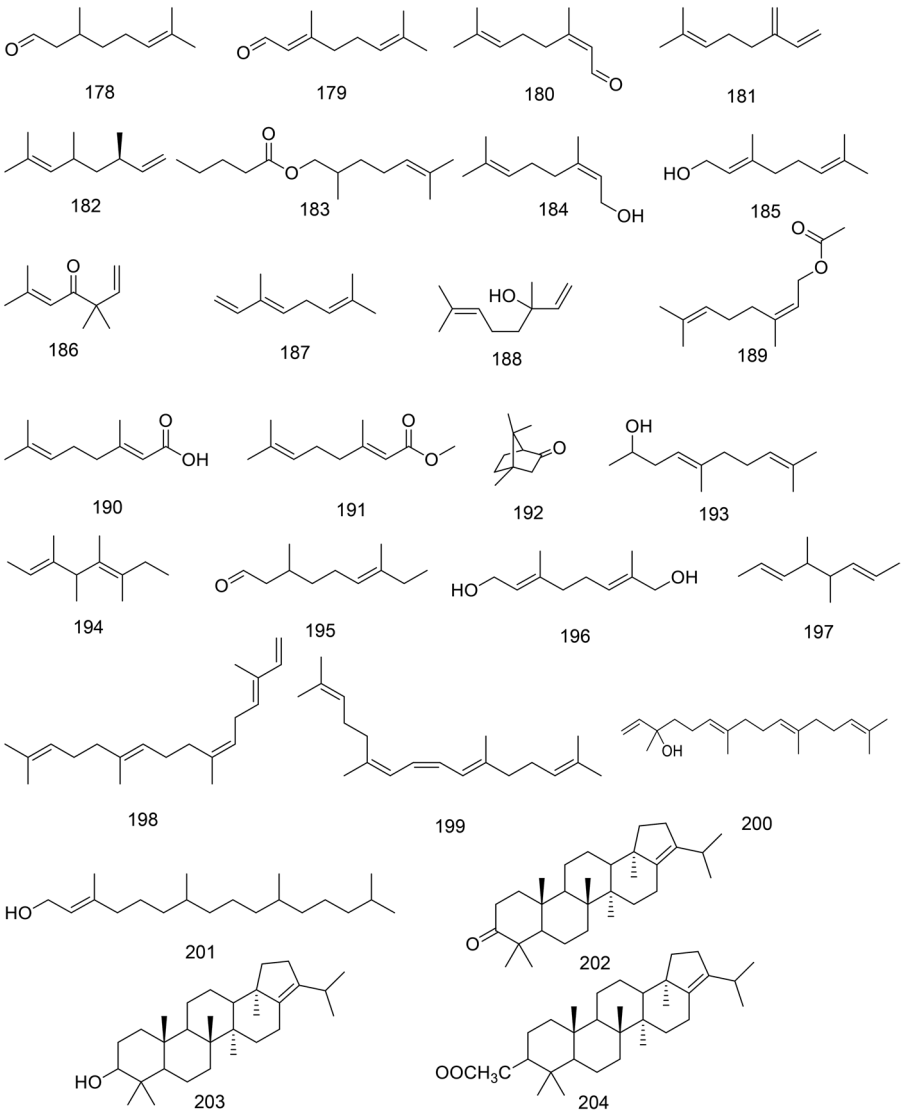


Figure 4.3 *(continued)*

To date, more than 50 curcuminoids including three characteristic subtypes have been identified: linear-curcuminoids, cyclic-curcuminoids and curcuminoids conjugated with monoterpenes or sesquiterpenes. Linear curcuminoids are the most common curcuminoids found in turmeric. The major curcuminoids in turmeric are three linear curcuminoids including CUR, DMC and BDMC. Two cyclic diarylheptanoids bearing a furan ring have also been isolated from turmeric. Recently, some unusual curcuminoids with the structures conjugating curcuminoids with monoterpenes or sesquiterpenes through a C–C or C–O–C bond were isolated from turmeric including derivatives from

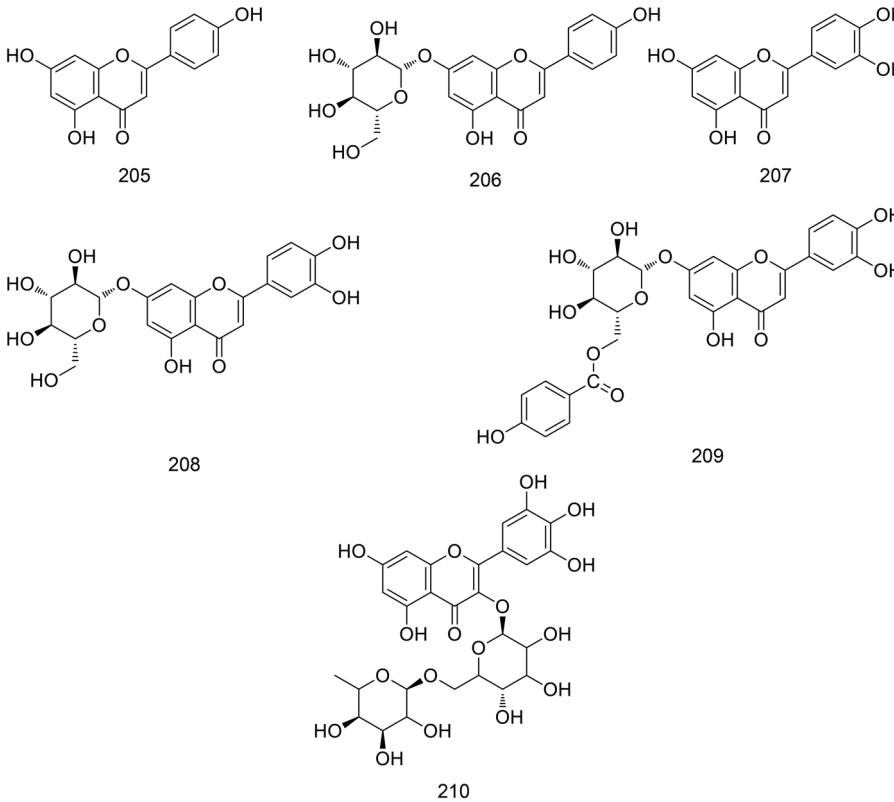


Figure 4.4 Chemical structures of flavonoids in turmeric.

the hybridization of curcuminoids and bisabolanes linked by a C–O bond, conjugated curcuminoids with sesquiterpenes through a C–C bond, and derivatives of bisdemethoxycurcumin connecting with a menthane monoterpene. Their structures and names are shown in Figure 4.1 and Table 4.1.[‡]

4.2.2 Terpenes

The aromatic flavor of turmeric is because of its essential oil, which is also one of the main component of turmeric. In comparison with curcuminoids, the chemistry of turmeric oil has not received much attention in the past. Terpenes are the major components in the volatile oil of turmeric. Phytochemical research elucidated about 67 sesquiterpenes (63–119), four norsesquiterpenes (120–123) and one norditerpene (124), isolated from ethanol/methanol extracts of turmeric.[7,16,23–26,28,30–36]

Sesquiterpenes are attributed to bisabolane-type sesquiterpenes (63–101), germacrane-type sesquiterpenes (102–104), elemane-type sesquiterpenes (105), guaiane-type sesquiterpenes (106–115), spironolactone-type sesquiterpenes (116–118), carane-type sesquiterpenes (119) and others (125–129); their structures are shown in Figure 4.2 and their IUPAC names are given in Table 4.2.

[‡]Adapted from ref. 112 with permission from Elsevier. Copyright 2018.

Table 4.1 Phenolic compounds (curcuminoids and their related components) in turmeric.

No.	Compound name	Compound type	Reference
1	Curcumin (Curcumin I)	Diarylheptanoid	7–12
2	Demethoxycurcumin (Curcumin II)	Diarylheptanoid	7–12
3	Bisdemethoxycurcumin (Curcumin III)	Diarylheptanoid	7–12
4	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione	Diarylheptanoid	8
5	(1*E*,6*E*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione	Diarylheptanoid	8
6	(1*E*,6*E*)-1,7-bis(3,4-dihydroxyphenyl) hepta-1,6-diene-3,5-dione	Diarylheptanoid	8
7	(1*E*,6*E*)-1-(3,4-dihydroxy-5-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione	Diarylheptanoid	8
8	(*E*)-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hept-1-ene-3,5-dione	Diarylheptanoid	13
9	(*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl) hept-1-ene-3,5-dione	Diarylheptanoid	13 and 14
10	(*E*)-1,7-bis(4-hydroxyphenyl) hept-1-ene-3,5-dione	Diarylheptanoid	13
11	(*E*)-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)hept-1-ene-3,5-dione	Diarylheptanoid	13
12	1,7-bis(4-hydroxy-3-methoxyphenyl) heptane-3,5-dione	Diarylheptanoid	15
13	(4*Z*,6*E*)-1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one	Diarylheptanoid	16
14	(4*Z*,6*E*)-1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) hepta-4,6-dien-3-one	Diarylheptanoid	16
15	(4*Z*,6*E*)-1,5-dihydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl) hepta-4,6-dien-3-one	Diarylheptanoid	16
16	(4*Z*,6*E*)-1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one	Diarylheptanoid	16
17	(*E*)-3-hydroxy-1,7-bis(4-hydroxyphenyl) hept-6-ene-1,5-dione	Diarylheptanoid	16
18	(1*E*,4*E*,6*E*)-1,7-bis(4-hydroxyphenyl) hepta-1,4,6-trien-3-one	Diarylheptanoid	14
19	(1*E*,4*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one	Diarylheptanoid	14
20	(4*Z*,6*E*)-5-hydroxy-1,7-bis(4-hydroxyphenyl) hepta-4,6-dien-3-one	Diarylheptanoid	8
21	(1*E*,4*E*)-1,5-bis(4-hydroxyphenyl) penta-1,4-dien-3-one	Diarylheptanoid	17
22	(1*E*,4*E*)-1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)penta-1,4-dien-3-one	Diarylheptanoid	16
23	(1*E*,4*E*)-1,5-bis(4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one	Diarylheptanoid	7
24	(*E*)-2-(4-hydroxyphenyl)-6-(4-hydroxystyryl)-2*H*-pyran-4(3*H*)-one	Diarylheptanoid	18

(*continued*)

Table 4.1 (*continued*)

No.	Compound name	Compound type	Reference
25	(*E*)-2-(4-hydroxy-3-methoxyphenyl)-6-(4-hydroxy-3-methoxystyryl)-2*H*-pyran-4(3*H*)-one [Cyclocurcumin]	Diarylheptanoid	3
26	(1*E*,4*Z*,6*E*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-(((1R,4S,6R)-4-hydroxy-3-methyl-6-((S)-6-methylhept-5-en-2-yl)cyclohex-2-en-1-yl)oxy)-3-methoxyphenyl)hepta-1,4,6-trien-3-one	Terpecurcumin A	19 and 20
27	(1*E*,4*Z*,6*E*)-5-hydroxy-7-(4-(((1R,4S,6R)-4-hydroxy-3-methyl-6-((S)-6-methylhept-5-en-2-yl)cyclohex-2-en-1-yl)oxy)phenyl)-1-(4-hydroxyphenyl)hepta-1,4,6-trien-3-one	Terpecurcumin S	20
28	(1*E*,4*Z*,6*E*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-(((1R,4S,6R)-4-hydroxy-3-methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-en-1-yl)oxy)-3-methoxyphenyl)hepta-1,4,6-trien-3-one	Bisabolocurcumin ether	19–21
29	(1*E*,4*Z*,6*E*)-5-hydroxy-7-(4-(((1R,4S,6R)-4-hydroxy-3-methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-en-1-yl)oxy)-3-methoxyphenyl)-1-(4-hydroxyphenyl)hepta-1,4,6-trien-3-one	Demethoxybis-abolocurcumin ether	19–21
30	(1*E*,4*Z*,6*E*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-(((1R,4S,6R)-4-hydroxy-3-methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-en-1-yl)oxy)phenyl)hepta-1,4,6-trien-3-one	Terpecurcumin U	20
31	(1*E*,4*Z*,6*E*)-5-hydroxy-7-(4-(((1R,4S,6R)-4-hydroxy-3-methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-en-1-yl)oxy)phenyl)-1-(4-hydroxyphenyl)hepta-1,4,6-trien-3-one	Didemethoxybis-abolocurcumin ether	19–21
32	(1*E*,4*Z*,6*E*)-5-hydroxy-7-(4-(((1S,4R,5R)-4-hydroxy-2-methyl-5-((S)-6-methylhept-5-en-2-yl)cyclohex-2-en-1-yl)oxy)-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one	Terpecurcumin B	19 and 20
33	(1*E*,4*Z*,6*E*)-5-hydroxy-7-(4-(((1S,2R,5R)-2-hydroxy-2-methyl-5-((S)-6-methylhept-5-en-2-yl)cyclohex-3-en-1-yl)oxy)-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one	Terpecurcumin C	19
34	(1*E*,4*Z*,6*E*)-5-hydroxy-7-(4-(((1S,2R,5R)-2-hydroxy-2-methyl-5-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-3-en-1-yl)oxy)-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one	Terpecurcumin D	19

35	(1*E*,4*Z*,6*E*)-5-hydroxy-7-(4-(((1S,4R,6S)-6-hydroxy-1,4-dimethyl-4-((S)-6-methylhept-5-en-2-yl)cyclohex-2-en-1-yl)oxy)-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one	Terpecurcumin E	19
36	(1*E*,4*Z*,6*E*)-5-hydroxy-7-(4-(((1S,4R,6S)-6-hydroxy-1,4-dimethyl-4-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-en-1-yl)oxy)-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one	Terpecurcumin F	19 and 20
37	(1*E*,4*Z*,6*E*)-5-hydroxy-7-(4-(((1S,4R,6S)-6-hydroxy-1,4-dimethyl-4-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-en-1-yl)oxy)phenyl)-1-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one	Terpecurcumin G	19 and 20
38	1-((2R,4S,4aS,6S,8aS)-6-hydroxy-6′-((*E*)-4-hydroxystyryl)-4,4′,4′,7-tetramethyl-3,3′,4,4a,4′,5,6,8a-octahydrospiro[chromene-2,2′-pyran]-5′-yl)-3-(4-hydroxyphenyl)propan-1-one	Terpecurcumin J	19 and 20
39	3-(4-hydroxy-3-methoxyphenyl)-1-((2R,4S,4aS,8aS)-6′-((*E*)-4-hydroxy-3-methoxystyryl)-4,4′,4′,7-tetramethyl-3,3′,4,4a,4′,5,6,-8a-octahydrospiro[chromene-2,2′-pyran]-5′-yl)propan-1-one	Terpecurcumin K	22
40	(2*Z*,4*E*)-3-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-1-((1R,2S,3S,4R)-3-(4-hydroxy-3-methoxyphenyl)-5-methyl-7-((S)-6-methylhept-5-en-2-yl)bicyclo[2.2.2]oct-5-en-2-yl)penta-2,4-dien-1-one	Terpecurcumin L	20 and 22
41	(2*Z*,4*E*)-3-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-1-((1R,2R,3R,4R)-3-(4-hydroxy-3-methoxyphenyl)-5-methyl-7-((S)-6-methylhept-5-en-2-yl)bicyclo[2.2.2]oct-5-en-2-yl)penta-2,4-dien-1-one	Terpecurcumin M	20 and22
42	(2*Z*,4*E*)-3-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-1-((1R,2R,3R,4R)-3-(4-hydroxy-3-methoxyphenyl)-6-methyl-8-((S)-6-methylhept-5-en-2-yl)bicyclo[2.2.2]oct-5-en-2-yl)penta-2,4-dien-1-one	Terpecurcumin N	20 and 22
43	(2*Z*,4*E*)-3-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-1-((1R,2S,3S,4R,8S)-3-(4-hydroxy-3-methoxyphenyl)-6-methyl-8-((S)-6-methylhept-5-en-2-yl)bicyclo[2.2.2]oct-5-en-2-yl)penta-2,4-dien-1-one	Terpecurcumin O	20 and 22
44	(6S)-6-((1R,4R,7R,8R)-8-(4-hydroxy-3-methoxyphenyl)-7-((2*Z*,4*E*)-3-hydroxy-5-(4-hydroxy-3-methoxyphenyl)penta-2,4-dienoyl)-5-methylbicyclo[2.2.2]oct-5-en-2-yl)-2-methylhept-2-en-4-one	Terpecurcumin P	20 and 22
45	(1*E*,6*E*)-4-((1R,2S,5S,6S,8S)-4,8-dimethyl-6-(2-methylprop-1-en-1-yl)bicyclo[3.3.1]non-3-en-2-yl)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione	Terpecurcumin Q	22

(*continued*)

Table 4.1 (*continued*)

No.	Compound name	Compound type	Reference
46	(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-((R,*E*)-2-methyl-6-((S)-4-methylenecyclohex-2-en-1-yl) hept-3-en-2-yl)hepta-1,6-diene-3,5-dione	Terpecurcumin R	22
47	(1*E*,4*Z*,6*E*)-5-hydroxy-7-(4-hydroxy-3-methoxy-5-((3R,4R)-2,2,6-trimethyl-1-oxaspiro[2.5]oct-5-en-4-yl) phenyl)-1-(4-hydroxy-3-methoxyphenyl) hepta-1,4,6-trien-3-one	Terpecurcumin V	20 and 22
48	(1*E*,4*Z*,6*E*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(3-methoxy-4-(((3R,4S)-2,2,6-trimethyl-1-oxaspiro[2.5] oct-5-en-4-yl)oxy)phenyl) hepta-1,4,6-trien-3-one	Terpecurcumin W	20 and 22
49	(1*E*,6*E*)-1-(4′,6-dihydroxy-5-methoxy-5′-methyl-2′-(6-methyl-4-oxohept-5-en-2-yl)-1′,2′,3′,4′-tetrahydro-[1,1′-biphenyl]-3-yl)-7-(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione	Bisabocurcumin	11
50	(1*E*,4*Z*,6*E*)-7-((1′S,2′R,4′S)-4′,6-dihydroxy-5-methoxy-5′-methyl-2′-((S)-6-methylhept-5-en-2-yl)-1′,2′,3′,4′-tetrahydro-[1,1′-biphenyl]-3-yl)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) hepta-1,4,6-trien-3-one	Terpecurcumin H	19 and 20
51	(1*E*,4*Z*,6*E*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-((1′S,2′R)-6-hydroxy-5-methoxy-5′-methyl-2′-((S)-6-methyl-4-oxohept-5-en-2-yl)-1′,2′,3′,4′-tetrahydro-[1,1′-biphenyl]-3-yl)hepta-1,4,6-trien-3-one	Terpecurcumin I	19
52	(1*E*,4*Z*,6*E*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-((1′S,2′R)-6-hydroxy-5-methoxy-5′-methyl-2′-((S)-6-methylhept-5-en-2-yl)-1′,2′,3′,4′-tetrahydro-[1,1′-biphenyl]-3-yl)hepta-1,4,6-trien-3-one	Terpecurcumin T	19 and 20
53	(2*Z*,4*E*)-3-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-1-((1′S,3S,4R,5S)-5-(4-hydroxy-3-methoxyphenyl)-4′-methyl-3-(2-methylprop-1-en-1-yl)-[1,1′-bi(cyclohexane)]-1,3′-dien-4-yl)penta-2,4-dien-1-one	Terpecurcumin X	20
54	2-(4-Hydroxy-3-methoxyphenyl)-6-((*E*)-4-hydroxy-3-methoxystyryl)-5-((1S,6R)-3-methyl-6-((S)-6-methylhept-5-en-2-yl) cyclohex-2-en-1-yl)-2*H*-pyran-4(3*H*)-one	Terpecurcumin Y	20
55	(*E*)-(*E*)-4-(4-hydroxy-3-methoxyphenyl)-2-oxobut-3-en-1-yl 3-(4-hydroxyphenyl)acrylate	Phenylpentanoid	23
56	(*E*)-(*E*)-4-(4-hydroxyphenyl)-2-oxobut-3-en-1-yl 3-(4-hydroxy-3-methoxyphenyl) acrylate	Phenylpentanoid	23
57	(*E*)-(*E*)-4-(4-hydroxy-3-methoxyphenyl)-2-oxobut-3-en-1-yl 3-(4-hydroxy-3-methoxyphenyl)acrylate [Calebin A]	Phenylpentanoid	15

58	(*E*)-4-(4-hydroxy-3-methoxyphenyl) but-3-en-2-one	Phenylpentanoid	7
59	(*E*)-3-(4-hydroxy-3-methoxyphenyl)acrylic acid [(*E*)-ferulic acid]	Phenylpentanoid	7
60	(*Z*)-3-(4-hydroxy-3-methoxyphenyl)acrylic acid [(*Z*)-ferulic acid]	Phenylpentanoid	7
61	4-hydroxy-3-methoxybenzoic acid [vanillic acid]	Phenolic	7
62	4-hydroxy-3-methoxybenzaldehyde [vanillin]	Phenolic	7

Table 4.2 Sesquiterpenes in turmeric.

No.	Compound name	Compound type	Reference
63	[6-Methyl-2-(4-methylcyclohex-3-en-1-yl) hepta-1,5-dien-4-one] β-Atlantone	Bisabolane-type sesquiterpene	7
64	[2-Methyl-6-(4-methylenecyclohex-2-en-1-yl) hept-2-en-4-one] Curlone	Bisabolane-type sesquiterpene	7 and 9
65	[3-Methyl-6-(6-methylhept-5-en-2-yl)cyclohex-2-enone] bisabolone	Bisabolane-type sesquiterpene	7 and 24
66	[3-Methyl-6-(6-methyl-4-oxohept-5-en-2-yl) cyclohex-2-enone] Bisabolone-9-one	Bisabolane-type sesquiterpene	7 and 9
67	[(R)-6-((S,*E*)-6-hydroxy-6-methylhept-4-en-2-yl)-3-methylcyclohex-2-enone] Curculonone A	Bisabolane-type sesquiterpene	7
68	[(S)-6-((S,*E*)-6-hydroxy-6-methylhept-4-en-2-yl)-3-methylcyclohex-2-enone] Curculonone B	Bisabolane-type sesquiterpene	7
69	[(6R)-6-((2S)-4-(3,3-dimethyloxiran-2-yl)butan-2-yl)-3-methylcyclohex-2-enone] Curculonone C	Bisabolane-type sesquiterpene	7
70	(S)-6-((1R,4S,5S)-5-hydroxy-4-methoxy-4-methylcyclohex-2-en-1-yl)-2-methylhept-2-en-4-one	Bisabolane-type sesquiterpene	25
71	[2-Methyl-5-(6-methylhept-5-en-2-yl)cyclohex-2-ene-1,4-diol] 2,5-Dihydroxybisabola-3,10-diene	Bisabolane-type sesquiterpene	26
72	[3-Methyl-6-(6-methylhept-5-en-2-yl)cyclohex-3-ene-1,2-diol] 4,5-Dihydroxybisabola-2,10-diene	Bisabolane-type sesquiterpene	26
73	[(1S,2S,5R)-5-((S,*E*)-6-hydroxy-6-methylhept-4-en-2-yl)-2-methylcyclohex-3-ene-1,2-diol] Curculonone D	Bisabolane-type sesquiterpene	7
74	(1S,2S,5R)-2-methyl-5-((S)-6-methylhept-5-en-2-yl)cyclohex-3-ene-1,2-diol	Bisabolane-type sesquiterpene	25
75	(S)-6-((1R,4R)-4-hydroxy-4-methylcyclohex-2-en-1-yl)-2-methylhept-2-en-4-one	Bisabolane-type sesquiterpene	25
76	(S)-6-((1R,4S,5S)-4,5-dihydroxy-4-methylcyclohex-2-en-1-yl)-2-methylhept-2-en-4-one	Bisabolane-type sesquiterpene	25

(*continued*)

Table 4.2 (*continued*)

No.	Compound name	Compound type	Reference
77	[(S)-6-((1S,4S,5R)-4,5-dihydroxy-4-methylcyclohex-2-en-1-yl)-2-methylhept-2-en-4-one] Bisacurone A	Bisabolane-type sesquiterpene	26 and 27
78	[(S)-6-((1S,4R,5S)-4,5-dihydroxy-4-methylcyclohex-2-en-1-yl)-2-methylhept-2-en-4-one] Bisacurone B	Bisabolane-type sesquiterpene	26 and 28
79	[(S)-6-((1S,4S,5S)-4,5-dihydroxy-4-methylcyclohex-2-en-1-yl)-2-methylhept-2-en-4-one] Bisacurone C	Bisabolane-type sesquiterpene	26
80	[(S)-6-((1S,4R,5R)-4,5-dihydroxy-4-methylcyclohex-2-en-1-yl)-2-methylhept-2-en-4-one] Bisacurone	Bisabolane-type sesquiterpene	7, 26 and 27
81	[(6S)-2-methyl-6-(*p*-tolyl)hept-2-en-4-ol] Ar-tumerol	Bisabolane-type sesquiterpene	9
82	6-(5-Hydroxy-2-methoxy-4-methylcyclohex-3-en-1-yl)-2-methylhept-2-en-4-one	Bisabolane-type sesquiterpene	27
83	1-(5-Hydroxy-3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran-2-yl)-3-methylbut-2-en-1-one	Bisabolane-type sesquiterpene	27
84	[(4R,6S)-4-hydroxy-3-methyl-6-(6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-enone] 5α-Hydroxyl-1β-bisabolon-9-one	Bisabolane-type sesquiterpene	29
85	[(4S,6S)-4-hydroxy-3-methyl-6-(6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-enone] 5β-Hydroxyl-1β-bisabolon-9-one	Bisabolane-type sesquiterpene	29
86	[(4S,6R)-4-hydroxy-3-methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-enone] Longpene C	Bisabolane-type sesquiterpene	28
87	[(4S,6S)-4-hydroxy-3-methyl-6-((R)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-enone] Longpene D	Bisabolane-type sesquiterpene	28
88	[6-(5-hydroxy-4-methylenecyclohex-2-en-1-yl)-2-methylhept-2-en-4-one] 4-Methylene-5-hydroxybisabola-2,10-diene-9-one	Bisabolane-type sesquiterpene	27
89	[(S)-6-((1R,5S)-5-hydroxy-4-methylenecyclohex-2-en-1-yl)-2-methylhept-2-en-4-one] Intermedin B	Bisabolane-type sesquiterpene	28
90	[(S)-6-((1S,5R)-5-hydroxy-4-methylenecyclohex-2-en-1-yl)-2-methylhept-2-en-4-one]	Bisabolane-type sesquiterpene	24
91	[(S)-2-methyl-6-(4-methylcyclohexa-1,3-dien-1-yl)hept-2-en-4-one] (S)-α-turmerone	Bisabolane-type sesquiterpene	9
92	[(S)-2-methyl-6-((R)-4-methylcyclohexa-2,4-dien-1-yl)hept-2-en-4-one] α-Turmerone	Bisabolane-type sesquiterpene	30
93	[(S)-2-methyl-6-(*p*-tolyl)heptan-4-one] Ar-dihydroturmerone	Bisabolane-type sesquiterpene	28
94	[(S)-2-methyl-6-(*p*-tolyl)hept-2-en-4-one] Ar-turmerone	Bisabolane-type sesquiterpene	7, 9 and 28
95	[(S)-6-(2-hydroxy-4-methylphenyl)-2-methylhept-2-en-4-one] Turmeronol B	Bisabolane-type sesquiterpene	9, 24, 28 and 31

96	[(S)-6-(3-hydroxy-4-methylphenyl)-2-methylhept-2-en-4-one] Turmeronol A	Bisabolane-type sesquiterpene	9, 24, 26–28, 31 and 32
97	[(5R,6R)-5-hydroxy-2-methyl-6-(*p*-tolyl)hept-2-en-4-one] 8-Hydroxyl-*ar*-turmerone	Bisabolane-type sesquiterpene	24
98	(S)-2-hydroxy-6-(3-hydroxy-4-methylphenyl)-2-methylheptan-4-one	Bisabolane-type sesquiterpene	32
99	(S)-6-(3-hydroxy-4-methylphenyl)-2-methoxy-2-methylheptan-4-one	Bisabolane-type sesquiterpene	32
100	6-(4-(hydroxymethyl) phenyl)-2-methylhept-2-en-4-one	Bisabolane-type sesquiterpene	33
101	(S)-4-(6-methyl-4-oxohept-5-en-2-yl) benzaldehyde	Bisabolane-type sesquiterpene	24
102	(1S,6*E*,10S)-6,10-dimethyl-3-(1-methylethylidene)-11-oxabicyclo[8.1.0] undec-6-en-4-one	Germacrane-type sesquiterpene	25
103	(6*E*,10S)-6,10-dimethyl-3-(1-methylethylidene)-6-cyclodecene-1,4-dione	Germacrane-type sesquiterpene	25
104	(2*E*)-2-[(3*E*,7*E*)-4,8-dimethyl-10-oxo-3,7-cyclodecadien-1-ylidene]-propanal	Germacrane-type sesquiterpene	25
105	[(5R,6R)-3,6-dimethyl-5-(prop-1-en-2-yl)-6-vinyl-6,7-dihydrobenzofuran-4(5*H*)-one] Curzerenone	Elemene-type sesquiterpene	28
106	[(3R,3aR,6S,8aR)-3,8-dimethyl-5-(propan-2-ylidene)-2,3,4,5,6,8a-hexahydro-1*H*-3a,6-epoxyazulen-6-ol] Curcumenol	Guaiane-type sesquiterpene	28 and 34
107	[(3S,3aS,6R,8aS)-3-methyl-8-methylene-5-(propan-2-ylidene)octahydro-1*H*-3a,6-epoxyazulen-6-ol] Isocurcumenol	Guaiane-type sesquiterpene	28 and 34
108	(3S,3aS,8aS)-3-hydroxy-3,8-dimethyl-5-(propan-2-ylidene)-1,3,3a,4,5,8a-hexahydroazulen-6(2*H*)-one	Guaiane-type sesquiterpene	25
109	(1R,3aS,8aR)-1-hydroxy-1-methyl-4-methylene-7-(propan-2-ylidene) octahydroazulen-6(2*H*)-one	Guaiane-type sesquiterpene	25
110	(3S,3aR,8aS)-3,3a-dihydroxy-3,8-dimethyl-5-(propan-2-ylidene)-1,3,3a,4,5,8a-hexahydroazulen-6(2*H*)-one	Guaiane-type sesquiterpene	25
111	[(3R,3aR,8aS)-3-hydroxy-3,8-dimethyl-5-(propan-2-ylidene)-1,3,3a,4,5,8a-hexahydroazulen-6(2*H*)-one] Procurcumenol	Guaiane-type sesquiterpene	28
112	[1,4-Dihydroxy-1,4-dimethyl-7-(propan-2 -ylidene)octahydroazulen-6(2*H*)-one] Zedoarondiol	Guaiane-type sesquiterpene	7
113	[(4aS,5S,7aS)-4a-hydroxy-8-(hydroxymethyl)-3,5-dimethyl-5,6,7,7a-tetrahydroazuleno[6,5-*b*]furan-4(4aH)-one] Zedoardiol	Guaiane-type sesquiterpene	28
114	[(4aS,5S)-5-hydroxy-3,5,8-trimethyl-4a,5,6,7-tetrahydroazuleno[6,5-*b*]furan-2(4*H*)-one] Zedoalactone F	Guaiane-type sesquiterpene	28

(*continued*)

Table 4.2 (*continued*)

No.	Compound name	Compound type	Reference
115	[(1R,8aS)-1-hydroxy-1,4-dimethyl-7-(propan-2-ylidene)-1,2,3,7,8,8a-hexahydroazulene-5,6-dione] 1,10-Dehydro-10-deoxy-9-oxozedoarondiol	Guaiane-type sesquiterpene	7
116	[(5S,6R,9S)-6-hydroxy-6-methyl-9-(prop-1-en-2-yl)-3-(propan-2-ylidene)-1-oxaspiro[4.4]nonan-2-one] 6α-Hydroxycurcumanolide A	Spironolactone-type sesquiterpene	7
117	[(5R,6S,9S)-6-methyl-9-(prop-1-en-2-yl)-3-(propan-2-ylidene)-1-oxaspiro[4.4]nonan-2-one] Curcumanolide A	Spironolactone-type sesquiterpene	7
118	[(5R,6S,9R)-6-methyl-9-(prop-1-en-2-yl)-3-(propan-2-ylidene)-1-oxaspiro[4.4]nonan-2-one] Curcumanolide B	Spironolactone-type sesquiterpene	7
119	(1S,6R,7R)-1-methyl-7-(3-oxobutyl)-4-(propan-2-ylidene)bicyclo[4.1.0]heptan-3-one	Carane-type sesquiterpene	25
120	4-(6-Methyl-4-oxohept-5-en-2-yl) cyclohex-2-enone	Norsesquiterpene	33
121	(S)-6-(4-hydroxyphenyl)-2-methylhept-2-en-4-one	Norsesquiterpene	32 and 33
122	(R)-3-methyl-6-((R)-5-methylhex-4-en-2-yl) cyclohex-2-enone	Norsesquiterpene	7
123	6-Methyl-7-(3-oxobutyl)bicyclo[4.1.0] heptan-3-one	Norsesquiterpene	34
124	[(*Z*)-2-(hydroxymethyl)-4-((1R,4aR,8aR)-5,5,8a-trimethyl-2-methylenedecahydronaphthalen-1-yl) but-2-enoic acid] Longpene A	Norditerpene	28
125	[(6S,7S,*E*)-3-(hydroxymethyl)-7-methyl-6-(3-methylbut-2-en-1-yl)cyclooct-2-enone] Longpene B	Sesquiterpene	28
126	[(4R,5R)-4-hydroxy-3,3,5-trimethyl-4-(*p*-tolyl) cyclohexanone] Curcumaone J	Sesquiterpene	35
127	(S)-6-(4-hydroxy-3-methylphenyl)-2-methylhept-2-en-4-one	Sesquiterpene	32 and 33
128	[(5R,6R)-5-hydroxy-2-methyl-6-((1S,5R)-4-methylbicyclo[3.1.0]hex-3-en-1-yl)hept-2-en-4-one] Bicycloturmeronol	Sesquiterpene	9
129	[2-Isopropyl-4,7-dimethyldecahydro-2,7-epoxynaphthalene] Curcumin L	Sesquiterpene	36

The structures of those sesquiterpenes in turmeric oil are very similar. Some of them are isomers and the structures differ from the position of the double bonds and some of them share the same skeleton but differ in numbers of double bonds.

Furthermore, 68 monoterpenes (130–197), four diterpenes (198–201) and three triterpenes (202–204) were also identified and their chemical structures are shown in Figure 4.3 and their chemical names are given in Table 4.3.

4.2.3 Flavonoids

Studies in recent years have shown that turmeric also contains flavonoids and their glycosides; their chemical structures are shown in Figure 4.4 and their chemical names are given in Table 4.4. This chemical characteristic can

Table 4.3 Terpenes in turmeric.

No.	Compound name	Compound type	Reference
130	[4-Isopropyltoluene] *p*-cymene	Monoterpene	37
131	[1-Isopropyl-3-methylbenzene] *m*-cymene	Monoterpene	38
132	[1-Methyl-4-propan-2-ylcyclohexa-1,3-diene] α-cymene	Monoterpene	38
133	[1-Methyl-4-propan-2-ylcyclohexa-1,4-diene] γ-terpinene	Monoterpene	38
134	[3-Methylidene-6-propan-2-ylcyclohexene] β-phellandrene	Monoterpene	38
135	[1-Methyl-4-propan-2-ylidenecyclohexene] *p*-mentha-1,4(8)-diene	Monoterpene	6
136	[1-Isopropyl-4-methylcyclohex-3-enol] 4-terpineol	Monoterpene	6
137	[1-Isopropyl-4-methylcyclohex-3-enol] terpinen-4-ol	Monoterpene	39
138	[1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene] limonene	Monoterpene	40
139	[1-Methyl-4-(propan-2-ylidene)cyclohex-1-ene] terpinolene	Monoterpene	40
140	[2-Isopropyl-5-methylphenol] thymol	Monoterpene	38
141	[4-Isopropylcyclohex-1-enecarbaldehyde] phellandrol	Monoterpene	37
142	[5-Isopropyl-2-methylphenol] carvacrol	Monoterpene	40
143	[2-Methyl-5-(prop-1-en-2-yl)cyclohex-2-enol] (*E*)-carveol	Monoterpene	40
144	[1-Methyl-4-(propan-2-ylidene)cyclohexanol] γ-terpineol	Monoterpene	6
145	[2-Isopropyl-5-methylcyclohexanol] menthol	Monoterpene	41
146	*o*-cymene	Monoterpene	42
147	2-Methyl-5-(prop-1-en-2-yl)cyclohex-2-enone	Monoterpene	42
148	2-Methyl-5-(prop-1-en-2-yl)cyclohexanone	Monoterpene	41
149	[5-Isopropyl-2-methylbicyclo[3.1.0]hex-2-ene] α-thujene	Monoterpene	6
150	[2-(4-methylcyclohex-3-en-1-yl)propan-2-ol] α-terpineol	Monoterpene	37
151	[2-(*p*-tolyl)propan-2-ol] *p*-Cymenol	Monoterpene	40
152	[2-Methyl-5-vinylcyclohexanone]	Monoterpene	38
153	[3-Isopropyl-6-methyl-7-oxabicyclo[4.1.0] heptan-2-one] Piperitone oxide	Monoterpene	37
154	[(R)-1-methyl-5-(prop-1-en-2-yl)cyclohex-1-ene] Diprene	Monoterpene	6
155	[3,6-Dimethyl-4,5,6,7-tetrahydrobenzofuran] Menthofuran	Monoterpene	6
156	(2-Methylprop-1-en-1-yl)benzene	Monoterpene	6
157	[(1R,4R)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one] Camphor	Monoterpene	6
158	[(2,3-Dimethyltricyclo[2.2.1.$0^{2,6}$]heptan-3-yl) methanol] Teresantalol	Monoterpene	37
159	1-(*sec*-butyl)-4-methylbenzene	Monoterpene	41
160	1-Methyl-4-(prop-1-en-2-yl) cyclohexa-1,3-diene	Monoterpene	41
161	[1-(*p*-tolyl)ethanone]	Monoterpene	41

(*continued*)

Table 4.3 (*continued*)

No.	Compound name	Compound type	Reference
162	[6-Isopropyl-3-methylcyclohex-2-enone] Piperitone	Monoterpene	41
163	[bicyclo[3.1.1]heptan-2-one] norpinone	Monoterpene	40
164	(1R,4R)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-ol	Monoterpene	38
165	[2,2-Dimethyl-3-methylenebicyclo[2.2.1]heptane] camphene	Monoterpene	40
166	[2,7,7-Trimethylbicyclo[3.1.1]hept-2-en-6-yl acetate]	Monoterpene	40
167	[(*Z*)-2-(but-2-en-1-yl)-3-methylcyclopent-2-enone]	Monoterpene	43
168	[(1S,3R,5S)-1-isopropyl-4-methylenebicyclo[3.1.0]hexan-3-ol] sabinol	Monoterpene	43
169	[2-(2,5-dihydroxy-4-methylcyclohex-3-en-1-yl)propanoic acid]	Monoterpene	27
170	[(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1] heptan-2-yl acetate] bornyl acetate	Monoterpene	38
171	[(1S,6R)-3,7,7-trimethylbicyclo[4.1.0] hept-3-ene]	Monoterpene	40
172	[(1S,6R)-3,7,7-trimethylbicyclo[4.1.0] hept-2-ene]	Monoterpene	37
173	[1-(*tert*-butyl)-4-methyl-2,3-dioxabicyclo[2.2.2]oct-5-ene]	Monoterpene	40
174	(5S,6R)-2,6-dimethyltricyclo[3.1.1.03,6] hept-1-ene	Monoterpene	37
175	[(5R)-6,6-dimethyl-2-methylenebicyclo[3.1.1] heptane] Pinene	Monoterpene	6
176	[1-Isopropyl-4-methyl-7-oxabicyclo[2.2.1] heptane] 1,4-Cineole	Monoterpene	6
177	[(*Z*)-3,7-dimethylocta-1,3,6-triene] (*Z*)-Ocimene	Monoterpene	38
178	[3,7-Dimethyloct-6-enal] Citronellal	Monoterpene	37
179	[(*E*)-3,7-dimethylocta-2,6-dienal] (*E*)-citral	Monoterpene	38
180	[(*Z*)-3,7-dimethylocta-2,6-dienal] (*Z*)-citral	Monoterpene	38
181	[7-Methyl-3-methyleneocta-1,6-diene] Myrcene	Monoterpene	38
182	(3R)-3,5,7-trimethylocta-1,6-diene	Monoterpene	6
183	2,6-Dimethylhept-5-en-1-yl pentanoate	Monoterpene	37
184	[(*Z*)-3,7-dimethylocta-2,6-dien-1-ol] *Z*-Geraniol	Monoterpene	37
185	[(*E*)-3,7-dimethylocta-2,6-dien-1-ol] *E*-Geraniol	Monoterpene	41
186	3,3,6-trimethylhepta-1,5-dien-4-one	Monoterpene	38
187	[(*E*)-3,7-dimethylocta-1,3,6-triene] ocimene	Monoterpene	38
188	[3,7-dimethylocta-1,6-dien-3-ol] linalool	Monoterpene	37
189	[(*Z*)-3,7-dimethylocta-2,6-dien-1-yl acetate] neryl acetate	Monoterpene	41

190	[(*E*)-3,7-dimethylocta-2,6-dienoic acid] geranic acid	Monoterpene	41
191	[(*E*)-methyl 3,7-dimethylocta-2,6-dienoate] Methyl geranate	Monoterpene	41
192	[(1S,4R)-4,7,7-trimethylbicyclo[2.2.1]heptan-2-one] 3-Bornanone	Monoterpene	41
193	[(*E*)-5,9-dimethyldeca-4,8-dien-2-ol]	Monoterpene	41
194	[(2*E*,5*E*)-3,4,5,6-tetramethylocta-2,5-diene]	Monoterpene	41
195	(*E*)-3,7-dimethylnon-6-enal	Monoterpene	41
196	(2*E*,6*E*)-2,6-dimethylocta-2,6-diene-1,8-diol	Monoterpene	41
197	(2*E*,6*E*)-4,5-dimethylocta-2,6-diene	Monoterpene	41
198	(3*E*,6*Z*,10*E*)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene	Diterpene	41
199	(6*Z*,8*Z*,10*E*)-2,6,11,15-tetramethylhexadeca-2,6,8,10,14-pentaene	Diterpene	41
200	(6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-1,6,10,14-tetraen-3-ol	Diterpene	41
201	[(*E*)-3,7,11,15-tetramethylhexadec-2-en-1-ol] Phytol	Diterpene	6
202	[(5aR,5bR,11aR,13bR)-3-isopropyl-5a,5b,8,8,11a,13b-hexamethyl-4,5,5a,5b,6,7,7a,8,10,11,11a,11b,12,13,13a,13b-hexadecahydro-1*H*-cyclopenta[*a*]chrysen-9(2*H*)-one] hopenone I	Triterpene	44
203	[(5aR,5bR,11aR,13bR)-3-isopropyl-5a,5b,8,8,11a,13b-hexamethyl-2,4,5,5a,5b,6,7,7a,8,9,10,11,11a,11b,12,13,13a,13b-octadecahydro-1*H*-cyclopenta[*a*]chrysen-9-ol] Hop-17(21)-en-3β-ol	Triterpene	44
204	[Methanedione compound with (5aR,5bR,11aS,13bR)-3-isopropyl-5a,5b,8,8,9,11a,13b-heptamethyl-2,4,5,5a,5b,6,7,7a,8,9,10,11,11a,11b,12,13,13a,13b-octadecahydro-1*H*-cyclopenta[*a*]chrysene (1 : 1)] Hop-17(21)-en-3β-yl acetate	Triterpene	44

be useful in various applications, particularly food, medicinal, pharmaceutical and nutraceutical research fields.

4.3 Other Important Components in Turmeric

The turmeric rhizomes contain various important components generally consisting of protein, fat, minerals, carbohydrates, starch and fibers *etc.*[46,47] Curcuminoids are considered as key active constituents of turmeric;[1] however, other components such as saccharides, proteins *etc.* from turmeric have been shown to possess various biological activities.

Table 4.4 Flavonoids in turmeric.

No.	Compound name	Compound type	Reference
205	[5,7-Dihydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one] Apigenin	Flavonoids	10
206	[5-Hydroxy-2-(4-hydroxyphenyl)-7-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one] Apigenin-7-*O*-β-D-glucopyranoside	Flavonoids	10
207	[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-chromen-4-one] Luteolin	Flavonoids	10
208	[2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one] Luteolin-7-*O*-β-D-glucopyranoside	Flavonoids	10
209	[((2R,3S,4S,5R,6S)-6-((2-(3,4-dihydroxyphenyl)-5-hydroxy-4-oxo-4*H*-chromen-7-yl)oxy)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-yl)methyl 4-hydroxybenzoate] Luteolin-7-*O*-(6"–*p*-hydroxybenzoyl-β-D-glucopyranoside	Flavonoids	10
210	[5,7-Dihydroxy-3-(((2S,5S)-3,4,5-trihydroxy-6-((((2R,4S,5R)-3,4,5-trihydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)methyl)tetrahydro-2*H*-pyran-2-yl)oxy)-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one] Myricetin-3-*O*-β-D-rutinoside	Flavonoids	45

4.3.1 Polysaccharides

The most abundant group of biopolymers – polysaccharides – have been found to assist in numerous biological processes, such as cell to cell communication,[48] embryogenesis, antibacterial,[49] anticancer, inflammation and humoral and cellular immunity.[50] Among the polar constituents of turmeric, the polysaccharides of turmeric were shown to possess phagocytosis activating, reticuloendothelial potentiating,[51] anticancer,[52] anti-hepatotoxic,[53] antidepressant,[54] anti-infertility,[54] insulin releasing[55] and anticomplementary activating activities.[51]

Interestingly, the immune-stimulatory and anti-inflammatory activities of an aqueous based extract of *C. longa* was studied by Chandrasekaran *et al.* and they confirmed the potential of a polysaccharide fraction toward inhibiting interleukins and prostaglandins in LPS (Lipopolysaccharide) stimulated splenocytes.[56] The polysaccharide enriched fraction of *C. longa* exhibited stimulatory effects on human peripheral blood mononuclear cells proliferation by the modulation of cytokine production, which revealed the

potential use of *C. longa* polysaccharide as an adjuvant supplement for cancer patients.[57] Moreover, in another study carried out by Illuri *et al.*,[58] the anti-inflammatory effects of the polysaccharide fraction of turmeric extract by the significant inhibition in carrageenan-induced paw edema with turmeric polysaccharide treatment was demonstrated.

4.3.2 Starch

Starch has a long history of use in food and food related products. Starch from turmeric may play a role as an alternative and sustainable starch source to the conventional available starches isolated from either corn, wheat, potato or cassava. Turmeric starch, with less information available, is not widely used in industry. However, with the high starch content and high viscosity values reported in turmeric starch, this plant species offers promise as a starch source of commercial interest to improve utilization beyond traditional usage.[59]

An investigation looking at new starches in the food industry was undertaken by Leonel *et al.*,[59] who evaluated two Curcuma species as a potential source for starch. Captivatingly, their study revealed low dry matter and high starch content, with amylose contents of 22%, which is similar to that of potato starch. Besides, the turmeric starch had high viscosity profiles with high thickening and gelling properties, along with high stability when agitated.[59] The ratio of amylose to amylopectin influences the rheological and textural properties, thereby having an impact on the applications of starch.[60] Further, Hornung *et al.*[61] developed a bioactive starch film with high levels of antioxidant capacity (DPPH, ABTS and ORAC). In addition, the starch film exhibited a smooth structural surface and strong resistance to tensile force, as well as maintaining its elasticity as measured by mechanical assays such as tensile strength and elongation at break.

4.3.3 Dietary Fiber

Dietary fiber comprises a unique blend of bioactive components including resistant starches, vitamins, minerals, phytochemicals and antioxidants; thus research efforts regarding the potential health benefit of dietary fiber have established substantial attention in the last several decades.[62] More importantly, epidemiological and clinical studies demonstrated the advantage of dietary fiber consumption, which is inversely related to obesity,[63] type II diabetes,[64] malignancies[65] and cardiovascular disease.[66] Promisingly, spent turmeric is rich in dietary fiber (45%), containing both insoluble fiber (43%) and soluble fiber (2%).[67] Moreover, an antidiabetic study of spent turmeric reported by Kumar *et al.*[68] demonstrated improved diabetic status in rats due to a high amount of dietary fibers in spent turmeric that facilitated slower absorption of glucose in the gastrointestinal tract.[68]

4.3.4 Cellulose

The turmeric dietary fiber consists of different important components such as hemicellulose, cellulose, lignin and pectin.[47] Cellulose, the most abundant organic compound on the globe, is a polydisperse linear polysaccharide consisting of β-1,4-glycosidic linked D-glucose units.[69] Cellulose, being hydrophilic, is insoluble in water and most organic solvents with a chiral structure. Moreover, it is biodegradable, and can be broken down chemically into its glucose units by treating it with concentrated acids at high temperature. Intriguingly, the characteristic β-acetal linkage in the cellulose structure differs from starch and is indigestible to humans due to an absence of appropriate enzymes to break down the beta acetal linkages.[70]

Cellulose in the nanometric form has tremendous potential as a component in bionanocomposites.[71] In a study, smooth cellulose fiber bundles from turmeric stems were obtained after treatment with an alkali solution. The natural fibers thus obtained had a cellulose content of about 50%, lignin content of 12% and about 10% ash. Further, with tensile strength similar to that of jute, it has antimicrobial activity against both gram-positive and gram-negative bacteria; thereby promising applications in medical (wound dressings) and textiles.[72] Recently, cellulose nanofibers derived from turmeric were reported as a promising biomaterial for application in medicinal and pharmacological fields. A phytogenic feed additive encapsulated onto turmeric nanofiber (TNF) reduced microbial content in cecum and enhanced bodyweight in broilers.[73] Furthermore, a gut health product containing TNF effectively improved typical inflammatory bowel disease symptoms and histological scores, attenuated inflammation and maintained intestinal integrity in dextran sulfate sodium induced ulcerative colitis.[74] The TNF based bionanocomposite films had distinctive physicochemical properties and pronounced biological activity, which is in sync with earlier reports of several properties including antimicrobial, biodegradability, renewability and biocompatibility.[75,76]

4.3.5 Pectin

Pectin is a type of structural fiber found in the cell wall and intracellular layer of plant cells with a linear structure in which polymeric monomeric units of galacturonic acid linked *via* an α-(1→4)-glycosidic bond forms the backbone. This biopolymer has an average molecular weight that ranges between 50 and 150 kDa.[77] Remarkably, pectin is considered as an emerging new bioactive food polysaccharide with health benefits associated with dietary fiber, particularly as an anticancer agent.[78]

Interestingly, pectic polysaccharide isolated from turmeric has patented bioactive functioning against metastasis. A study on anti-ulcer potentials of pectic polysaccharides isolated from turmeric revealed a highly potent promising anti-ulcerous activity, including inhibition of excess acid production, preservation of the gastric mucus layer, anti-inflammatory action,

modulation of antioxidant mechanisms and inhibition of *H. pylori* growth and adherence.[79,80]

4.3.6 Turmeric Protein

Turmerin (~14 kDa), a water-soluble antioxidant turmeric protein, is known to be an effective antioxidant and anti-inflammatory agent.[81] It is a heat stable, noncyclic peptide containing 40 amino acid residues, with a blocked N-terminal and leucine at the C-terminal. Turmeric contains ~0.1% of turmerin, which offers 80% protection to membranes and DNA against oxidative injury[82] and inhibits HIV-infected T-cell proliferation with increased cell viability.[83]

β-turmerin, the antioxidant protein (~34 kDa), is named to differentiate it from turmerin (14 kDa). It is a single chain hydrophobic glycoprotein with amino sugars that is available ~0.021% in turmeric.[84] The antioxidant properties of this protein have been compared with standard antioxidants such as BHA and α-tocopherol. The inhibition rate of β-turmerin was found to be 3200 times more efficient than the standard antioxidants, which proves that β-turmerin can be an effective bioprotective antioxidant agent to cellular components.[85]

4.4 Biological Activities of the Active Ingredients of Turmeric

The chemical composition of turmeric is diverse, primarily containing in the region of 235 terpenes and phenolic compounds,[2] which are obtained through various extraction procedures. The discovery and chemical composition, including the structure and physicochemical properties of these bioactive compounds, provide valuable knowledge about each component. Various researchers intensively studied and explained the fact that as well as curcumin, turmeric contains numerous other active ingredients that exhibit various biological activities that are distinct from curcumin, whereas other compounds' activities are similar to those of curcumin.[1,2,86,87] In addition, certain active ingredients other than curcuminoids have shown similarities in their chemical structures as curcuminoids such as diarylheptanoid (4–25) and terpecurcumin (26–54). Most of the active ingredients other than curcuminoids such as cyclocurcumin (25), calabin A (57), β-atlantone (63), curlone (64), bisabolone (65), bisacurones (77–80), *ar*-turmerol (81), turmerones and turmeronol (91–97) can be comparable to curcuminoids due to their significant biological activities, which were explained by various researchers.[88–105]

Extensive research within the last half century has proven that most of the biological activities, once associated with turmeric, are due to CUR. Even though, it is interesting to maintain that synergistic effect of a mixture of compounds in turmeric that has been observed in a bioavailability study of CUR.[3] A research scientist from Aurea Biolabs developed the bioavailable

curcuminoid – 'Cureit™' based on the recreation of the complete natural turmeric matrix (CNTM) with active curcuminoids (~50%) by a method known as polar-nonpolar-sandwich (PNS) technology, a patent pending formulation. The PNS technology can be used to preserve functional properties, improve the stability of compounds, enhance health benefits, control the release of bioactive compounds at a desired time and for a specific target, and increase the bioavailability of bioactive compounds.§ This CNTM contains not only curcuminoids, but also other active ingredients of turmeric in the form of turmeric water extract and turmeric essential oil.[87,106] The presence of active ingredients other than curcuminoids in Cureit™ increases the physical stability and facilitates sustained drug delivery as well as protecting drug molecules from degradation.[87] The anti-inflammatory properties of CNTM containing curcuminoids and other active ingredients of turmeric CNTM considerably improve the symptoms and biomarkers of rheumatoid arthritis patients.[107] This CNTM can also be used as an anti-ageing, antioxidant and anticancer agent.[108–111] Indeed, it is a successful system for the synergistic combination of other active ingredients of turmeric with curcumin, which ameliorate the bioavailability and enhance the various biological activities without rendering any adverse effects.

4.5 Conclusion

Turmeric (*Curcuma longa* L.) is one of the most extensively phytochemically investigated plant species. More than 235 compounds have been isolated from *Curcuma longa*, among those, active ingredients such as 22 diarylheptanoids, 26 terpecurcumin, polypentanoids, other phenolic compounds, 67 sesquiterpenes, 68 monoterpenes, four diterpenes, two triterpenes and six flavonoids and their chemical names and structures are explained in detail.

Acknowledgements

The contributors gratefully thank the management of Plant Lipids (P) Ltd., Cochin, India, for their support and encouragement. We wish to express our appreciation to our laboratory members for their active help and cooperation.

References

1. A. Amalraj, A. Pius, S. Gopi and S. Gopi, *J. Tradit., Complement. Med.*, 2017, **7**, 205.
2. B. B. Aggarwal, W. Yuan, S. Li and S. C. Gupta, *Mol. Nutr. Food Res.*, 2013, **57**, 1529.
3. S. Li, W. Yuan, G. Deng, P. Wang, P. Yang and B. B. Aggarwal, *Pharm. Crops*, 2011, **2**, 28.
4. S. N. Garg, R. P. Bansal, M. M. Gupta and S. Kumar, *Flavour Fragrance J.*, 1999, **14**, 315.

§Adapted from ref. 87 with permission from Elsevier. Copyright 2017.

5. A. C. Manzan, F. S. Toniolo, E. Bredow and N. P. Povh, *J. Agric. Food Chem.*, 2003, **51**, 6802.
6. X. Ma and D. R. Gang, *J. Agric. Food Chem.*, 2006, **54**, 9573.
7. J. J. Chen, C. S. Tsai, T. L. Hwang, P. C. Shieh, J. F. Chen and P. J. Sung, *Food Chem.*, 2010, **119**, 974.
8. T. T. Dao, P. H. Nguyen, H. K. Won, E. H. Kim, J. Park, B. Y. Won and W. K. Oh, *Food Chem.*, 2012, **134**, 21.
9. D. Del Prete, E. Millán, F. Pollastro, G. Chianese, P. Luciano, J. A. Collado, E. Munoz, G. Appendino and O. Taglialatela-Scafati, *J. Nat. Prod.*, 2016, **79**, 267.
10. M. H. Shabana and M. S. Afifi, *J. Med. Plants Res.*, 2014, **8**, 1.
11. Y. C. Xiao, J. Xie, M. Yu, M. Liu, J. Ran, Z. Xi, W. Li and J. Huang, *Chin. Chem. Lett.*, 2011, **22**, 1457.
12. J. H. Yi, Y. Chen, B. G. Li and G. L. Zhang, *Nat. Prod. Res. Dev.*, 2003, **15**, 98.
13. T. Kita, S. Imai, H. Sawada and H. Seto, *Biosci., Biotechnol., Biochem.*, 2009, **73**, 1113.
14. S. Y. Park and D. S. L. H. Kim, *J. Nat. Prod.*, 2002, **65**, 1227.
15. B. S. Park, G. J. Kim, M. R. Kim, S. E. Lee, G. R. Takeoka, K. B. Oh and J. H. Kim, *J. Agric. Food Chem.*, 2005, **53**, 9005.
16. W. Li, S. S. Wang, J. T. Feng, Y. S. Xiao, X. Y. Xue, H. Zhang, Y. Q. Wang and X. M. Liang, *Magn. Reson. Chem.*, 2009, **47**, 902.
17. L. Y. Wang, M. Zhang, C. F. Zhang and Z. T. Wang, *Biochem. Syst. Ecol.*, 2008, **36**, 476.
18. P. Chavalittumrong, W. Jirawattanapong and W. Thai, *J. Pharm. Sci.*, 1992, **16**, 165.
19. X. H. Lin, S. Ji, R. Li, Y. H. Dong, X. Qiao, H. B. Hu, W. Z. Yang, D. A. Guo, P. F. Tu and M. Ye, *J. Nat. Prod.*, 2012, **75**, 2121.
20. X. Qiao, X. H. Lin, S. Ji, Z. X. Zhang, T. Bo, D. A. Guo and M. Ye, *Anal. Chem.*, 2016, **88**, 703.
21. Y. C. Xiao, J. Lei, M. Liu, M. Yu, J. Ran, J. Xie, W. Li and J. Huang, *Helv. Chim. Acta*, 2012, **95**, 327.
22. X. H. Lin, S. Ji, X. Qiao, H. B. Hu, N. Chen, Y. H. Dong, Y. Huang, D. A. Guo, P. F. Tu and M. Ye, *J. Org. Chem.*, 2013, **78**, 11835.
23. Y. C. Zeng, F. Qiu, K. Takahashi, J. M. Liang, G. X. Qu and X. S. Yao, *Chem. Pharm. Bull.*, 2007, **55**, 940.
24. Y. Zeng, J. Liang and G. Qu, *Chin. J. Med. Chem.*, 2007, **17**, 238.
25. M. Ohshiro, M. Kuroyanagi and A. Ueno, *Phytochemistry*, 1990, **29**, 2201.
26. L. Y. Wang, M. Zhang, C. F. Zhang and Z. T. Wang, *Acta Pharmacol. Sin.*, 2008, **43**, 724.
27. W. Li, J. T. Feng, Y. S. Xiao, Y. Q. Wang, X. Y. Xue and X. M. Liang, *J. Asian Nat. Prod. Res.*, 2009, **11**, 569.
28. J. Xu, F. F. Ji, J. Kang, H. Wang, S. Li, D. Q. Jin, Q. Zhang, H. W. Sun and Y. Q. Guo, *J. Agric. Food Chem.*, 2015, **63**, 5805.
29. W. Y. Zhang, H. F. Wang, G. Chen, O. Zhang, S. Bai and Y. H. Pei, *J. Asian Nat. Prod. Res.*, 2014, **16**, 271.

30. B. T. Golding and E. Pombo-Villar, *J. Chem. Soc., Perkin Trans.*, 1992, **1**, 1519.
31. S. Imai, M. Morikiyo, K. Furihata, Y. Hayakawa and H. Seto, *Agric. Biol. Chem.*, 1990, **54**, 2367.
32. J. Li, H. F. Wang, G. Chen, S. D. Huang, W. Y. Zhang, H. M. Hua and Y. H. Pei, *Magn. Reson. Chem.*, 2015, **53**, 536.
33. Y. H. Cui, H. F. Wang, J. Li, G. Chen and Y. H. Pei, *J. Shenyang Pharm. Univ.*, 2016, **33**, 198.
34. J. H. Yi, Y. Chen, B. G. Li and G. L. Zhang, *Nat. Prod. Res. Dev.*, 2003, **15**, 98.
35. Z. H. Wu, Y. L. Cao and C. J. Gao, *Chin. Tradit. Herb. Drugs*, 2010, **41**, 1234.
36. C. Liu, B. Sun, J. Huang, H. Gao, S. Wen and L. Wu, *Asian J. Tradit. Med.*, 2007, **2**, 82.
37. B. Gopalan, M. Goto, A. Kodama and T. Hirose, *J. Agric. Food Chem.*, 2000, **48**, 2189.
38. L. A. Usman, A. A. Hamid, O. C. George, O. M. Ameen, N. O. Muhammad, M. F. Zubair and A. Lawal, *World J. Chem.*, 2009, **4**, 178.
39. Y. C. Zeng, J. M. Liang, G. X. Qu and F. Qiu, *Acta Pharm. Sin.*, 2007, **17**, 738.
40. A. L. Chassagnez-Me'ndez, N. T. Machado, M. E. Araujo, J. G. Maia and M. A. A. Meireles, *Ind. Eng. Chem. Res.*, 2000, **39**, 4729.
41. J. U. Chowdhury, N. C. Nandi, M. N. I. Bhuiyan and M. H. Mobarok, *Bangladesh J. Sci. Ind. Res.*, 2008, **43**, 259.
42. N. K. Leela, A. Tava, P. M. Shafi, S. P. John and B. Chempakam, *Acta Pharm.*, 2002, **52**, 137.
43. B. B. Aggarwal, I. D. Bhatt, H. Ichikawa, K. S. Ahn, G. Sethi, S. K. Sandur, C. Sundaram, N. Seeram and S. Shishodia, in *Turmeric: The Genus Curcuma*, ed. P. N. Ravindran, K. Nirmal Babu and K. Sivaraman, CRC Press, New York, 2007, p. 297.
44. S. M. Mohamed, S. E. El-Gengaihi and H. M. Motawe, *Egypt. J. Pharm. Sci.*, 2003, **43**, 139.
45. R. Sahu and J. Saxena, *World Pharm. Res.*, 2014, **3**, 740.
46. K. J. Kamble, V. M. Ingale and D. P. Kaledhonkar, *Afr. J. Food Sci.*, 2011, **5**, 780.
47. S. Gopi, A. Amalraj, S. Jude, K. T. Benson, P. Balakrishnan, J. T. Haponiuk and S. Thomas, *J. Macromol. Sci., Part A*, 2019, **56**, 327.
48. A. Varki, *Glycobiology*, 1993, **3**, 97.
49. C. L. Cooke, H. J. An, J. Kim, J. V. Solnick and C. B. Lebrilla, *Anal. Chem.*, 2007, **79**, 8090.
50. D. H. Dube and C. R. Bertozzi, *Nat. Rev. Drug Discovery*, 2005, **4**, 477.
51. R. Gonda, M. Tomoda, N. Ohara and K. Takada, *Biol. Pharm. Bull.*, 1993, **16**, 235.
52. S. S. Deshpande, A. D. Ingle and G. B. Maru, *Cancer Lett.*, 1998, **123**, 35.
53. L. Subramanian and R. Selvam, *Nutr. Res.*, 1999, **19**, 429.

54. R. K. Mishra and S. K. Singh, *Contraception*, 2009, **79**, 479.
55. S. Mohankumar and J. R. McFarlane, *Phytother Res.*, 2011, **25**, 396.
56. C. V. Chandrasekaran, K. Sundarajan, J. R. Edwin, G. M. Gururaja, D. Mundkinajeddu and A. Agarwal, *Pharmacogn. Res.*, 2013, **5**, 71.
57. G. G. Yue, B. C. Chan, P. M. Hon, E. J. Kennelly, S. K. Yeung, B. R. Cassileth, K. P. Fung, P. C. Leung and C. B. Lau, *Int. J. Biol. Macromol.*, 2010, **47**, 342.
58. R. Illuri, B. Bethapudi, S. Anandakumar, S. Murugan, J. A. Joseph, D. Mundkinajeddu, A. Agarwal and C. V. Chandrasekaran, *Antiinflamm. Antiallergy Agents Med. Chem.*, 2015, **14**, 53.
59. M. Leonel, S. B. S Sarmento and M. P. Cereda, *Carbohydr. Polym.*, 2003, **54**, 385.
60. J. W. Lawton (Retired), in *Encyclopedia of Food Grains*, ed. C. Wrigley, H. Corke, K. Seetharaman and J. Faubion, Academic Press, 2nd edn, 2016, p. 274.
61. P. S. Hornung, K. Masisi, L. N. Malunga, T. Beta and R. H. Ribani, *Polym. Bull.*, 2018, **75**, 4735.
62. J. M. Lattimer and M. D. Haub, *Nutrients*, 2010, **2**, 1266.
63. L. A. Tucker and K. S. Thomas, *J. Nutr.*, 2009, **139**, 576.
64. K. Esposito, F. Nappo, F. Giugliano, C. Di Palo, M. Ciotola, M. Barbieri, G. Paolisso and D. Giugliano, *Am. J. Clin. Nutr.*, 2003, **78**, 1135.
65. FDA, Code of Federal Regulations, *Food and Drug Administration; Health Claims: Fiber-containing Grain Products, Fruits and Vegetables and Cancer*, Silver Spring, MD, USA, 2008, vol. 2.
66. FDA, Code of Federal Regulations, *Food and Drug Administration; Health Claims: Fruits, Vegetables, and Grain Products that Contain Fiber, Particularly Soluble Fiber, and Risk of Coronary Heart Disease*, Silver Spring, MD, USA, 2008, vol. 2.
67. G. S. Kumar and P. V. Salimath, *J. Diabetes Metab. Disord.*, 2014, **13**, 78.
68. S. G. Kumar, A. K. Shetty, K. Sambaiah and P. V. Salimath, *Nutr. Res.*, 2005, **25**, 1021.
69. E. Rudnik, in *Handbook of Biopolymers and Biodegradable Plastics*, ed. S. Ebnesajjad, William Andrew Publishing, 2013, p. 189.
70. Y. Dahman, in *Nanotechnology and Functional Materials for Engineers*, ed. Y. Dahman, Elsevier, 2017, p. 47.
71. D. S. Le Corre, N. Tucker and M. P. Staiger, in *Natural Fibre Composites*, ed. A. Hodzic and R. Shanks, Woodhead Publishing, 2014, p. 115.
72. M. Ilangovan, V. Guna, C. Hu, G. S. Nagananda and N. Reddy, *Ind. Crop. Prod.*, 2018, **112**, 556.
73. S. Gopi, A. Amalraj, K. Varma, S. Jude, P. B. Reddy, C. Divya, J. T. Haponiuk and S. Thomas, *Int. J. Polym. Mater. Polym. Biomater.*, 2018, **67**, 581.
74. S. Gopi, A. Amalraj, S. Jude, K. Varma, T. R. Sreeraj, J. T. Haponiuk and S. Thomas, *Mater. Sci. Eng., C*, 2017, **81**, 20.
75. S. Gopi, A. Amalraj, N. Kalarikkal, J. Zhang, S. Thomas and Q. Guo, *Mater. Sci. Eng., C*, 2019, **97**, 723.

76. S. Gopi, A. Amalraj, S. Jude, S. Thomas and Q. Guo, *J. Taiwan Inst. Chem. Eng.*, 2019, **96**, 664.
77. D. Mudgil, in *Dietary Fiber for the Prevention of Cardiovascular Disease*, ed. R. A. Samaan, Academic Press, US, 2017, p. 35.
78. E. G. Maxwell, N. J. Belshaw, K. W. Waldron and V. J. Morris, *Trends Food Sci. Technol.*, 2012, **24**, 64.
79. M. R. Harsha, S. V. Chandra Prakash and S. M. Dharmesh, *Carbohydr. Polym.*, 2016, **138**, 143.
80. H. M. Rajagopal, S. B. Manjegowda, C. Serkad and S. M. Dharmesh, *Int. J. Biol. Macromol.*, 2018, **118**, 864.
81. M. Chethankumar and L. Srinivas, *Biol. Chem.*, 2008, **389**, 299.
82. L. Srinivas, V. K. Shalini and M. Shylaja, *Arch. Biochem. Biophys.*, 1992, **292**, 617.
83. H. H. P. Cohly, S. Asad, S. K. Das, M. F. Angel and M. Rao, *Int. J. Mol. Sci.*, 2003, **4**, 22.
84. S. Smitha, B. L. Dhananjaya, R. Dinesha and L. Srinivas, *Biochimie*, 2009, **91**, 1156.
85. M. Sivapriya and L. Srinivas, *Food Chem.*, 2007, **104**, 510.
86. A. Nair, A. Amalraj, J. Jacob, A. B. Kunnumakkara and S. Gopi, *Biomolecules*, 2019, **9**, 13.
87. A. Amalraj, S. Jude, K. Varma, J. Jacob, S. Gopi, O. S. Oluwafemi and S. Thomas, *Mater. Sci. Eng., C*, 2017, **75**, 359.
88. H. B. Cheng, L. C. Wu, Y. C. Hsieh, C. H. Wu, Y. J. Chan, L. H. Chang, C. M. J. Chang, S. L. Hsu, C. L. Teng and C. C. Wu, *J. Agric. Food Chem.*, 2012, **60**, 9620.
89. D. Kim, Y. Suh, H. Lee and Y. Lee, *Int. J. Mol. Med.*, 2013, **31**, 386.
90. G. G. L. Yue, L. Jiang, H. F. Kwok, J. K. M. Lee, K. M. Chan, K. P. Fung, P. C. Leung and C. B. S. Lau, *J. Funct. Foods*, 2016, **22**, 565.
91. K. S. Mukunthan, R. S. Satyan and T. N. Patel, *J. Ethnopharmacol.*, 2017, **209**, 82.
92. X. S. Wu, T. Xiea, J. Lina, H. Z. Fana, H. J. Huang-Fua, L. F. Nia and H. F. Yan, *J. Pharm. Pharmacol.*, 2009, **61**, 1653.
93. Z. Shu, J. Liua, X. J. Hub, B. Jina, X. J. Qua, K. Z. Houa and Y. P. Liua, *J. Pharm. Pharmacol.*, 2011, **64**, 146.
94. S. Y. Zhao, J. Wu, F. Zheng, Q. Tang, L. J. Yang, L. Li, W. Y. Wu and S. S. Hann, *J. Cell. Mol. Med.*, 2015, **19**, 630.
95. J. S. Liu, S. C. He, Z. L. Zhang, R. Chen, L. Fan, G. L. Qiu, S. Chang, L. Li and X. M. Che, *Oncol. Rep.*, 2014, **32**, 2635.
96. A. Kumar and U. Bora, *Interdiscip. Sci.: Comput. Life Sci.*, 2014, **6**, 1.
97. W. S. Liou, C. Lin and P. S. Lee, *J. Funct. Foods*, 2016, **26**, 781.
98. A. K. Tyagi, S. Prasad, M. Majeed and B. B. Aggarwal, *Arch. Biochem. Biophys.*, 2016, **593**, 80.
99. D. I. Suna, I. T. Nizamutdinova, Y. M. Kim, X. F. Cai, J. J. Lee, S. S. Kang, Y. S. Kim, K. M. Kang, G. Y. Chai, K. C. Chang and H. J. Kim, *Int. Immunopharmacol.*, 2008, **8**, 1272.

100. P. Prakash, A. Misra, W. R. Surin, M. Jain, R. S. Bhatta, R. Pal, K. Raj, M. K. Barthwal and M. Dikshit, *Thromb. Res.*, 2011, **127**, 111.
101. A. K. Tyagi, S. Prasad, W. Yuan, S. Li and B. B. Aggarwal, *Invest. New Drugs*, 2015, **33**, 1175.
102. C. Guan, W. Liu, Y. Yue, H. Jin, X. Wang and X. J. Wang, *Int. J. Clin. Exp. Pathol.*, 2014, **7**, 3948.
103. Y. Dong, L. Li, L. Wang, T. Zhou, J. W. Liu and Y. J. Gao, *Genet. Mol. Res.*, 2015, **14**, 2347.
104. J. Chen, T. Wang, S. Xu, P. Zhang, A. Lin, L. Wu, H. Yao, W. Xie, Z. Zhu and J. Xu, *Eur. J. Med. Chem.*, 2017, **135**, 414.
105. M.-J. Lee, Y.-J. Tsai, M.-Y. Lin, H.-L. You, N. Kalyanam, C.-T. Ho and M.-H. Pan, *Phytomedicine*, 2019, **57**, 377.
106. S. Gopi, J. Jacob, K. Varma, S. Jude, A. Amalraj, C. A. Arundhathy, R. George, T. R. Sreeraj, C. Divya, A. B. Kunnumakkara and S. J. Stohs, *Phytother. Res.*, 2017, **31**, 1883.
107. A. Amalraj, K. Varma, J. Jacob, C. Divya, A. B. Kunnumakkara, S. J. Stohs and S. Gopi, *J. Med. Food*, 2017, **20**, 1022.
108. S. Gopi, R. George, M. Thomas and S. Jude, *Asian J. Pharm. Technol. Innovation*, 2015, **3**, 92.
109. S. Gopi, R. George, S. Jude and V. T. Sriraam, *J. Chem. Pharm. Res.*, 2014, **6**, 96.
110. S. Gopi, R. George and V. T. Sriraam, *Asian J. Pharm. Technol. Innovation*, 2014, **2**, 123.
111. S. Gopi, R. George and V. T. Sriraam, *Int. J. Curr. Res.*, 2014, **6**, 8473.
112. F.-C. Meng, Y.-Q. Zhou, D. Ren, R. Wang, C. Wang, L.-G. Lin, X.-Q. Zhang, W.-C. Ye and Q.-W. Zhang, *Natural and Artificial Flavoring Agents and Food Dyes*, 2018, pp. 299–350.

CHAPTER 5

Curcuminoids – Isolation, Formulations and Bioavailability Problems

BERND-MICHAEL LÖFFLER*[a], SHINTU JUDE[b], AUGUSTINE AMALRAJ[b] AND SREERAJ GOPI[b]

[a]Institute of Mitochondrial Medicine, Pfalzburger Str. 43/44, 10717 Berlin, Germany; [b]R&D Centre, Aurea Biolabs (P) Ltd, Kolenchery, Cochin–682 311, Kerala, India
*E-mail: loeffler@imm.institute

5.1 Introduction

Natural products have been used in traditional medicines for thousands of years, and have shown promise as sources of components for the development of new drugs.[1,2] Turmeric (*Curcuma longa* Linn) is a member of the Zingiberaceae family and is cultivated in tropical and subtropical regions around the world. It originates from India, Southeast Asia and Indonesia.[3] Turmeric powder is used extensively as a coloring and flavoring agent in curries and mustards. Turmeric has been used in India to maintain oral hygiene.[4] It has traditionally been used for medical purposes for many centuries in countries such as India and China for the treatment of jaundice and other liver ailments.[5,6] Turmeric is one of the most popular medicinal herbs, with a wide range of pharmacological activities due to its antioxidant,[7] anti-protozoal,[8]

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

anti-venom activities,[9] anti-microbial,[10] anti-malarial,[11] anti-inflammatory,[12] anti-proliferative,[13] anti-angiogenic,[14] anti-tumor[15] and anti-aging[16] properties. It has also been used to treat ulcers, parasitic infections, various skin diseases, auto-immune diseases and to cure the symptoms of colds and flu.[17] Chemical analyses have shown that the dried rhizomes of turmeric contains carbohydrates (69.4%), moisture (13.1%), protein (6.3%), fat (5.1%) and minerals (3.5%). The essential oil (5.8%) obtained by steam distillation of the rhizomes contains α-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%).[18]

Two active parts of turmeric are the volatile oil and curcuminoids and both are present in the oleoresin extracted from the turmeric root. The essential oils are composed mainly of sesquiterpenes, many of which are specific for the Curcuma genus. The aroma of this spice is principally derived from α- and β-turmerones and aromatic turmerone (Ar-turmerone).[19]

Turmeric contains more than 200 biologically active constituents, which are just recently becoming a focus of research.[20] However, the pharmacological activity of turmeric has been attributed mainly to curcuminoids consisting of curcumin (CUR) and two related compounds named desmethoxycurcumin (DMC) and bisdesmethoxycurcumin (BDMC).[3] CUR itself appears as a crystalline compound with a bright orange-yellow color. Curcuminoids are commonly used as coloring agents as well as food additives. The World Health Organization (WHO) stated the acceptable daily intake of curcuminoids as a food additive is in the range of 0–3 mg kg^{-1} bodyweight. Curcuminoids and turmeric products have been characterized as safe by the Food and Drug Administration (FDA) in the USA. The average daily intake of turmeric in the Indian diet is approximately 2–2.5 g, which corresponds to a daily intake of approximately 60–100 mg of CUR.[21] Curcuminoids have achieved potential therapeutic interest to cure immune related, metabolic diseases and cancer due to a vast number of biological targets and virtually no side effects.[17,21]

5.2 Discovery of Curcumin

Curcumin is the major active ingredient of the dietary spice turmeric and is extracted from the rhizomes of *Curcuma longa* Linn, a plant in the Zingiberaceae family. It was first discovered about two centuries ago when Vogel and Pelletier reported the isolation of a 'yellow coloring matter' from rhizomes of *Curcuma longa* Linn and named it curcumin.[22] It has been characterized by Milobedeska *et al.*[23] and first synthesized by Lampe *et al.*[24]

5.3 Isolation of Curcumin

Curcumin is insoluble in water, so an organic solvent is used for its isolation. Anderson *et al.*[25] developed a technique for isolating CUR from ground turmeric. They magnetically stirred the ground turmeric in dichloromethane

and heated at reflux for 1 hour. The mixture was suction-filtered, and the filtrate was concentrated in a hot-water bath maintained at 50 °C. The reddish-yellow oil residue was triturated with hexane and the resulting solid was collected by suction filtration. Further TLC analysis (3% methanol and 97% dichloromethane) showed the presence of all three components.[26] Bagchi explained extraction of CUR from turmeric powder with the use of a solvent consisting of a mixture of ethanol and acetone.[19]

5.4 Physical, Chemical and Molecular Properties of Curcuminoids

Curcumin (3–6%) is responsible for the yellow-orange color of turmeric.[18] The chemical structures of curcuminoids make them much less soluble in water at acidic and neutral pH, but soluble in methanol, ethanol, dimethyl sulfoxide and acetone. The curcuminoids give a yellow-orange coloration to turmeric powder due to the wide electronic delocalization inside the molecules that exhibit strong absorption between 420 and 430 nm in an organic solvent. As stated above, the curcuminoids are a mixture of curcumin, chemically a diferuloylmethane [1,7-bis(4-hydroxy-3-methoxy-phenyl)-hepta-1,6-diene-3,5-dione], with its two derivatives, DMC [1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione] and BDMC [1,7-bis(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione], defining the chemical formulae as $C_{21}H_{20}O_6$, $C_{20}H_{18}O_5$ and $C_{19}H_{16}O_4$, respectively.[17] The chemical structures of these quantitative most important constituents present in turmeric are given in Figure 5.1 They share the same structure with two benzenemethoxy rings, joined by an unsaturated chain. It has three important functions: an aromatic methoxy phenolic group, α,β-unsaturated β-diketo linker and keto–enol tautomerism. All these compounds exist in the trans–trans keto–enol form. The aromatic groups provide hydrophobicity and the linker gives flexibility. The tautomeric structures also influence the hydrophobicity and polarity. The hydrophobicity of curcuminoids makes them

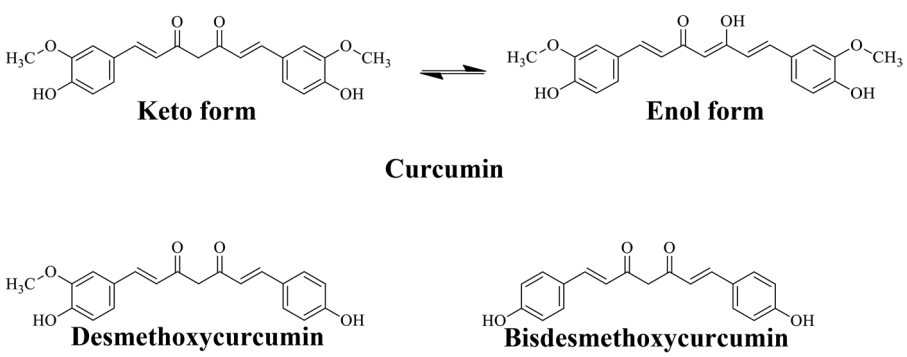


Figure 5.1 Chemical structures of curcuminoids.

poorly soluble in water. Three acidity constants (p*K*a) were measured for curcuminoids as follows, p*K*a1 = 8.38 ± 0.04, p*K*a2 = 9.88 ± 0.02 and p*K*a3 = 10.51 ± 0.01.[27] They are chemically unstable under alkaline conditions but much more stable under acidic conditions in an aqueous environment. Typical curcuminoids composition of popular Indian Curcuma species was found to be in the range of CUR 52–63%, DMC 19–27% and BDMC 18–28%.[17]

5.5 Why are Formulations Needed for Curcumin?

Curcuminoid is a yellow-colored phenolic natural extract derived from the rhizome of the spice herb *Curcuma longa* Linn, widely known as turmeric, and its commercially available natural form (commonly referred to as 'standard curcumin') with a purity of ≥95%, is a mixture of three curcuminoids CUR (72 to 78%), DMC (12 to 18%) and BDMC (3 to 8%).[18] This relatively stable composition as a result from a standardized extraction process, which is used in the vast majority of clinical 'curcumin' studies in different formulations, will be referred to in this chapter as curcuminoid, for the sake of simplicity. In recent decades, curcuminoid draws great attention for its broad spectrum of therapeutic actions and potential ability in preventing many diseases.[7,12,13,15,18,28–30] Besides, it has a superior safety profile determined by clinical studies that as high as 8 g day^{-1} of dosage would not induce any observable adverse effects.[31] Even so, one should also critically reflect on these data: (1) They have been obtained with unformulated standard curcuminoid. Thus, their significances are limited to the already named pure systemic bioavailability of unformulated curcuminoid and does reflect only limited systemic safety. (2) The gastrointestinal acceptance for curcuminoid differs, for example, between Europeans and Indians. Some Europeans tend to develop temporary side effects such as *e.g.* diarrhea, probably because their gastrointestinal tracts are not by their nutritional habits 'trained' for the immediate acceptance of higher curcuminoid amounts. (3) The data available so far, especially taking into account unformulated and differently formulated curcuminoid preparations, are scattered and limit the predication of 'safety'. Nevertheless, the safety profile accorded with unformulated curcuminoid has been reflected by the continuous increase of preparations based on curcuminoid marketed as a food ingredient or constituent of dietary supplements in an increasingly wide range of formulations in which curcuminoid is also combined with other biologically active ingredients. However, the functional applications of curcuminoid have been seriously limited by its very low systemic bioavailability, attributable to poor absorption, fast metabolic alterations and rapid elimination.[32,33] Available evidence indicates that only minute amounts of curcuminoid reach the circulation after high-dose oral administration in animals and humans (this will be discussed in more detail later in this chapter). So far, the vast majority of studies base their conclusions on the serum levels measured in humans and animals. Whether this is appropriate or not we will discuss later. The majority of orally administered curcuminoid is excreted in feces and urine, with very little being detected

in the systemic circulation.[34] Curcuminoid has very low solubility in aqueous media due to inter and intra hydrogen bonding.[35] Higher solubility was observed in alkaline solution, when dissolved curcuminoid quickly degraded into vanillin, ferulic acid and feruloyl methane.[36] Other environmental factors such as UV irradiation and heating also contribute to the decomposition of this yellowish polyphenolic compound.[37–39] These dramatically affect the absorption and bioavailability of this active molecule with consequent unsatisfactory pharmacokinetic profile and reduced efficacy. However, it had been shown that (a) curcuminoid is more stable to degradation as CUR, (b) CUR and curcuminoid are more stable in a water/lipid environment, and (c) CUR and curcuminoid stability can be majorly increased in the presence of proteins, *e.g.* albumin.[40–42] Thus, the classical 'presentation' of turmeric as in the Indian diet has considerable impact on the bioavailability of curcuminoid. This, logically, should have effects on health and at the same time presents a principal question on the design and logic of the majority of modern bioavailability studies, where unformulated curcuminoid – following the narrative of classical pharmacokinetic studies – are administered in a fasting state, to be then compared to curcuminoid, which has been (in one way or another) stabilized to achieve higher bioavailability.

To increase its water solubility, stability, bioavailability and potential applications as well as to improve clinical efficacy, different methods have been proposed and investigated. Several strategies such as incorporation of adjuvants, nanoparticles, liposomes, micronization, encapsulation, solid dispersions and complexation with phospholipids and cyclodextrins have been developed and investigated to improve the bioavailability of curcumin/curcuminoid.[43–47] Different formulations and their discrimination are depicted in Figure 5.2.

5.6 Different Formulations

As mentioned earlier, many strategies have been introduced for the enhancement of curcuminoid bioavailability and the potency of them has been assured with different modes of analyses ranging from *in vitro* studies to human trials. This is a crucial point of understanding, which is so far not taken properly in common understanding. As the main interest is centered on the therapeutic value of CUR in different medical applications, the development of oral formulations is centered on curcuminoid. Curcuminoid is more stable in acidic environments due to its composition of CUR + DMC + BDMC, as CUR alone and as a mixture is more stable in the presence of proteins, *e.g.* albumin. All of these factors will influence the outcome in a multiparametric way – they are difficult to control and compare, from study to study – and might, at least to some extent, be the background for the conflicting results reported.[40–42] While cell-line studies and animal studies ensure efficiency and provide grounds for further developments, reliable and compatible results produced from human trials, including the systemic concentration of the curcuminoid, enable one to extrapolate the data towards real

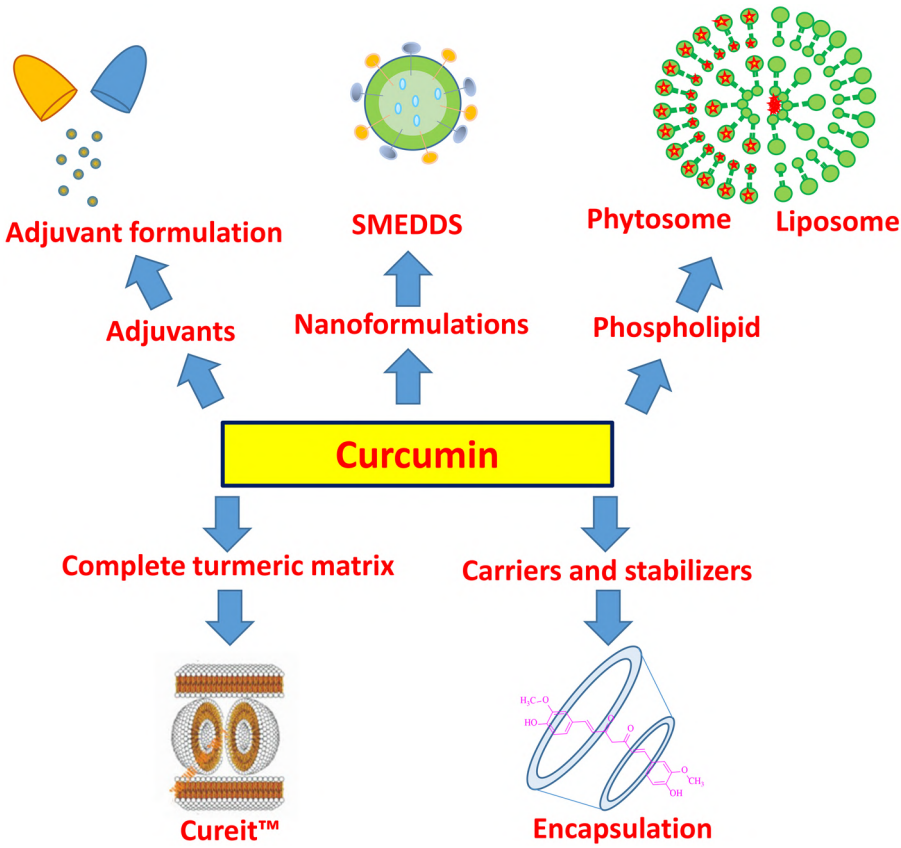


Figure 5.2 Schematic representation of different bioavailability formulations.

contexts. We have considered these studies in the discussion in this chapter, and their features are given in Table 5.1.

5.6.1 Adjuvants for Bioavailability Enhancement

The activities and bioavailability of curcuminoid can be positively altered by the co-administration of many compounds. One important factor among the formulations is the co-administration of piperine, as an adjuvant. Piperine was evidenced to inhibit the glucuronidation of many drugs and thus enhances their bioavailability. The influence of this record is reflected in the formulations of curcumin incorporated with piperine. Curcumin–piperine combinations are prepared in many formats, and almost all of them exhibit an enhanced oral bioavailability. Curcumin–piperine simple co-administration,[48] nano-formulations of curcumin–piperine complex[49,50] curcumin–piperine complex loaded in zein–chitosan nanoparticles[51] are different fabrications for this purpose. The bioavailability provided by the

Table 5.1 Different formulations and their bioavailability studies.[a]

	Administered dose		Study details				Ratio of pharmacokinetic parameters against control			
Formulations	Control	Test	Subject	Mode of administration	No. of subjects	Control used	C_{max}	AUC	T_{max}	Reference
CUR–piperine complex	2g kg^{-1}	2 g kg^{-1} + 20 mg kg^{-1}	Rats	Oral	96	Free curcumin	1.33	1.54	1.55	48
	2g	2g	Human	Oral	10	Free curcumin	30	20	0.69	48
CUR–piperine cubosome	100 mg kg^{-1}	100 mg kg^{-1} + 5 mg kg^{-1}	Mice	Oral	10	Free curcumin	7.95	16.648	50.34	50
Curcuminoid with hydrophilic carrier, cellulosic derivatives and antioxidants	1800 mg	376 mg	Human	Oral	15	Standardized curcumin	6.7	9.6	0.179	55
CUR–SMEDDS	50 mg kg^{-1}	50 mg kg^{-1}	Rats	Oral	NA	Curcumin suspension	17.52	13.93	1	57
CUR–SMEDDS containing EIE	50 mg kg^{-1}	50 mg kg^{-1}	Rats	Oral	12	Curcumin aqueous suspension	26.45	73.93	1	58
CUR–PLGA nanoparticles	50 mg kg^{-1}	50 mg kg^{-1}	Rats	Oral	10	Curcumin aqueous suspension	2.9	15.63	4	60
CUR–PLGA–PEG nanoparticles	50 mg kg^{-1}	50 mg kg^{-1}	Rats	Oral	10	Curcumin aqueous suspension	7.4	55.46	6	60
CUR nanosuspension	15 mg kg^{-1}	15 mg kg^{-1}	Rabbits	Oral	8	Curcumin solution	3.14	4.81	NA	61

Curcuminoid in colloidal nanoparticles	50 mg kg^{-1}	50 mg kg^{-1}	Rats	Oral stomach intubation	12	Curcumin powder	58.76	43.99	0.5	66
	30 mg	30 mg	Human	Oral	14	Curcumin powder	16.38	27.56	0.167	66
Curcuminoid in colloidal nanoparticles liquid	30 mg	30 mg	Human	Oral	24	Three other commercial products	1.71–3.8	1.52–4.04	NA	67
Micronized curcuminoid	500 mg	500 mg	Human	Oral	23	Native powder curcumin	10.16	11.05	1.17	72
MicroActive curcuminoid	500 mg	500 mg	Human	Oral	10	95% Curcumin powder	NA	9.7	NA	73
Curcuminoid microspheres with ascorbic acid	100 mg kg^{-1}	100 mg kg^{-1} + 5 mg kg^{-1}	Rats	Oral gavage	24	Curcumin powder	2.22	7.33	NA	75
CUR microspheres with ethyl cellulose and citric acid	50 mg kg^{-1}	50 mg kg^{-1}	Rats	Oral	40	Curcumin solution	3.2	12.07	16	76
Curcuminoid with soluble fenugreek dietary fiber microgranulates	250 mg kg^{-1}	250 mg kg^{-1}	Rats	Oral gavage	16	Curcumin powder	35	19.99	1.67	78
	1000 mg	600 mg	Human	Oral	8	Curcumin powder	16.8	15.88	2	78
CUR-phytosome-loaded chitosan microspheres	100 mg kg^{-1}	100 mg kg^{-1}	Rats	Oral gavage	20	Natural curcumin	4.155	7.49	3.33	79
Curcuminoid–cyclodextrine complex	1800 mg	376 mg	Human	Oral	12	Standard curcumin extract	18.5	7.45	0.25	80

(*continued*)

Table 5.1 (*continued*)

	Administered dose		Study details				Ratio of pharmacokinetic parameters against control			
Formulations	Control	Test	Subject	Mode of administration	No. of subjects	Control used	C_{max}	AUC	T_{max}	Reference
CUR-hydroxypropyl-β-cyclodextrin	10 mg kg^{-1}	10 mg kg^{-1}	Rats	Iv	NA	Free curcumin	NA	1.59	NA	82
	50 mg kg^{-1}	50 mg kg^{-1}	Rats	Oral gavage	NA	Free curcumin	NA	2.77	NA	82
Curcuminoid liquid micelles	500 mg	500 mg	Human	Oral	23	Native powder curcumin	404.17	173.02	0.147	72
Curcuminoid–phosphatidylcholine phytosome complex	1799 mg	376 mg	Human	Oral	9	Curcuminoids mixture	14.37	6.58	0.39	90
	340 mg kg^{-1}	340 mg kg^{-1}	Rats	Oral gavage	12	Unformulated commercial curcumin	5.138	5.56	0.5	91
Solid-lipid curcuminoid particle	>390 mg	130–195 mg	Human	Oral	6	95% curcuminoids extract	22.43	178.44/NA	2.4/NA	92
Curcuminoid-turmeric oil	NA	2 g	Human	Oral	11	Curcumin powder	3.049	6.93	1.72	98
Curcuminoid in complete turmeric matrix	500 mg	250 mg	Human	Oral	12	Curcumin powder	10.075	5.08	1.16	109
	80.5 mg, 351 mg	180 mg	Human	Oral	45	Two other curcuminoid formulations	3.57, 2.44	7.03, 4.40	1.24, 1.41	112

[a]The results produced in the table are not normalized with respect to the dose of administration; but given the actual values as obtained in the respective studies. Names are given as in the studies. NA: not available in the study.

synergism of the two natural components was evaluated for the pharmacokinetic disposition of curcumin[48,50] in many studies, which resulted in a 20-fold increase in the bioavailability. However, a negative impression was left by the examination of the inhibition of drug metabolism depending on human CYP3A, CYP2C9, UGT and SULT enzymes, by the administration of curcumin–piperine preparation. No considerable effect was recorded in the plasma compared with the placebo.[52] Also, various studies have provided the toxicity of piperine in test animals. Piperine supports bioavailability enhancement by interacting with the enzymes involved in drug metabolism and hence results in a lack of metabolism and an increased concentration of drugs, without any transformation.[53] In addition, at least in an animal model, piperine seems to compromise the antioxidant and anti-diabetic efficacy of curcuminoid.[54]

Water solubility of curcuminoid is improved in a formulation, by the addition of a hydrophilic carrier, cellulosic derivatives and natural antioxidants along with purified turmeric extract. With a different view on the relative absorption of curcuminoid, it was shown to have a significant 45.9-fold higher value of curcuminoid appearance than standard curcumin. Besides, the formulation exerted a better anti-depressant-like activity by the enhancement of neurotransmitter levels compared with the standard curcumin.[55,56] Moreover, the effects of quercetin, genistein, terpineol, eugenol *etc.* in modulating curcuminoid bio-efficacy have been reported in various studies.[53]

5.6.2 Enhancement through Nanotechnology

Nanoformulations find a distinct set in the realm of bioavailability enhancement. Many nanoformulations have been reviewed as potential multifunctional and composite strategies for bioavailability enhancement. Self-microemulsifying drug delivery systems (SMEDDS) were constructed through the liquid and pellet formulations of CUR, which have been made efficiently with almost 99% CUR loading, and proved to have a 10–14 fold absorption in rats, compared with the same dose of free standard curcumin.[57] By a similar formulation, a 26-fold curcumin absorption was achieved *in vivo* oral bioavailability, with strong anticancer activities.[58] CUR encapsulated in PLGA nanoparticles by a nanoprecipitation technique has exhibited a better cellular uptake and anticancer potential in A2780CP ovarian and MDA-MB-231 breast cancer cells, compared to the free curcumin.[59] Even better pharmacokinetic properties were exhibited by PLGA and PLGA-PEG blend nanoparticles loaded with CUR. Oral administration of them in rats resulted in 15.6 and 55.4-fold increased bioavailability than an aqueous suspension of CUR.[60] CUR nanosuspension was another promising formulation, which ameliorated the plasma concentration of curcumin by 4.8-fold in rabbits than a curcumin solution, after an intravenous administration. The intravenous administration of the same formulation in mice resulted in the increased appearance of CUR in lungs, liver, spleen *etc.*[61] Bioconjugates and glucosides were prepared out of CUR to boost solubility.[62,63] PEGylated and

water soluble conjugates are a more advanced format of conjugated CUR formulations, with proven bioactivity, especially towards cancer cells.[64]

Curcuminoid dispersed with colloidal nanoparticles prepared from gum ghatti, glycerine and water forms one major commercialized bio-enhanced curcuminoid. The bioavailability profile of the product dominates in a dose dependent manner,[65] with recorded values 44 and 27 times higher than curcuminoid powder in rats and humans, respectively.[66] A drinkable preparation, made out of this formulation was checked for plasma concentrations, which delivered 4-fold higher values than three other curcuminoid beverages.[67] Co-solvent liquid formulations have been prepared for improving the solubility and enhancing the bioavailability, and have been examined for their solubility and pharmacokinetic properties.[68] Intravenous administration of one of the formulations exhibited a 28-fold increase in the bioavailability of CUR.[69] Although various bioavailability enhancing nanosystems are not suitable for different applications in foods, drugs and other related applications due to many unanswered questions regarding their biocompatibility.[70,71]

5.6.3 Micronized Formulations

Micronization, as the name indicates, is a process of reducing the average size of particles of a material. By theory, the reduced size increases the surface area and thereby increases the dissolution. In the case of curcuminoid, it was proposed to increase the absorption. A micronized curcumin containing 17.2% curcuminoid, produced 14- and 5-fold increases in the curcumin bioavailability in female and male candidates, respectively.[72] Another micronized formulation comprising 25% curcuminoid exhibited 9.7 times better bioavailability than the unformulated curcumin in human volunteers.[73] However, the range of applications of micronized formulations of drug particles have been restricted by their lower stability in the presence of moisture, and they are not easy to pack and store in bulk production.[74]

5.6.4 Encapsulation

A protective sheath is fabricated for curcumin by encapsulation. Depending on the conditions and compounds used, the protection can be from pH, chemical degradation, *etc.* Curcuminoid encapsulated along with ascorbic acid in a chitosan microsphere is an example. A delayed absorption and 7.3-fold higher bioavailability and better C_{max} values decipher the potential of the system.[75] Hollow microspheres prepared with ethyl cellulose-citric acid-CUR combination is another delivery system, which on oral administration produced more elevated values for all the pharmacokinetic parameters than free curcumin, in rats.[76] A nano-encapsulation of CUR was achieved with whey protein, which accomplished better bioavailability and cytotoxicity.[77] Fenugreek-derived soluble dietary fiber microgranulates, impregnated with curcuminoid, were prepared on the grounds of protection provided by the fibers to curcuminoid from degrading enzymes. The formulation's efficiency

of bioavailability was verified from 20-fold and 15.8-fold higher curcuminoid absorption than unformulated curcuminoid in rats and humans, respectively.[78] Encapsulation of curcumin phytosomes in polymeric chitosan microspheres were developed by utilizing the synergic effect of two systems, polymer and lipid, and it is evidenced that their pharmacokinetic properties outstand the formulations utilizing either of the systems alone.[79]

Inclusion complexes of lipophilic compounds with cyclodextrins are a proven technology for bioavailability enhancement. The specific ring structure of the cyclic oligosaccharides, cyclodextrines, is such that the hydrophilic glucose units face outwards, protecting the lipophilic compounds inside, which are released under suitable conditions. One such product has shown a 39-fold increased bioavailability of total curcuminoid.[80] Association of curcuminoid with cyclodextrin produced a ten-fold increase in the area under the curve (AUC).[81] CUR-hydroxypropyl-β-cyclodextrin (HP-β-CD) inclusion complex remarkably increased the drug water solubility and the oral bioavailability was enhanced 2.77-fold compared to the free standard curcumin.[82]

5.6.5 Curcumin Formulations Incorporating Lipid Matrices

5.6.5.1 Micellar Formulations

A 'lesser amount of curcumin in an effective matrix' was the concept behind a liquid micellar curcumin formulation, which incorporated 7% curcumin powder with 93% Tween 80®.[77] The oral bioavailability data were incorporated with gender differences, which have concluded that the curcumin absorption efficiency is greater in women than men. Sticking to that fact, female subjects exhibited 277-fold better bioavailability, while male subjects showed a 114-fold compared to native curcumin.[72] However, the study itself showed a huge gender difference in the mean plasma concentration post drug administration, which further needs optimization and possibly a second formulation. The same study also reported a gender difference for a micronized curcuminoid formulation. In this study, the two test populations also showed a significant difference in body weight (the male subjects had, on average, a significantly higher body weight than the female subjects), *i.e.* also a significant higher blood volume and volume of distribution, which questions the interpretation of a gender dependent difference in bioavailability so far.

The same liquid micellar curcumin formulation was tested for its serum as well as intra-tumoral concentrations, and thereby its clinical tolerance was evaluated but showed so far no specific effect on metabolism in glioblastoma multiforme (GBM) patients.[83] Sophorolipid-coated curcuminoid nanoparticles, prepared using a pH driven method, offered a better bioavailability than free crystal as determined by the curcumin glucuronide concentration.[84] As seen in many studies, lipid matrices might degrade in the stomach before reaching their targeted sites, hence compromising the bioavailability of the active ingredient.[85]

5.6.5.2 *Phospholipid Formulations*

The polar groups present in the curcumin molecule enable the complex formation with phospholipids through hydrogen bonding and dipole interactions. This feature found a way for two formulations: liposomes and phytosomes. The spherical vesicles of liposomes can shield the active molecules inside a bilayer lipid membrane, and at the same time can interact with the aqueous moieties and thus improve the solubility.[86] Curcuminoid liposomes coated with chitosan derivatives are promising controlled curcumin releasing vehicles.[87] Curcuminoid-loaded long circulating liposomes are yet another improved version. It exhibited improved pharmacokinetic parameters, even better than simple curcumin liposomes.[88]

Phytosomes are a slightly different category of product formulation, where the active molecules are complexed with phospholipids, which provide the perfect balance between hydrophilicity and lipophilicity for the complexes and serve as delivery systems, which allow the solubilization of the incorporated drug/phytochemical into the intestinal fluids with a good biomembrane crossing capacity. The peculiar properties of the formulation are achieved by the arrangement of active functionalities in such a way that the interactions between these active and phospholipid moieties are possible.[89] These properties contribute towards a 20-fold increase of curcumin absorption in an animal study and a 29-fold increase in the total curcuminoid absorption in a human study for a curcumin–phosphatidylcholine (lecithin) complex, than the corresponding unformulated curcumin matrix.[90,91] The curcuminoid availability in plasma and liver was significantly higher than the unformulated curcuminoid, but a reverse trend was observed in the curcuminoid levels of the gut mucosa.[91]

Solid lipid curcuminoid particles were prepared by a combination of soy lecithin, docosahexaenoic acid (DHA), vegetable stearic acid, ascorbyl esters together with 20–30% curcuminoid. In a human study, the formulation exhibited considerable bioavailability, compared to the 'nil' results obtained for standard curcuminoid.[92] Another curcuminoid–phospholipid complex delivered a 3.3-fold increase in the serum concentration of curcumin in rats.[93] By complexing with phospholipids, CUR was proved to possess better solubility and antioxidant activity, together with improved bioavailability. Moreover, even the CCl_4 induced liver damage in rats was healed by such a complex, prepared out of CUR.[94]

A powder form of the phospholipid vehicle was formulated with a nanofiber fabrication *via* nanofiber weaving technology by high-pressure homogenization and removal of water through a spray drying process to improve the bioavailability of curcumin and improve the stability of the liposomal curcumin powder. The encapsulation efficiency, loading capacity, *in vitro* release, and DPPH activity revealed that the liposomal curcuminoid powder could be a promising drug delivery system for curcuminoid due to the use of nanofiber weaving technology and spray drying process.[95] A complex preparation with a small amount of curcuminoid (or any other lipophilic

compound) is preferred, because a higher amount may result in saturation of the shielding points and the availability of free bioactive to be exposed to the degradation.[76] However, one of the major shortcomings of liposomes as drug carriers are their quick elimination from blood circulation and reticulo-endothelial system macrophages. In addition, the physical and chemical instability of liposomes leads to problems such as aggregation, degradation, hydrolysis and oxidation of phospholipids.[75,84]

5.6.6 Reconstitution with Non-curcuminoids

Reconstitution of non-curcuminoid turmeric components is another successful bioavailability enhancing method. One of the formulations followed this by assimilating curcuminoid with turmeric sesquiterpenoids, the major constituents of turmeric oil. A 7-fold increase in bioavailability was marked for the formulation in dogs, compared with the control curcuminoid.[96] A curcuminoid absorption of 96% was noted for the product in rats, while the absorption was 58% for the standard curcuminoid.[97] In human studies, the product gives rise to a 7-fold increase compared to standard curcuminoid.[98]

5.6.7 Complete Natural Turmeric Matrix

Most of the best performing curcuminoid formulations produced so far have provided no more than a ten-fold increase in bioavailability compared to unformulated curcuminoid.[99] Whilst each of these novel delivery strategies offers significant promise, there are still limitations to their potential use in food/medicine. In addition, most of these technologies are not able to accommodate high loading of curcumin/curcuminoid, thus limiting the bioactivity of the finished products. Most of the delivery systems have limited application for use as a powder formulation as their stability will be affected when converted to powder.[85] Most of these formulations are lacking a clear explanation of the mode of actions. Another important concern regarding these formulations is the greater availability of other ingredients than the active compound. As an example, we shall offer here the use of Tween 80®. Tween 80® approved in small amounts as a food additive (*e.g.* 20 mg kg^{-1} ice cream in the European Union) mounts up to 450 mg in one 500 mg capsule of a formulation sold on the market under different names *e.g.* Curcumin-Loges® or Curosol® in Germany. Tween 80® has been shown in animal experiments to promote colitis and metabolic syndrome.[100] In another study it has been shown to induce low grade intestinal inflammation and promote colon cancer.[101] These are certainly unwanted side effects of a strategy to improve curcuminoid bioavailability, which shall in fact produce opposite effects.[102–104] The role and fate of synthetic/modified compounds incorporated in these formulations are not explained anywhere. In this regard, natural matrix based formulations without sophisticated fabrication and chemical modification have been investigated for delivering curcumin/curcuminoid.

The researchers at Aurea Biolabs developed an innovative bioavailable curcuminoid (Cureit™) based on the recreation of the complete natural turmeric matrix with active curcuminoid by using polar-nonpolar-sandwich (PNS) technology.[105] PNS technology is one of the most promising techniques among various methods used to improve the dissolution of poorly soluble curcuminoid. Moreover, the technology attracts attention as it is simple, cost effective and commercially attractive for industrial production. Indeed, PNS technology is proven to improve bioavailability and bioefficacy, in which the target molecules are well protected by polar and nonpolar entities as in a sandwich. The polar and non-polar interactions of the molecules stabilize the target molecules to attain a specific site. PNS technology can be used to preserve functional properties, improve the stability of compounds, enhance health benefits, control the release of bioactive compounds at a desired time and specific target sites and increase the bioavailability of bioactive compounds. The power of the natural turmeric matrix is completely utilized by recreating the same, incorporated with purified curcuminoid. The product has already demonstrated efficacy from different studies on antioxidant, antineoplastic, elastase inhibition, anti-aging and anti-rheumatoid arthritis activities.[106–111]

Acceptance and delivery of curcuminoid in body tissues are perceptible and important for its biological activities. The bioavailability of curcuminoid was assessed in a detailed pharmacokinetic study of the aforementioned product, where it was compared with the formulations available commercially and proved to be the most promising in terms of bioavailability, longer retention, higher values for C_{max} *etc.*, which clearly demonstrated the synergism of curcuminoid with other bioactive molecules of turmeric, and showed that PNS technology made a positive impact on generating a high concentration of free curcuminoid in the blood plasma, which could play a significant role in therapeutic applications.[112] The improved bioavailability was verified by the increased amounts of curcumin metabolites, post drug administration.[113] The consumption of curcumin in a complete natural turmeric matrix led to improved recovery and reduction of delayed onset muscle soreness (DOMS) without any side effects due to the enhancement of the bioavailable form of curcumin.[85] Also, the positive effects on rheumatoid arthritis and osteoarthritis are verified in human studies.[114,115]

5.7 The Narrative of Bioavailability

In classical pharmacological bioavailability studies, the compound of interest can be given in appropriate amounts as (a) an intravenous bolus application, or (b) orally in a certain formulation. The pharmacokinetic of both administrations is measured, and the key parameters $T_{1/2}$, T_{max}, C_{max}, AUC, AUC per mg of the compound applied, the total bioavailability, volume of distribution (*e.g.* plasma compartment, plasma + interstitial fluid compartment, plasma + interstitial + intracellular compartment), metabolism and so

on is calculated. This classical way is so far not possible in the field of curcumin/curcuminoid, especially not in humans, as CUR/curcuminoid is not applicable as an intravenous bolus injection. Therefore, in the whole field of oral curcuminoid application, 'relative bioavailability improvements' (RBIs) are claimed by the comparison of plasma concentrations (specifically, AUCs) of total curcuminoid and/or individual CUR, DMC and BDMC (as the main components of curcuminoid) of an unformulated curcuminoid preparation (as a standard) *versus* a formulated curcuminoid preparation. This comprises several problems when different pharmacokinetic and bioavailability studies are compared with each other and thereby the superiority/inferiority of one formulation over another shall be evaluated.

1. The amounts of unformulated curcuminoid differ considerably between the reported pharmacokinetic studies as well as the amounts of formulated curcuminoid. Also, the ratio of the relative amounts of unformulated/formulated curcuminoid differs considerably between studies where in most studies the amount of unformulated curcuminoid exceed that of formulated because of the expected lower absorption of the unformulated curcuminoid. Thus, to make different studies more comparable, the reported values for C_{max} as well as AUC have to be normalized to C_{max}/AUC per mg curcuminoid applied.
2. Many pharmacokinetic studies are performed with amounts of formulated curcuminoid that are much higher than those finally used in clinical applications. To give just one example, in the PK-study of Schiborr,[72] three curcuminoid preparations (standard, micronized and micelles) have been applied at amounts of 500 mg total curcuminoid (410 mg curcumin), leading for the micellar preparation to an application of 500 mg curcuminoid with 6.64 g Tween 80®, whereas the compared micronized formulation contained just 2.41 g additives and the standard curcuminoid contained no additive at all. Despite the potential harmful effects of such a high concentration of Tween 80® on the gastrointestinal tract, its potential unphysiological impact on the observed 'bioavailability' and the disparities between compared curcuminoid preparations presented for oral absorption may influence the pharmacokinetic results produced here and have only little to do with the finally marketed formulation in 500 mg capsules, which contain only 35 mg curcuminoid solubilized in 465 mg Tween 80® for the micellar formulation.
3. The different studies also differ with respect to the detection technologies used and the time-frame of 0 to *x*-hours across which the plasma concentrations of total curcuminoid, CUR, DMC and BDMC (as the main components of interest) are measured.
4. The studies also differ with respect to the time points chosen and number of time points measured to establish an absorption profile. Both the time points as such and the number of time points will affect the calculation of AUCs and to a large extend, the C_{max}. As an example,

the PK-study reported by Jäger[55] shows data for 0–12 hours where the amounts of CUR, DMC and BDMC did not return to the point of origin at the 12 hour time point, whereas in the study of Gopi[112] (0–24 hours) the measured concentrations of total curcuminoid of the different compared formulations returned to the point of origin.

5. Studies in which a high number of time points have been measured, as *e.g.* Gopi,[112] report not just one C_{max} point, but rather two to three. This raises the so far mostly unanswered question, whether the curcuminoid absorbed from the gastrointestinal tract may be delayed, or whether the curcuminoid measured in plasma is subjected to a complex distribution/redistribution kinetics between different compartments. In this context, the finding of Asher[120] is interesting. His group observed an inverse correlation between the plasma concentrations and tissue concentrations of curcuminoid, while applying a curcuminoid–phosphatidylcholine complex in contrast to standard curcuminoid. In addition, the tissue concentrations of curcuminoid remained stable for unformulated curcuminoid at around 50 ng mg^{-1} tissue over a large range of plasma AUCs (0–24 h) whereas those for the curcuminoid–phosphatidylcholine complex dropped to zero at higher plasma AUCs. This finding raises several questions, one at least is, whether the simple observation of higher C_{max}/AUC values for a given formulation in plasma as compared to another really translates to a higher clinical efficacy.
6. Another question of concern is that of the 'conversion stability' of CUR after gastrointestinal uptake. In Table 5.2 the relative composition CUR : DMC : BDMC of the orally applied 95% curcuminoid (same for formulated/unformulated curcuminoid) with those reported in plasma after oral uptake varies considerably for non-formulated curcuminoid compared to that for different formulations compared by Jäger[55] and Schiborr.[72] These data give rise to the assumption that formulations do not just improve oral bioavailability of curcuminoid but also influence (after uptake) the conversion of CUR into DMC and BDMC. This can be more or less favorable for curcumin.
7. The PK studies reported also differ technically, not just in detection technologies but also whether curcuminoid is measured with (total plasma curcuminoid: free and protein bound) or without enzymatic hydrolysis (free curcuminoid) of the plasma samples subjected to analysis. We think that it is clear that a table summarizing the present scientific knowledge about the efficacy of different curcuminoid formulations is hampered to some extent by this inherent complexity. We do not claim here completeness for all formulations available up to now, and we intentionally do not refer to all the parameters available but rather concentrate on the AUCs for curcuminoid (CUR + DMC + BDMC) and claim RBI (relative bioavailability increase) as compared to unformulated standard 95% curcuminoid and on the normalization of these values to 1 mg curcuminoid applied (Table 5.3).

Table 5.2 Metabolization of unformulated and formulated 95% curcuminoid after oral application.[a]

Jäger[55]			
Curcuminoid composition used for formulations CUR : DMC : BDMC = 1.00 : 0.200 : 0.067[(1)]			
Formulation	CUR	DMC	BDMC
Unformulated	1.00	1.70	0.86
Curcuminoid with turmeric oil	1.00	0.38	0.44
Curcuminoid phosphatidylcholine phytosome	1.00	0.96	0.23
Curcuminoid with hydrophilic carriers, cellulosic derivatives and natural antioxidants.	1.00	0.18	0.03
Schiborr[72]			
Curcuminoid composition used for formulations CUR : DMC : BDMC = 1.00 : 0.195 : 0.024[(2)]			
Unformulated	1.00	0.133	0.049
Micronisate	1.00	0.423	0.055
Micelles	1.00	0.101	0.003

[a]The mean composition of 95% curcuminoid is 75% CUR (72–78%), 15% DMC (12–18%) and 5% BDMC (3–8%) summing up to 95%, or relative to CUR 1.00 : 0.200 : 0.067. [(1)] The composition of the 95% curcuminoid used by Jäger[55] was not given. Therefore, the average composition was assumed. [(2)] The composition of 95% curcuminoid used by Schiborr[72] was reported to be 82% CUR, 16% DMC and 2% BDMC, summing up to 100% and a relative composition of 1.00 : 0.195 : 0.024. The relative composition of CUR : DMC : BDCM has been calculated from the respective $AUC_{0\text{-}t}$s reported by Jäger[55] and Schiborr.[72] The plasma pharmacokinetic was performed for 0 to 12 hours Jäger[55] and 0 to 24 hours Schiborr.[72] In the pharmacokinetics reported by Jäger,[55] the values of the measured components had not returned to the origin (undetectable), which certainly influences the reported AUCs and might influence also the relative proportions of CUR, DMC and BDMC found in plasma.

The mean normalized AUC for unformulated 95% curcuminoid across 13 studies was found to be 0.25 ± 0.28 ng mL^{-1} h^{-1} per mg curcuminoid. It is not first expected that the AUC for unformulated curcuminoid varies by a factor of 91-fold comparing the lowest with the higher AUC reported for unformulated 95% curcuminoid[67,118] (Table 5.3) between these studies. This is only explainable if considering the differences between the used unformulated curcuminoid preparations (despite a generally quite homogenous composition of CUR, DMC and BDMC). Such differences could be the different physicochemical properties with respect to the capability of the different 95% curcuminoid preparations used for their dispersion in the gastrointestinal tract or differences in application mode (*e.g.* in water or as in the C. Schiborr *et al.* study[72] in 50 g of woodruff syrup).

It is well known from classical pharmacology that the degree of dispersion of a pharmaceutical agent influences tremendously its gastrointestinal absorption. This is the everyday experience of a physician prescribing an originally patented product, *e.g.* antihypertensive, in comparison to its cheaper option. The original, highly sophisticated formulated product (to achieve high and reproducible oral bioavailability) normally shows higher efficacy, lower side effects and better tolerability, compared to the simple formulated (but cheaper) option. The degree of dispersion of unformulated curcuminoid

Table 5.3 An evaluation of different studies for their pharmacokinetic parameters and efficacy reports.[a]

Formulation	Curcuminoid dose (mg)	Curcumin dose (mg)	$AUC_{0\text{-}t}$ (ng^{-1} mL h^{-1}) curcuminoid	$AUC_{0\text{-}t}$ (ng^{-1} mL h^{-1}) curcumin	RBI (claimed)	$AUC_{0\text{-}t}$ (ng^{-1} mL h^{-1}) per mg curcuminoid applied	$AUC_{0\text{-}t}$ (ng^{-1} mL h^{-1}) per mg curcumin	Clinical studies and results	Reference
Values for standard 95% purified curcuminoid									
95% Curcuminoid	380	323	4.16	3.36[(3)]	1	0.0109[(3)]	0.0104	Clinical results for	118
95% Curcuminoid	391	307	49.87	ng	1	0.1275	ng	unformulated	119
95% Curcuminoid	97.7	76.8	38.8	ng	1	0.3971	ng	curcuminoid	119
95% Curcuminoid	30	ng	4.1	ng	1	0.1367	ng	has been not	66
95% Curcuminoid	4000	2920	1999.9	731.6	1	0.4999	0.251	included even if	120
95% Curcuminoid	2000	ng	461.86	ng	1	0.2309	ng	existing	98
95% Curcuminoid	1799	1295	202.8	122.5	1	0.1127	0.0946		90
95% Curcuminoid	1800	ng	38.5	10.8	1	0.0214	ng		55
95% Curcuminoid	1945.2	1774.2	52.1	19.7	1	0.0268	0.0101		80
95% Curcuminoid	500	410	28.55	24.17	1	0.057	0.059		72
95% Curcuminoid	30 (drink)	ng	30.1	ng	1	0.997	ng		67
95% Curcuminoid	1000	ng	510	ng	1	0.51	ng		78
95% Curcuminoid	500	ng	165.7	ng	1	0.3314	ng		109
95% Curcuminoid	2000	ng	91.82	ng	1	0.046	ng		73
Mean + SD						*0.250 ± 0.278*	*0.085 + 0.099*		
Values for formulated 95% curcuminoid for formulations which have been reported several times									
Curcuminoid	30 (drink)	ng	121.2	ng	4.0	4.04	ng	Yes, positive; [122–127]	67
in colloidal	30	ng	113	ng	27.3	3.7667	ng		66
nanoparticles	182	ng	2778	ng	ng	15.264	ng		119
	150	ng	2648	ng	ng	17.653	ng		65
	210	ng	3648	ng	ng	17.371	ng		65
Mean + SD						*11.62 + 7.10*			
Curcuminoid	391	307	2274	ng	45.5	5.816	ng	Yes, positive; [128 and 129]	119
with soluble	97.7	76.8	963	ng	24.8	9.857	ng		119
fenugreek	250	100	6587	ng	12.9	26.348	ng		78
dietary fiber	1500	600	8100		15.9	5.4	ng		78
microgranulates									

Mean + SD					*24.8 + 14.7*	*11.86 + 9.87*			
Curcuminoid-turmeric oil	279.4	ng	305.6	ng	ng	1.094	ng	Yes, positive not double blind; [130]	121
	376	ng	16.6	5.8	2.6	0.044	ng		55
	392.4	355.2	12.1	6.7	1.1	0.0308	0,0189		80
	2000	ng	3975	ng	8.61	1.988	ng		98
	2000	ng	3201.28	ng	6.37	1.601	ng		98
Mean + SD					*4.67 + 3.44*	*0.95 + 0.89*			
Curcuminoid–phosphatidylcholine phytosome complex	152.5	ng	638.9	ng	ng	4.190	ng	Yes, positive studies are not double blind; [131–137]	121
	376	297	1336.0	538.8	31.5	3.553	1.814		90
	209	165	640.2	272.6	27.2	3.063	1.652		90
	376	ng	63.4	28.7	12.7	0.169	ng		55
	385	303	946.4	669.4	8.8	2.458	2.21		120
	382.2	354.0	86.9	35.1	8.5	0.2274	0.0992		80
	99.0	80.5	187.3	ng	ng	1.892	ng		112
Mean + SD					*17.7 +10.8*	*2.22 + 1.57*	*1.44 + 0.93*		
Complete natural turmeric matrix (Cureit/Acumin)	232.5	180	824.9	ng	ng	3.548	ng	Yes, positive double blind; [85, 114 and 115]	112
	245	190	904	ng	10	3.690	ng		109
Values for formulated 95% curcuminoid for formulations which have been reported just once									
MicroActive curcumin	2000	ng	887,48	ng	9.7	0.4437	ng	No	73
Micronized curcumin	500	410	315.55	214.65	11.1 (8.9)[(1)]	0,6311	0.524	No	72
Curcuminoid-Tween80® micelles	500	410	4939.53	4474.98	173 (185)[(1)]	9.879	10.915	Yes, negative; [117]	72

(*continued*)

Table 5.3 (*continued*)

Formulation	Curcuminoid dose (mg)	Curcumin dose (mg)	$AUC_{0\text{-}t}$ (ng^{-1} mL h^{-1}) curcuminoid	$AUC_{0\text{-}t}$ (ng^{-1} mL h^{-1}) curcumin	RBI (claimed)	$AUC_{0\text{-}t}$ (ng^{-1} mL h^{-1}) per mg curcuminoid applied	$AUC_{0\text{-}t}$ (ng^{-1} mL h^{-1}) per mg curcumin	Clinical studies and results	Reference
Curcumin liquid droplet micromicellar formulation	76	64.6	391.5	351	472.6 (522)[3]	5.151 (5.433)[3]	Ng	No	118
Curcuminoid-cyclodextrine complex	371.4	348.0	388.6	327.7	39.1	1.046 (0.942)	0.942	No	80
Solid lipid curcuminoid particle	650	ng	95.3	Ng	100[2]	0.147	Ng	Yes, positive; [138–142]	92
Curcuminoid with hydrophilic carrier, cellulosic derivatives and antioxidants	376	ng	372.3	307.6	45.9 (136.3)[1]	1.011	Ng	Yes, negative; [143]	55
Curcu-gel ultra	429.5	351	117.3	Ng	ng	0.273	Ng		112
Curcuminoid–phospholipid–piperine	2000	ng	461,86	Ng	ng	0.231	Ng	No	98

[a]ng, not given;[1] the RBIs in parenthesis are for curcumin (CUR), the RBIs for curcuminoid have been calculated from C+DMC+BDMC data given in the reference.[2] The RBI given by Jamwal[144] is not conclusive as in the publication of Gota;[92] no AUC for unformulated curcuminoid could be measured. [3] There are some minor problems with the data given by Stohs.[118] The RBI of 522 [118] given in the table is that for CUR. Also, the normalized AUCs for unformulated (0.0104) as that for the formulation (5.433). The normalized AUC for the formulation for total curcuminoid can be calculated from the applied curcuminoid amounts as (unformulated/formulated) given as (76/64.6) and the total curcuminoid AUC for the formulation is 391.5 and the normalized value of the same is 5.151. However, the total AUC for CUR for the unformulated control is not given, but can be calculated to be 3.36, and the normalized AUC for unformulated curcuminoid (0.0104) cannot be calculated from the table, because the given total AUC of 4.16/323 mg CUR would result in a normalized AUC for unformulated curcuminoid of 0.129. For total curcuminoid thus results an RBI of 472.6, which is still the highest reported to far. The reported AUC for unformulated curcuminoid of 4.16 is according to discussion that for total curcuminoid, the total for unformulated CUR can be calculated to be 3.36.

may be severely affected by the differences in batches used, and/or application conditions. However, these effects might be much smaller for the formulated curcuminoid analyzed in the same study. These differences could at least result, in part, in the improved RBI claimed for the formulation, as well as to the limited reproducibility of these claim. For this, refer to Table 5.3 where the reported RBI, *e.g.* for the formulation with turmeric oil, varies between 1.1 and 8.6-fold and that of curcumin–phosphatidylcholine phytosome complex is 8.5 to 31.5-fold. The same is true for the respectively reported normalized AUCs. We are lacking complete information about these aspects. In addition, it seems to be highly questionable to base pharmacokinetic studies of curcuminoid preparations on fasting conditions. Not just that this is far from the 'real life' application of curcuminoid preparations under clinical conditions, it is already – looking back into the historical use of turmeric – illogical to apply unformulated curcuminoid fasting instead with or after a standard meal providing a mixture of protein, fat and carbohydrate for stabilizing as well as dispersing the curcuminoid, irrespective of whether it is formulated or not. We have discussed these aspects already in brief. Every curry, as it is daily food praxis in India, delivers turmeric in this way. With this background taken into consideration, it might not be so astonishing anymore that the formulations reported to have the highest relative improvement in oral bioavailability over standard curcuminoid – micelles (173/185-fold),[72] micro micellar formulation (472/522-fold)[118] – report at the same time smaller AUCs for unformulated curcuminoid (Table 5.3).The curcuminoid formulation with hydrophilic carrier and cellulosic derivatives, which has been reported in the original publication of Jäger *et al.*[55] with an RBI of 45.9 compared to curcuminoid, was reported in the review of Jamwal[144] with an RBI of 136-fold for curcumin. The latter difference is due to the base of comparison: total curcuminoid or curcumin. The formulation-dependent difference of conversion as already discussed and presented in Table 5.2 might be an explanation for these differences. However, to what extent such effects translate into clinical/improved clinical efficacy remains open. The solid lipid curcuminoid particle is referred to by Jamwal[144] with an RBI of 100-fold, even so that in the original report from Gota *et al.*[92] as well as in the review of Jamwal,[144] the AUC of the control standard curcuminoid was reported as not measurable at an applied dose of 650 mg curcuminoid. Thus, it is necessary to look closely at RBIs.

It is at least remarkable that the authors claiming the highest RBIs, at the same time, report the lowest AUCs for the measured control standard unformulated curcuminoid in their respective studies (Table 5.3). To compare different studies, it seems to be mandatory first to normalize the AUCs (formulated and non-formulated) to 1 mg curcuminoid applied. In Figure 5.3, we have plotted the reported RBIs of the different studies summarized in Table 5.3 and applied a best fit regression analysis to the data. Figures 5.3A and B show the results for either all (A) or after exclusion of the highest RBIs (522, 185) (B). In both cases, we obtained a significant inverse correlation between RBI and AUC of standard curcuminoid. In Figures 5.3C and D, we

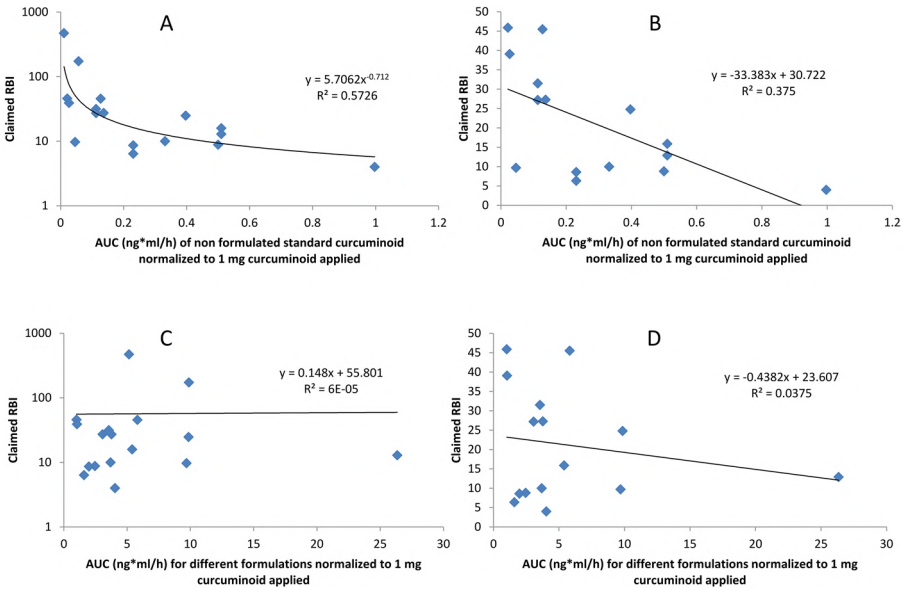


Figure 5.3 The relationship between the claimed relative bioavailability increase and the AUC of unformulated as well as formulated total curcuminoid. Data used are summarized in Table 5.3. AUC, $AUC_{0\text{-}t}$ of 13 different formulations of 95% enriched curcuminoid per mg of curcuminoid applied or their respective unformulated control are given. RBI (relative bioavailability increase, calculated as $AUC_{formulated}/AUC_{unformulated}$ curcuminoid) are plotted as per the published data. 5.3(A) all 15 reported RBIs plotted *versus* the respective AUCs of the non-formulated control 95% curcuminoid; 5.3(B) as 5.3(A), but just 13 reported RBIs excluding those of Schiborr[72] and Stohs[116] are plotted; 5.3(C) all 15 reported RBIs plotted *versus* the respective plasma AUCs of those RBIs; 5.3(D) as 5.3(C), but just 13 reported RBIs excluding those of Schiborr[72] and Stohs[116] are plotted. The RBI for 'curcuminoid with hydrophilic carrier, cellulosic derivatives and antioxidants', reported by Jäger[55] included in this analysis is the one reported for curcuminoid (45.9) not that for curcumin (136.3). The RBI for micelles included here is not that reported by Schiborr[72] for curcumin, but that calculated from the reported AUCs for CUR, DMC and BDMC (173).

did the same analysis but plotted the RBIs *versus* the achieved plasma AUCs of the formulated curcuminoid normalized to the 1 mg curcuminoid applied. Now there can be no correlation between the claimed RBIs and achieved normalized plasma AUCs of the different formulations observed anymore. We conclude from these correlations that it is misleading to rank different formulations according to the reported RBIs at least if the goal is to obtain information concerning the potential clinical efficacy improvement of a given formulation in comparison to another one. Second, it seems to be more useful not to deal anymore with 'relative bioavailable' improvements over the largely variable unformulated curcuminoid controls but rather compare

the reported AUCs of the different formulated curcuminoid normalized to 1 mg curcuminoid applied with each other. Doing this, the bioavailability improvements of different formulations give a largely different impression. For example, the reported and reproduced mean normalized AUC for a complete natural turmeric matrix formulation is 3.6 ng mL^{-1} h^{-1},[109,112] that of phosphatidylcholine formulation is 2.2 ng mL^{-1} h^{-1} [55,80,90,120,121] and that of fenugreek dietary fiber microgranulates formulation is 9.7 ng mL^{-1} h^{-1} [78,119] whereas for a micellar preparation it is 9.9 ng mL^{-1} h^{-1} [72] and that of curcumin liquid droplet micromicellar formulation it is 5.1 ng mL^{-1} h^{-1} [118] with reported RBIs of 10, 17, 29, 185(173) and 522(473)-fold. Thus, these four formulations differ based on normalized AUC (ng mg^{-1} h^{-1}) 4.4-fold but based on RBIs 52-fold and the fenugreek dietary fiber microgranulates formulation (RBI 29) achieves practically the same normalized AUC as the micellar formulation (RBI 185). These inconsistencies should provide reasons for a new assessment of the reported results. Finally, it seems to be important to compare different formulations with respect to the reported clinical efficacy of these formulations at compound amounts really applied under clinical conditions and not at compound amounts used in pharmacokinetic studies. Doing so one has to accept that for, *e.g.* the curcuminoid–phosphatidylcholine formulation, there are a number of clinical studies available showing clinical efficacy,[131–137] or for the complete natural turmeric formulation[114,115] but not so far for the micellar formulation.[117] What we are lacking so far is completely clinical head-to-head studies for different formulations at best double-blind placebo controlled.

Last but not least, to estimate 'bioavailability' on the presence and concentrations of CUR, DMC and BDMC in plasma as has been done up to now by the used detection technologies is most likely a tremendous oversimplification of the real situation if we want to evaluate the clinical and therapeutic value of curcumin. As has been discussed briefly earlier, curcumin tends to bind to proteins, in the plasma most likely predominantly to albumin. As we know from other biological active compounds, for the sake of simplicity we name here testosterone and 25-hydroxy-vitamin-D, only the free fractions of these compounds are able to release a biological effect. In the case of testosterone only the free fraction can bind to a cell surface receptor or enter the cell. In the case of 25-hydroxy-vitamin-D only the free fraction can pass the cell membrane and thereafter interact either as 25-hydroxy-vitamin-D directly or after lysosomal metabolization to 1,25-dihydroxy-vitamin-D with the vitamin D receptor (VDR) in the cell nucleus. In both cases the albumin bound testosterone or the vitamin D binding protein (VDBP)-bound 25-hydroxy-vitamin-D are biologically inactive. However in all three cases: testosterone, 25-hydroxy-vitamin D and curcuminoid (CUR, DMC, BDMC), we are talking about lipophilic compounds, and as has been shown at least for testosterone and vitamin D, the equilibrium between the protein bound and free compound is dependent as well on the total concentration and on the concentration of the free compound, and the protein bound fraction can be liberated if the concentration of the

free drops, *e.g.* because of cellular uptake or binding of free compound to cell surface acceptors. Transposing this general knowledge to curcumin, a very important as well as so far mostly unanswered question is, how much free curcumin is available to enter the cells of therapeutic interest and to what extent is this interfered by 'solubility facilitators' such as Tween 80® or Cyclodextrin. In a clinical study, Stohs *et al.* showed that the curcumin amounts measured in plasma with and without enzymatic hydrolysis differ significantly.[116] This is of particular interest in the light of the use of, *e.g.* Tween 80® or cyclodextrins, and questions arise: (a) how is the distribution of curcumin influenced by such unphysiological solvent facilitators, (b) to what extent can curcumin 'escape' from them, (c) how do such facilitators influence the distribution of curcumin within the components of the blood, and (d) how do they interfere with the interaction/uptake of curcumin with cells? These so far disregarded potential effects of solvent facilitators may explain why the micellar formulation[72] with a pharmacokinetic proven, superior plasma AUC did not show any clinical effect, even astonishing the authors themselves.[117]

From the latter question arises another. Do we – as done so far in the majority of pharmacokinetic studies – really get a true reflection of the systemic uptake of curcumin or curcuminoid or would it not be much more essential to measure at least in full blood or even better in plasma and the blood cell sediment in parallel?

As it can be seen, the AUCs for curcuminoid (unformulated as well as formulated) between studies vary considerably (Table 5.3). In addition, several of the available pharmacokinetic studies do not measure the formulated curcuminoid in concentrations that are later to be applied under clinical conditions, but rather in much bigger amounts. The study of Schiborr *et al.*[72] may serve as an example. They performed their pharmacokinetic study with 500 mg curcuminoid in a formulation of 7% curcuminoid + 93% Tween 80®. As has been discussed, such high concentrations of Tween 80® could disrupt the continuity of the gastrointestinal barrier and thereby interfere substantially with the pharmacokinetic results. Considering this, it might be not surprising that this formulation resulted in a later performed 'clinical safety study' with no effect of curcumin on the measured clinical parameters (blood lipids, inflammation markers, glucose) even though several other clinical curcuminoid trials did show curcuminoid to be effective on these parameters.[117] Besides, in this trial the formulation was applied in high amounts of 294 mg curcumin (345 mg curcuminoid) per day for six weeks equivalent to an administration of 3 × 3 capsules of the marketed product. Also, the already named study of Dützmann *et al.*[83] did not report antitumor efficacy despite providing evidence for high plasma as well as intratumoral concentrations of curcumin. The already discussed classical combination of curcuminoid with piperine to improve oral bioavailability is suspicious to deprive curcumin of several of its potentially clinically useful effects.[54] Thus, the goal cannot simply be 'to measure' the enhancement of the relative bioavailability.

5.8 Conclusion

The absolute values produced by different studies cannot be considered for comparison between themselves, because many parameters affecting the values such as the experimental subjects, analytical method, study design and administration of the product, *etc.* may interfere, and the resulting data by comparison will not be meaningful. One important factor in these analyses was the moiety analyzed. Some studies deal with the metabolites of curcumin, while other studies concentrated on the free curcumin itself. Yet other studies are available, which analyzed other particular metabolites. One curious thing found while looking through the papers is that, in many cases, standard curcumin doesn't produce any results, even with highly sophisticated instruments, while in some other studies it did. So, a relevant comparison can be made on the ratios of actual comparative results produced at the time of the experiment. This chapter has discussed the enhancement in pharmacokinetic properties provided by different formulations, with relevant examples along with the required conditions and the significant factors related to them. In fact, there are many more formulations available for systemic bioavailability, discussion of which may be beyond a book itself. Moreover, not all curcumin formulations are meant for bioavailability enhancement. There are trials for targeted drug delivery, food and cosmetic purposes, *etc.* Also, the bioavailable amount of curcumin needed for an effective impact in cellular and other body functions is another questionable factor. There is not much proof available on the relation between curcumin bioavailability and its bio-efficacy. Then too, for certain, the improved versions of bioavailable curcuminoid perform therapeutic functions at lower doses than those which are unformulated.

References

1. D. J. Newman, G. M. Cragg and K. M. Snader, *J. Nat. Prod.*, 2003, **66**, 1022.
2. S. Pandit, H. Kim, J. Kim and J. Jeon, *Food Chem.*, 2011, **126**, 1565.
3. M. Paramasivam, R. Poi, H. Banerjee and A. Bandyopadhyay, *Food Chem.*, 2009, **113**, 640.
4. T. P. Chaturvedi, *Indian J. Dent. Res.*, 2009, **20**, 107.
5. A. Mukerjee and J. K. Vishwanatha, *Anticancer Res.*, 2009, **29**, 3867.
6. T. Perko, M. Ravbe, Z. Knez and M. Skerget, *J. Supercrit. Fluids*, 2015, **103**, 48.
7. R. W. Kalpravidh, N. Siritanaratkul, P. Insain, R. Charoensakdi, N. Panichkul, S. Hatairaktham, S. Srichairatanakool, C. Phisalaphong, E. Rachmilewitz and S. Fucharoen, *Clin. Biochem.*, 2010, **43**, 424.
8. C. Changtam, H. P. de Koning, H. Ibrahim, M. S. Sajid, M. K. Gould and A. Suksamrarn, *Eur. J. Med. Chem.*, 2010, **45**, 941.
9. H. S. Lim, S. H. Park, K. Ghafoor, S. Y. Hwang and J. Park, *Food Chem.*, 2011, **124**, 1577.

10. L. Peret-Almeida, A. P. F. Cherubino, R. J. Alves, L. Dufosse and M. B. A. Gloria, *Food Res. Int.*, 2005, **38**, 1039.
11. N. P. Aditya, G. Chimote, K. Gunalan, R. Banerje, S. Patankar and B. Madhusudhan, *Exp. Parasitol.*, 2012, **131**, 292.
12. M. A. Khan, R. El-Khatib, K. D. Rainsford and M. W. Whitehouse, *Bioorg. Chem.*, 2012, **40**, 30.
13. G. G. L. Yue, B. C. L. Chan, P. M. Hon, E. J. Kennelly, S. K. Yeung, B. R. Cassileth, K. P. Fung, P. C. Leung and C. B. Lau, *Int. J. Biol. Macromol.*, 2010, **47**, 342.
14. A. Tapal and P. K. Tiku, *Food Chem.*, 2012, **130**, 960.
15. Y. Panahi, A. Saadat, F. Beiraghdar, S. M. H. Nouzari, H. R. Jalalian and A. Sahebkar, *J. Funct. Foods*, 2014, **6**, 615.
16. P. Y. Zhan, X. H. Zeng, H. M. Zhang and H. H. Li, *Food Chem.*, 2011, **129**, 700.
17. A. Siviero, E. Gallo, V. Maggini, L. Gori, A. Mugelli, F. Firenzuoli and A. Vannacci, *J. Herb. Med.*, 2015, **5**, 57.
18. A. Amalraj, A. Pius, S. Gopi and S. Gopi, *J. Tradit., Complementary Med.*, 2016, **7**, 205.
19. A. Bagchi, *IOSR J. Environ. Sci., Toxicol. Food Technol.*, 2012, **1**, 1.
20. A. Nair, A. Amalraj, J. Jacob, A. B. Kunnumakkara and S. Gopi, *Biomolecules*, 2019, **2**, 13.
21. K. Mahmood, K. M. Zia, M. Zuber, M. Salman and M. N. Anjum, *Int. J. Biol. Macromol.*, 2015, **81**, 877.
22. H. Vogel and J. Pelletier, *J. Pharm.*, 1815, **2**, 50.
23. J. Milobedeska, S. Kostanecki and V. Lampe, *Ber. Dtsch. Chem. Ges.*, 1910, **43**, 2163.
24. V. Lampe and J. Milobedeska, *Ber. Dtsch. Chem. Ges.*, 1913, **46**, 2235.
25. A. M. Anderson, M. S. Mitchell and R. S. Mohan, *J. Chem. Educ.*, 2000, **77**, 359.
26. P. N. Ravindran, K. Nirmal Babu and K. Sivaraman, *Turmeric: The genus Curcuma*, CRC Press, Taylor & Francis Group, 2007, p. 235.
27. M. Bernabe-Pineda, M. T. Ramírez-Silva, M. Romero-Romo, E. Gonzalez-Vergara and A. Rojas-Hernandez, *Spectrochim. Acta, Part A*, 2004, **60**, 1091.
28. A. S. Darvesh, R. T. Carroll, A. Bishayee, N. A. Novotny, W. J. Geldenhuys and C. J. Van der Schyf, *Expert Opin. Invest. Drugs*, 2012, **21**, 1123.
29. P. Basnet and N. Skalko-Basnet, *Molecules*, 2011, **16**, 4567.
30. R. K. Basniwal, H. S. Buttar, V. K. Jain and N. Jain, *J. Agric. Food Chem.*, 2011, **59**, 2056.
31. A. L. Cheng, C. H. Hsu, J. K. Lin, M. M. Hsu, Y. F. Ho, T. S. Shen, J. Y. Ko, J. T. Lin, B. R. Lin, M. S. Wu, H. S. Yu, S. H. Jee, G. S. Chen, T. M. Chen, C. A. Chen, M. K. Lai, Y. S. Pu, M. H. Pan, Y. J. Wang, C. C. Tsai and C. Y. Hsieh, *Anticancer Res.*, 2001, **21**, 2895.
32. C. Jantarat, *Int. J. Pharm. Pharm. Sci.*, 2013, **5**, 493.
33. S. Prasad, A. K. Tyagi and B. B. Aggarwal, *Cancer Res. Treat.*, 2014, **46**, 2.
34. M. H. Pan, T. M. Huang and J. K. Lin, *Drug Metab. Dispos.*, 1999, **27**, 486.

35. M. Heger, R. F. van Golen, M. Broekgaarden and M. C. Michel, *Pharmacol. Rev.*, 2014, **66**, 222.
36. H. H. Tonnesen and J. Karlsen, *Z. Lebensm.-Unters. Forsch.*, 1985, **180**, 132.
37. H. H. Tonnesen, J. Karlsen and G. B. Vanhenegouwen, *Z. Lebensm.-Unters. Forsch.*, 1986, **183**, 116.
38. E. I. Paramera, S. J. Konteles and V. T. Karathanos, *Food Chem.*, 2011, **125**, 913.
39. J. Li, G. H. Shin, I. W. Lee, X. Chen and H. J. Park, *Food Hydrocolloids*, 2015, **56**, 41.
40. O. Naksuriya, M. J. van Steenbergen, J. S. Torano, S. Okonogi and W. E. Hennink, *AAPS J.*, 2016, **18**, 777.
41. M. Kharat, Z. Du, G. Zhang and D. J. McClements, *J. Agric. Food Chem.*, 2017, **65**, 1525.
42. F. Mirzaee, L. Hosseinzadeh, M. R. Ashrafi-Kooshk, S. Esmaeili, S. Ghobadi, M. H. Farzaei, M. R. Zad-Bari and R. Khodarahmi, *Protein Pept. Lett.*, 2019, **26**, 132.
43. M. C. Bergonzi, R. Hamdouch, F. Mazzacuva, B. Isacchi and A. R. Bilia, *LWT–Food Sci. Technol.*, 2014, **59**, 148.
44. S. Chaurasia, R. R. Patel, P. Chaubey, N. Kumar, G. Khan and B. Mishra, *Carbohydr. Polym.*, 2015, **130**, 9.
45. N. Sanoj Rejinold, P. R. Sreerekha, K. P. Chennazhi, S. V. Nair and R. Jayakumar, *Int. J. Biol. Macromol.*, 2011, **49**, 161.
46. C. Righeschi, M. C. Bergonzi, B. Isacchi, C. Bazzicalupi, P. Gratteri and A. R. Bilia, *LWT–Food Sci. Technol.*, 2016, **66**, 475.
47. P. R. Sarika, N. R. James, P. R. Anil Kumar and D. K. Raj, *Mater. Sci. Eng., C*, 2016, **68**, 251.
48. G. Shoba, D. Joy, T. Joseph, M. Majeed, R. Rajendran and P. Srinivas, *Planta Med.*, 1998, **64**, 353.
49. C. Moorthi, K. Krishnan, R. Manavalan and K. Kathiresan, *Asian Pac. J. Trop. Biomed.*, 2012, **2**, 841.
50. Y. S. Tu, J. W. Fu, D. M. Sun, J. J. Zhang, N. Yao, D. E. Huang and Z. Q. Shi, *J. Microencapsulation*, 2014, **31**, 551.
51. Y. Baspinar, M. Üstündas, O. Bayraktar and C. Sezgin, *Saudi Pharm. J.*, 2018, **26**, 323.
52. L. P. Volak, M. J. Hanley, G. Masse, S. Hazarika, J. S. Harmatz, V. Badmaev, M. Majeed, D. J. Greenblatt and M. H. Court, *Br. J. Clin. Pharmacol.*, 2013, **75**, 450.
53. P. Anand, A. B. Kunnumakkara, R. A. Newman and B. B. Aggarwal, *Mol. Pharm.*, 2007, **4**, 807.
54. C. A. Arcaro, V. O. Gutierres, R. P. Assis, T. F. Moreira, P. I. Costa, A. M. Baviera and I. L. Brunetti, *PLoS One*, 2014, **9**, e113993.
55. R. Jäger, R. P. Lowery, A. V. Calvanese, J. M. Joy, M. Purpura and J. M. Wilson, *Nutr. J.*, 2014, **13**, 11.
56. S. K. Kulkarni, K. K. Akula and J. Deshpande, *Pharmacology*, 2012, **89**, 83.
57. S. Setthacheewakul, S. Mahattanadul, N. Phadoongsombut, W. Pichayakorn and R. Wiwattanapatapee, *Eur. J. Pharm. Biopharm.*, 2010, **76**, 475.

58. D. M. Dhumal, P. R. Kothari, R. S. Kalhapure and K. G. Akamanchi, *RSC Adv.*, 2015, **5**, 90295.
59. M. M. Yallapu, B. K. Gupta, M. Jaggi and S. C. Chauhan, *J. Colloid Interface Sci.*, 2010, **351**, 19.
60. N. M. Khalil, T. C. F. do Nascimento, D. M. Casa, L. F. Dalmolin, A. C. de Mattos, I. Hoss, M. A. Romano and R. M. Mainardes, *Colloids Surf., B*, 2013, **101**, 353.
61. Y. Gao, Z. Li, M. Sun, H. Li, C. Guo, J. Cui, A. Li, F. Cao, Y. Xi, H. Lou and G. Zhai, *Drug Dev. Ind. Pharm.*, 2010, **36**, 1225.
62. S. K. Dubey, A. K. Sharma, U. Narain, K. Misra and U. Pati, *Eur. J. Med. Chem.*, 2008, **43**, 1837.
63. Y. Kaminaga, A. Nagatsu, T. Akiyama, N. Sugimoto, T. Yamazaki, T. Maitani and H. Mizukami, *FEBS Lett.*, 2003, **555**, 311.
64. M. M. Yallapu, M. Jaggi and S. C. Chauhan, *Drug Discovery Today*, 2012, **17**, 71.
65. M. Kanai, A. Imaizumi, Y. Otsuka, H. Sasaki, M. Hashiguchi, K. Tsujiko, S. Matsumoto, H. Ishiguro and T. Chiba, *Cancer Chemother. Pharmacol.*, 2012, **69**, 65.
66. H. Sasaki, Y. Sunagawa, K. Takahashi, A. Imaizumi, H. Fukuda, T. Hashimoto, H. Wada, Y. Katanasaka, H. Kakeya, M. Fujita and K. Hasegawa, *Biol. Pharm. Bull.*, 2011, **34**, 660.
67. T. Morimoto, Y. Sunagawa, Y. Katanasaka, S. Hirano, M. Namiki, Y. Watanabe, H. Suzuki, O. Doi, K. Suzuki, M. Yamauchi and T. Yokoji, *Biol. Pharm. Bull.*, 2013, **36**, 1708.
68. V. Thakkar, R. Dhankecha, M. Gohel, P. Shah, T. Pandya, T. Gandhi and V. Thakkar, *Int. J. Drug Delivery*, 2016, **8**, 77.
69. M. K. John, H. Xie, E. C. Bell and D. Liang, *Anticancer Res.*, 2013, **33**, 4285.
70. J. K. Patra, G. Das, L. F. Fraceto, E. V. R. Campos, M. del Pilar Rodriguez-Torres, L. S. Acosta-Torres, L. A. Diaz-Torres, R. Grillo, M. K. Swamy, S. Sharma and S. Habtemariam, *J. Nanobiotechnol.*, 2018, **16**, 71.
71. D. Lombardo, M. A. Kiselev and M. T. Caccamo, *J. Nanomater.*, 2019, **2019**, 3702518.
72. C. Schiborr, A. Kocher, D. Behnam, J. Jandasek, S. Toelstede and J. Frank, *Mol. Nutr. Food Res.*, 2014, **58**, 516.
73. D. Madhavi and D. Kagan, *Integr. Med. A Clin. J.*, 2014, **13**, 24.
74. H. S. Ali, R. S. Suliman, B. M. A. Elhaj and R. Suliman, *Int. J. Pharm. Chem. Res.*, 2019, **11**, 1.
75. P. G. Karade and N. R. Jadhav, *J. Microencapsulation*, 2018, **35**, 372.
76. C. Pi, J. Yuan, H. Liu, Y. Zuo, T. Feng, C. Zhan, J. Wu, Y. Ye, L. Zhao and Y. Wei, *Int. J. Biol. Macromol.*, 2018, **115**, 1046.
77. G. K. Jayaprakasha, K. N. C. Murthy and B. S. Patil, *Eur. J. Pharmacol.*, 2016, **789**, 291.
78. I. M. Krishnakumar, A. Ravi, D. Kumar, R. Kuttan and B. Maliakel, *J. Funct. Foods*, 2012, **4**, 348.
79. J. Zhang, Q. Tang, X. Xu and N. Li, *Int. J. Pharm.*, 2013, **448**, 168.
80. M. Purpura, R. P. Lowery, J. M. Wilson, H. Mannan, G. Münch and V. Razmovski-Naumovski, *Eur. J. Nutr.*, 2017, **57**, 929.

81. P. K. Mohan, G. Sreelakshmi, C. Muraleedharan and R. Joseph, *Vib. Spectrosc.*, 2012, **62**, 77.
82. N. Li, N. Wang, T. Wu, C. Qiu, X. Wang, S. Jiang, Z. Zhang, T. Liu, C. Wei and T. Wang, *Drug Dev. Ind. Pharm.*, 2018, **44**, 1966.
83. S. Dützmann, C. Schiborr, A. Kocher, U. Pilatus, E. Hattingen, J. Weissenberger, F. Geßler, J. Quick-Weller, K. Franz, V. Seifert and J. Frank, *Nutr. Cancer*, 2016, **68**, 943.
84. S. Peng, Z. Li, L. Zou, W. Liu, C. Liu and D. J. McClements, *J. Agric. Food Chem.*, 2018, **66**, 1488.
85. A. Amalraj, C. Divya and S. Gopi, *J. Med. Food*, 2020, **23**, 1.
86. N. Karimi, B. Ghanbarzadeh, H. Hamishehkar, F. Keyvani, A. Pezeshki and M. M. Gholian, *Appl. Food Biotechnol.*, 2015, **2**, 17.
87. A. Karewicz, D. Bielska, A. Loboda, B. Gzyl-Malcher, J. Bednar, A. Jozkowicz, J. Dulak and M. Nowakowska, *Colloids Surf., B*, 2013, **109**, 307.
88. J. You, D. B. Dai, W. J. He, G. Li, S. C. Song, Y. H. Wei, F. Z. Li and X. L. Xu, *China J. Chin. Mater. Med.*, 2014, **39**, 1238.
89. A. Semalty, M. Semalty, M. S. M. Rawat and F. Franceschi, *Fitoterapia*, 2010, **81**, 306.
90. J. Cuomo, G. Appendino, A. S. Dern, E. Schneider, T. P. McKinnon, M. J. Brown, S. Togni and B. M. Dixon, *J Nat Prod.*, 2011, **74**, 664.
91. T. H. Marczylo, R. D. Verschoyle, D. N. Cooke, P. Morazzoni, W. P. Steward and A. J. Gescher, *Cancer Chemother. Pharmacol.*, 2007, **60**, 171.
92. V. S. Gota, G. B. Maru, T. G. Soni, T. R. Gandhi, N. Kochar and M. G. Agarwal, *J. Agric. Food Chem.*, 2010, **58**, 2095.
93. A. Liu, H. Lou, L. Zhao and P. Fan, *J. Pharm. Biomed. Anal.*, 2006, **40**, 720.
94. K. Maiti, K. Mukherjee, A. Gantait, B. P. Saha and P. K. Mukherjee, *Int. J. Pharm.*, 2007, **330**, 155.
95. S. Gopi, A. Amalraj, J. Jacob, N. Kalarikkal, S. Thomas and Q. Guo, *New J. Chem.*, 2018, **42**, 5117.
96. R. Butchin, *J. Vet. Intern. Med.*, 2009, **23**, 767.
97. B. Antony, M. Benny and S. Rao, *Spice India*, 2005, **23**, 11.
98. B. Antony, B. Merina, V. S. Iyer, N. Judy, K. Lennertz and S. Joyal, *Indian J. Pharm. Sci.*, 2008, **70**, 445.
99. L. Zhongfa, M. Chiu, J. Wang, W. Chen, W. Yen, P. Fan-Havard, L. D. Yee and K. K. Chan, *Cancer Chemother. Pharmacol.*, 2012, **69**, 679.
100. B. Chassaing, O. Koren, J. K. Goodrich, A. C. Poole, S. Srinivasan, R. E. Ley and A. T. Gewirtz, *Nature*, 2015, **519**, 92–96.
101. E. Viennois, D. Merlin, A. T. Gewirtz and B. Chassaing, *Cancer Res.*, 2017, **77**, 27.
102. W. Yue, Y. Liu, X. Li, L. Lv, J. Huang, J. Liu and J. Turk, *Gastroenterol*, 2019, **30**, 290.
103. S. Toden, A. L. Theiss, X. Wang and A. Goel, *Sci. Rep.*, 2017, **11**, 814.
104. L. Zhang, H. Xue, G. Zhao, C. Qiao, X. Sun, C. Pang and D. Zhang, *Mol. Med. Rep.*, 2019, **19**, 3053.
105. A. Amalraj, S. Jude, K. Varma, J. Jacob, S. Gopi, O. S. Oluwafemi and S. Thomas, *Mater. Sci. Eng., C*, 2017, **75**, 359.
106. S. Gopi, R. George, S. Jude and V. T. Sriraam, *J. Chem. Pharm. Res.*, 2014, **6**, 96.

107. S. Gopi, R. George and V. T. Sriraam, *Br. Biomed. Bull.*, 2014, **2**, 545.
108. S. Gopi, R. George and V. T. Sriraam, *Asian J. Pharm. Technol. Innovation*, 2014, **2**, 123.
109. S. Gopi, R. George, M. Thomas and S. Jude, *Asian J. Pharm. Technol. Innovation*, 2015, **3**, 92.
110. S. Gopi, R. George and V. T. Sriraam, *Int. J. Curr. Res.*, 2014, **6**, 8473.
111. A. Amalraj, K. Varma, J. Jacob, C. Divya, A. B. Kunnumakkara, S. J. Stohs and S. Gopi, *J. Med. Food*, 2017, **20**, 1022.
112. S. Gopi, J. Jacob, K. Varma, S. Jude, A. Amalraj, C. A. Arundhathy, R. George, T. R. Sreeraj, C. Divya, A. B. Kunnumakkara and S. J. Stohs, *Phytother. Res.*, 2017, **31**, 1883.
113. S. Jude, A. Amalraj, A. B. Kunnumakkara, C. Divya, B.-M. Löffler and S. Gopi, *Molecules*, 2018, **23**, 2415.
114. A. Amalraj, K. Varma, J. Jacob, C. Divya, A. B. Kunnumakkara, S. J. Stohs and S. Gopi, *J. Med. Food*, 2017, **20**, 1022.
115. A. Amalraj, J. Jacob, K. Varma, A. B. Kunnumakkara, C. Divya and S. Gopi, *J. Herb. Med.*, 2019, **17**, 100276.
116. S. J. Stohs, C. Y. O. Chen, H. G. Preuss, S. D. Ray, L. R. Bucci, J. Ji and K. J. Ruff, *BMC Complementary Altern. Med.*, 2019, **4**, 293.
117. A. Kocher, L. Bohnert, C. Schiborr and J. Frank, *Mol. Nutr. Food Res.*, 2016, **60**, 1555.
118. S. J. Stohs, J. Ji, L. R. Bucci and H. G. Preuss, *J. Am. Coll. Nutr.*, 2018, **37**, 51.
119. D. Kumar, D. Jacob, P. S. Subash, A. Maliakkal, N. M. Johannah, R. Kuttan, B. Maliakel, V. Konda and I. M. Krishnakumar, *J. Funct. Foods*, 2016, **22**, 578.
120. G. N. Asher, Y. Xie, R. Moaddel, M. Sanghvi, K. S. Dossou, A. D. Kashuba, R. S. Sandler and R. L. Hawke, *J. Clin. Pharmacol.*, 2017, **57**, 185.
121. Y. Sunagawa, S. Hirano, Y. Katanasaka, Y. Miyazaki, M. Funamoto, N. Okamura, Y. Hojo, H. Suzuki, O. Doi, T. Yokoji and E. Morimoto, *J. Nutr. Sci. Vitaminol.*, 2015, **61**, 37–44.
122. Y. A. Shin, M. H. Suk, H. S. Jang and H. J. Choi, *J. Exercise Rehabil.*, 2017, **13**, 684.
123. M. Kanai, Y. Otsuka, K. Otsuka, M. Sato, T. Nishimura, Y. Mori, M. Kawaguchi, E. Hatano, Y. Kodama, S. Matsumoto, Y. Murakami, A. Imaizumi, T. Chiba, J. Nishihira and H. Shibata, *Cancer Chemother. Pharmacol.*, 2013, **71**, 1521.
124. Y. Nakagawa, S. Mukai, S. Yamada, M. Matsuoka, E. Tarumi, T. Hashimoto, C. Tamura, A. Imaizumi, J. Nishihira and T. Nakamura, *J. Orthop. Sci.*, 2014, **19**, 933.
125. M. Funamoto, Y. Sunagawa, Y. Katanasaka, Y. Miyazaki, A. Imaizumi, H. Kakeya, H. Yamakage, N. Satoh-Asahara, M. Komiyama, H. Wada, K. Hasegawa and T. Morimoto, *Int. J. Chronic Obstruct. Pulm. Dis.*, 2016, **11**, 2029.
126. G. W. Small, P. Siddarth, Z. Li, K. J. Miller, L. Ercoli, N. D. Emerson, J. Martinez, K. P. Wong, J. Liu, D. A. Merrill, S. T. Chen, S. M. Henning,

N. Satyamurthy, S. C. Huang, D. Heber and J. R. Barrio, *Am. J. Geriatr. Psychiatry*, 2018, **26**, 266.
127. Y. Tanabe, S. Maeda, N. Akazawa, A. Zempo-Miyaki, Y. Choi, S. G. Ra, A. Imaizum, Y. Otsuka and K. Nosaka, *Eur. J. Appl. Physiol.*, 2015, **115**, 1949.
128. S. P. Sudheeran, D. Jacob, J. N. Mulakal, G. G. Nair, A. Maliakel, B. Maliakel, R. Kuttan and K. Im, *J. Clin. Psychopharmacol.*, 2016, **36**, 236.
129. M. S. Campbell, A. Ouyang, I. M. Krishnakumar, R. J. Charnigo, P. M. Westgate and B. S. Fleenor, *Nutrition*, 2019, **62**, 135.
130. B. Antony, R. Kizhakedath, M. Benny and B. T. Kuruvilla, *Osteoarthrotic Cartilage*, 2011, **19**, 145.
131. G. Belcaro, M. R. Cesarone, M. Dugall, L. Pellegrini, A. Ledda, M. G. Grossi, S. Togni and G. Appendino, *Altern. Med. Rev.*, 2010, **15**, 337.
132. E. Antiga, V. Bonciolini, W. Volpi, E. Del Bianco and M. Caproni, *BioMed Res. Int.*, 2015, **2015**, 283634.
133. G. Belcaro, M. Dugall, R. Luzzi, A. Ledda, L. Pellegrini, M. R. Cesarone, M. Hosoi and M. Errichi, *Eur. Rev. Med. Pharmacol. Sci.*, 2014, **18**, 3959.
134. G. Appendino, G. Belcaro, U. Cornelli, R. Luzzi, S. Togni, M. Dugall, M. R. Cesarone, B. Feragalli, E. Ippolito, B. M. Errichi, L. Pellegrini, A. Ledda, A. Ricci, P. Bavera, M. Hosoi, S. Stuard, M. Corsi, S. Errichi and G. Gizzi, *Panminerva Med.*, 2011, **53**, 43.
135. D. Pastorelli, A. S. C. Fabricio, P. Giovanis, S. D'Ippolito, P. Fiduccia, C. Soldà, A. Buda, C. Sperti, R. Bardini, G. Da Dalt, G. Rainato, M. Gion and F. Ursini, *Pharmacol. Res.*, 2018, **132**, 72.
136. F. Mazzolani, S. Togni, L. Giacomelli, R. Eggenhoffner and F. Franceschi, *Eur. Rev. Med. Pharmacol. Sci.*, 2018, **22**, 3617.
137. F. Di Pierro, P. Zacconi, A. Bertuccioli, S. Togni, R. Eggenhoffner, L. Giacomelli and S. Scaltrini, *Eur. Rev. Med. Pharmacol. Sci.*, 2017, **21**, 4935.
138. P. A. Gupte, S. A. Giramkar, S. M. Harke, S. K. Kulkarni, A. P. Deshmukh, L. L. Hingorani, M. P. Mahajan and S. S. Bhalerao, *J. Inflammation Res.*, 2019, **12**, 145.
139. J. R. Santos-Parker, T. R. Strahler, C. J. Bassett, N. Z. Bispham, M. B. Chonchol and D. R. Seals, *Aging*, 2017, **9**, 187.
140. K. H. Cox, A. Pipingas and A. B. Scholey, *J. Psychopharmacol.*, 2015, **29**, 642.
141. B. K. McFarlin, A. S. Venable, A. L. Henning, J. N. Sampson, K. Pennel, J. L. Vingren and D. W. Hill, *BBA Clin.*, 2016, **5**, 72.
142. V. K. Hazarey, A. R. Sakrikar and S. M. Ganvir, *J. Oral Maxillofac. Pathol.*, 2015, **19**, 145.
143. R. Jäger, M. Purpura and C. M. Kerksick, *Nutrients*, 2019, **11**, 1692.
144. R. Jamwal, *J. Integr. Med.*, 2018, **16**, 367.

CHAPTER 6

Curcumin Pharmacokinetics and Plasma Determination

SIDNEY J. STOHS*[a], HARRY G. PREUSS[b], JIN JI[c], C. Y. OLIVER CHEN[d], KEVIN J. RUFF[e], SIDHARTHA D. RAY[f] AND LUKE R. BUCCI[g]

[a]School of Pharmacy and Health Professions, Creighton University Medical Center, Omaha, NE, USA; [b]Department of Biochemistry, Georgetown University Medical Center, Washington, DC, USA; [c]PulchriBio Intl., Mérieux NutriSciences, Cambridge, MA, USA; [d]Biofortis Research, Addison, IL, USA; [e]Stratum Nutrition, Carthage, MO, USA; [f]Department of Pharmaceutical and Biomedical Sciences, Touro College of Pharmacy, Manhattan, NY, USA; [g]Innerpath Nutrition, Reno, NV, USA
*E-mail: sid.stohs9@gmail.com

6.1 Introduction

Curcumin is the primary active polyphenolic constituent in turmeric derived from the rhizomes of *Curcuma longa*. Curcumin [(1*E*,6*E*) 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] exhibits many beneficial effects including anti-inflammatory, antioxidant, chemoprotective, metabolism regulating, immuno-modulating, antibacterial, anti-fungal, antiviral, and anti-depressant as well as antineoplastic properties.[1–12] Unformulated curcumin is poorly soluble in water and poorly absorbed, therefore it exhibits poor bioavailability which greatly limits

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

its effects and usefulness. As a consequence, various formulations have been developed to enhance the bioavailability of curcumin and consequent bio-efficacy.

Formulations that have been developed and studied include curcumin: in a liquid droplet nano-micellar formulation containing Gelucire® and polysorbate 20 (BioCurc®); in a natural turmeric matrix formulation composed of carbohydrates, proteins, fiber and volatile oil (Acumin®/Cureit®); co-administered with piperine (Curcumin C^3 Complex®); micronized plus turmeric oil (BCM-95®; BioCurcumax®; Curcugreen™); complexed with fenugreek-derived galactomannan fiber (CurQfen®); formulated with phosphatidylcholine from soy lecithin and microcrystalline cellulose (Meriva®); in a solid lipid curcumin particle composed of soy lecithin, docosahexaenoic acid (DHA), stearic acid and ascorbyl esters (vitamin C) (Longvida®); complexed with a hydrophobic carrier, cellulosic derivatives and natural antioxidants (CurcuWIN®); in a micro-particle surface-controlled colloidal dispersion using ghatti gum and glycerin (Theracurmin®); complexed with γ-cyclodextrin (Cavacurmin®); and in a matrix consisting of glycerol esters of fatty acids, medium chain triglycerides, hydroxymethylcellulose and sodium alginate (MicroActive Curcumin™).[13–32]

Several commercial formulations have claimed enhanced water solubility but have not demonstrated enhanced bioavailability through published human pharmacokinetic studies. These products include curcumin in a mixture of surfactants, polar lipids and solvents (Hydrocurc™); in a complex of triacetin and Panodan® spray-dried on porous silicon dioxide (Micronized Curcumin™); and in a whey–protein–curcumin conjugate (CurcuminPro®), in proprietary microcapsules (Curcushine™); in a turmeric oleoresin (Curcugen™); and in a mixture of rice flour, stearic acid, silica and magnesium stearate (CurcuFresh™). In addition, various other nano-formulations as well as conjugates and chemically modified variations of curcumin have been developed, and in some cases clinically tested, but without pharmacokinetic studies to determine the degree to which absorption and bioavailability were enhanced.[33,34]

This chapter discusses the metabolism of curcumin, various formulations designed to enhance bioavailability, the inappropriate use of hydrolysis to prepare plasma samples for analysis, and various human pharmacokinetic studies involving formulated curcumin products.

6.2 Curcumin Metabolism

In order to discuss the pharmacokinetics of curcumin, an understanding of the metabolism of curcumin is essential (Figure 6.1). Like most polyphenols, when consumed orally, curcumin is rapidly conjugated *via* phase

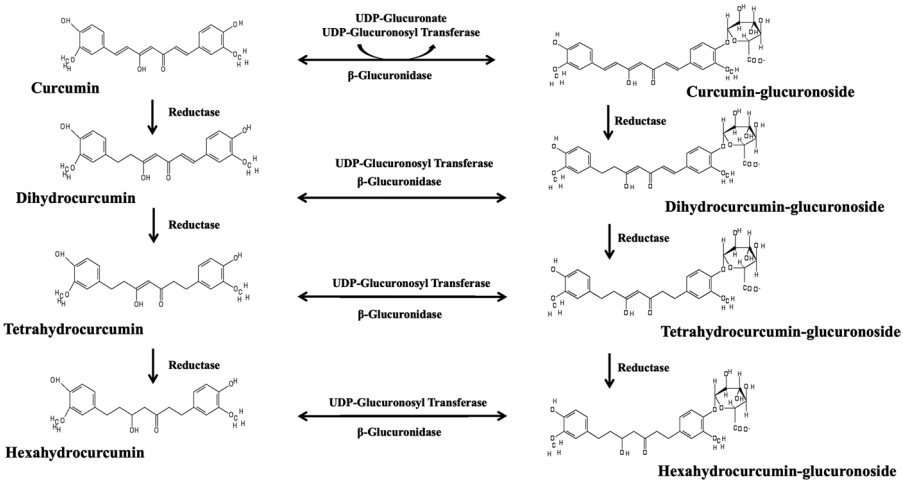


Figure 6.1 Metabolic pathways of curcumin.

II metabolism in the small intestine, liver and kidneys to curcumin glucuronides and curcumin sulfates, which undergo rapid excretion in the urine and feces.[4,5,7,11,14,16,35,36] Thus, relatively little free, bioactive curcumin occurs in the blood, and is predominantly present as the physiologically and pharmacologically inactive glucuronide and sulfate conjugates. Curcumin also undergoes extensive metabolic reduction to dihydrocurcumin, tetrahydrocurcumin and hexahydrocurcumin by intestinal microorganisms, particularly in the colon.[5,11–13,15,34–36] These reduction products of curcumin further undergo rapid conjugation, converting them into physiologically inactive constituents that are excreted in the urine and feces (Figure 6.1).[5,11-14,16,35-37] The inactive, conjugated tetrahydrocurcumin (as glucuronide and sulfate) constitute the metabolites in the highest concentrations in the plasma.[38] Similar metabolism exists for the minor curcuminoids demethoxycurcumin and bis-demethoxycurcumin.

6.3 Hydrolysis *vs.* No Hydrolysis of Plasma Samples

Because curcumin is much more physiologically active as compared to its conjugated forms and conjugated metabolites, determining free curcumin most accurately reflects its true bioavailability and bio-efficacy.[5,11–14,23] The major pharmacokinetic index used for determining the extent of absorption of various curcumin formulations is a plot of blood plasma concentration of the active constituent(s) against time, yielding the area

under the concentration–time curve (AUC). However, if plasma samples are enzymatically hydrolyzed with glucuronidase/sulfatase to free curcumin from its conjugates as opposed to measuring all curcumin metabolites including free curcumin in non-hydrolyzed plasma samples, the resulting data does not reflect free, bioactive curcumin. As a consequence, curcumin data reported from hydrolyzed plasma samples provide grossly misleading results.[38,39]

Early curcumin pharmacokinetic studies used high performance liquid chromatography in conjunction with photo detection.[40] These detection methods have now been supplanted by much more highly sensitive methods such as triple quadrupole mass spectrometry, which can detect curcumin and its metabolites in the nanogram range.[14,40] Thus, curcumin and its metabolites can be readily detected without first hydrolyzing the plasma samples to remove phase II conjugate moieties.

Various products make claims regarding enhanced absorption based on a comparison with unformulated curcumin. Since hydrolysis has generally been used, although enhanced absorption of total curcumin can be demonstrated, no information is provided regarding the enhanced absorption of free, bioactive curcumin based on the formulation.

Another common issue is the fact that both the curcumin load and the material (administered) dose of formulated products vary greatly, and therefore, it is very difficult to make comparisons between various formulated products concerning absorbability and bioavailability. However, data from pharmacokinetic studies of various products can be compared by normalizing the results based on AUC per mg and C_{max} per mg of administered curcumin, which provides a means for making comparisons based on the amount of curcumin that was ingested.[14,15] The AUC provides an estimate of the amount (extent) of curcumin absorbed over a finite period of time. The C_{max} denotes the maximum concentration of curcumin in the plasma after dosing.

For some products only a single pharmacokinetic study has been published while for other products as well as unformulated curcumin multiple pharmacokinetic studies have been reported. A very wide variation in the pharmacokinetic results is apparent for published data of unformulated curcumin. The reason for the wide variance in the normalized results for unformulated curcumin is not clear. However, the results may have been due to differences in quantification protocols, such as in analytical sensitivity and accuracy, and extraction techniques and solvents as well as the physicochemical nature and purity of the administered curcumin.[39] In addition, protection of plasma samples from light is essential due to the light sensitivity of curcumin. Furthermore, such a large variation may be a result of widely varied metabolic capacity toward curcumin and other polyphenols among study subjects.

When one compares AUC per mg and C_{max} per mg of administered curcumin from various enhanced absorption curcumin formulations, the data indicate that the greatest absorption and bioavailability are produced with a novel liquid droplet nano-micellar formulation.[14] This product (BioCurc®) provides approximately 5–10-fold better absorption (AUC per mg) than most other products based on published studies. This comparison was made based on the assumption that similar methods for plasma curcumin quantification were employed. However, as discussed below, most studies involving curcumin formulations that hydrolyzed their plasma samples provided total curcumin and not free curcumin.

The lack of a demonstrable increase in free, bioactive curcumin in blood has been a major pitfall of curcumin formulations for which no pharmacokinetic studies have been conducted as well as for products for which pharmacokinetic studies have been reported.[16–31] With respect to pharmacokinetic studies, with relatively few exceptions,[14,15,17,20] as a step in the analytical protocol plasma samples are routinely hydrolyzed with the enzymes β-glucuronidase and sulfatase which are most commonly derived from *Helix pomatia* snail extracts. Hydrolysis results in the generation of total curcumin because curcumin glucuronide and curcumin sulfate are the predominant circulating but physiologically inactive conjugates of curcumin.[16,18.19,21–32] As a consequence, the resulting conclusions do not provide a clear understanding of the potential pharmacokinetic benefits of the formulations with respect to an increase in free, bioactive curcumin. Failure to assess free curcumin while only determining total curcumin provides no indication of the amount of bioactive curcumin, thus greatly limiting conclusions that can be drawn regarding the potential efficacy and bioavailability of curcumin in the products.

For nine studies testing unformulated 95% curcumin, the average AUC per mg curcumin was approximately 0.030.[14,15] All of these studies involved enzymatic hydrolysis of the plasma samples prior to quantification.[14,15] It is not always easy to determine if the enzymatic hydrolysis step was used in some studies based on a description of the published procedures employed. However, when bioavailability results from these studies involving 95% unformulated curcumin are in the same range of the results of studies with hydrolysis, sample hydrolysis is a likely explanation, particularly when results from non-hydrolyzed samples are at least an order of magnitude lower.[14,15]

In a pharmacokinetic study in which direct extraction without enzymatic hydrolysis was used to determine plasma curcumin from unformulated 95% curcumin, the AUC per mg curcumin administered was 0.0104. These results suggest that hydrolysis increased the amount of measured curcumin in the plasma by a factor of approximately 29-fold because one is comparing free curcumin with total curcumin.[14] These results provide an indication of the misleading information provided by enzymatic hydrolysis of plasma samples. A pilot study assessed curcumin content in human plasma with and without hydrolysis, and determined that the curcumin content after hydrolysis was

approximately 14-fold higher than the free curcumin content determined without hydrolysis.[39]

In a follow-up study involving eight human subjects who received a single dose of a liquid droplet nano-micellar formulation (BioCurc®), blood samples were collected over time. Plasma was prepared from each sample and aliquots were treated with glucuronidase and sulfatase enzymes.[38] Hydrolyzed and non-hydrolyzed samples were analyzed for free curcumin, curcumin glucuronide, curcumin sulfate, tetrahydrocurcumin, demethoxycurcumin and bis-demethoxycurcumin by high performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS). Free curcumin equivalents were calculated to determine the actual free curcumin quantities (corrected) based on the molecular weights of each form (free curcumin being 100%) for each analyte.

In this follow-up study,[38] direct extraction of plasma samples resulted in the detection and determination of free curcumin, free demethoxycurcumin, free bis-demethoxycurcumin, curcumin glucuronide and curcumin sulfate. The total curcuminoids were calculated. Following enzymatic hydrolysis, curcumin, tetrahydrocurcumin, demethoxycurcumin and bis-demethoxycurcumin could all be quantified. The results indicated that enzymatic hydrolysis increased by a factor of approximately 31-fold the amount of total curcuminoids detected in plasma as compared to free curcumin in unhydrolyzed plasma samples. Curcumin glucuronide constituted the primary curcuminoid metabolite detected in non-hydrolyzed plasma while tetrahydrocurcumin was the primary metabolite in hydrolyzed samples.[38]

No tetrahydrocurcumin was detected in non-hydrolyzed plasma, while it constituted the majority of total curcuminoids detected in enzymatically hydrolyzed plasma. Due to the lack of a commercially available standard for tetrahydrocurcumin glucuronide, this metabolite in non-hydrolyzed plasma samples was not determined. Hydrolysis of plasma samples relative to non-hydrolyzed plasma samples resulted in an average C_{max} value for total curcuminoids that increased by a factor of approximately 19-fold,[38] again demonstrating the very misleading impact of plasma sample hydrolysis. These results unequivocally demonstrate that enzymatic hydrolysis of plasma samples prior to solvent extraction and analysis greatly exaggerates the levels of curcumin that are detected.

6.4 Pharmacokinetic Studies

A limited number of human pharmacokinetic studies have been conducted with various formulations designed to enhance the bioavailability of curcumin. As previously noted, the comparative bioavailability of these formulations based on AUC per mg and C_{max} per mg administered curcumin have been reported.[14,15] Without normalization of the pharmacokinetic data to mg of administered curcumin, it is not possible to make meaningful comparisons. Furthermore, the fact that data are reported in terms of either

curcumin or curcuminoids adds to the confusion. A review article summarized pharmacokinetic studies in humans associated with various curcumin formulations.[41] However, this review did not differentiate between studies that analyzed plasma levels of curcuminoids with and without hydrolysis.

A randomized, crossover, double-blind, comparator-controlled pharmacokinetic study was performed in healthy adult subjects to determine the appearance of free curcumin and its metabolites curcumin sulfate and curcumin glucuronide in plasma after a single dose of a novel proprietary curcumin liquid droplet nano-micellar formulation (BioCurc®), which contained 64.6 mg curcumin.[14] Blood samples were drawn and plasma was analyzed for curcumin and its two conjugates without enzymatic hydrolysis by liquid chromatography/tandem mass spectroscopy. Free curcumin reached a maximum level of 2 ng mL^{-1} at 1.5 hours with a small secondary free curcumin peak occurring at the 12-hour time point and a tertiary 1.5 ng mL^{-1} curcumin peak occurred at 24 hours. The second and third curcumin peaks may reflect lymphatic uptake and enterohepatic recycling with deconjugation of phase II conjugates, respectively.[5,6] Plasma levels of curcumin glucuronide and curcumin sulfate after 1.5 hours were approximately 300 ng mL^{-1} and 20 ng mL^{-1}, respectively. However, these two metabolites were not detected and were below the limits of detection at the 12- and 24-hour time points. The biological relevance of these inactive conjugates is unclear.

The oral absorption of 180 mg curcumin in a natural turmeric matrix (Acumin®/Cureit®) was compared in human subjects in a parallel-arm study with 80.5 mg of curcumin in Meriva® and 351 mg of curcumin in BCM-95®.[15] A single material dose of 500 mg of each product was given and blood samples were collected over a 24-hour time frame. In this study, plasma samples were not hydrolyzed and peak blood levels of curcuminoids were observed at approximately 0.5 and 3 hours for all products. The AUC per mg administered curcumin for Acumin®/Cureit® was approximately 5-fold and 2-fold greater than for Meriva® and BCM-95®, respectively. At the 12-hour time point, curcuminoid blood levels for Acumin®/Cureit® were approximately 60-fold and 30-fold higher as compared to Meriva® and BCM-95®, respectively.

A pharmacokinetic study was conducted with a formulation of curcumin with fenugreek dietary fiber (CurQfen®).[20] Doses of approximately 98 and 391 mg of curcumin were given in material doses of 250 mg and 1000 mg, respectively, in order to investigate the relative bioavailability and pharmacokinetics of the free curcuminoids.[20] Blood samples from human subjects were collected over 12 hours, and aliquots of the samples were directly extracted without enzymatic hydrolysis and with enzymatic hydrolysis.

At both doses the primary plasma peak of curcuminoids occurred at approximately one-hour post-administration.[20] Only a single extended peak in plasma curcuminoids was detected with measurable levels at the 12-hour time point.[20] Of interest was the observation that enzymatic hydrolysis only increased the total amount of measurable curcuminoids by approximately 20%, suggesting that the hydrolysis of the curcuminoid conjugates might not have been complete prior to extraction. As previously noted, other

studies that have specifically looked at the effects of enzymatic hydrolysis of measurable curcumin levels have shown that hydrolysis results up to a 31-fold increase in the amount of detectable curcumin due to cleavage of the glucuronide and sulfate conjugates.[38,39] The authors also suggested that the formulation facilitated better blood levels of free curcuminoids,[20] but they failed to take into account the extensive metabolism of curcumin to tetrahydrocurcumin and its rapid conjugation.[38]

A human pharmacokinetic study with a solid lipid curcumin particle formulation (Longvida®) that contained approximately 25% curcumin was conducted.[17] Plasma samples were collected over 12 hours following a single dose of 650 mg of the product, which contained 163 mg curcumin, and extracted for analysis without hydrolysis. A single plasma curcumin peak was reported with a C_{max} at the 2-hour time point.

A number of studies have examined the pharmacokinetics of a curcumin phosphatidylcholine phytosome complex of soy lecithin with microcrystalline cellulose (Meriva®), which have been compared and contrasted with other enhanced absorption products.[14,15] Doses of curcumin ranged from 152–382 mg curcumin while the material (administered) weights ranged from 500–2000 mg. Over a 20-fold variation existed in the reported AUC per mg administered curcumin between the various studies. The reason for the marked differences in the reported bioavailability may be due to differences in the dose of curcumin, differences in the material (administered) weights, or variations in sample handling and analytical methods. The typical analytical techniques that were used included high performance liquid chromatography (HPLC) coupled with tandem mass spectrometry or electrospray mass spectrometry.[15,19,25,27,28] Furthermore, most but not all of the studies hydrolyzed the plasma samples prior to extraction and analysis. Therefore, the actual levels of free, bioactive curcumin in the plasma were not determined in hydrolyzed samples, which greatly exaggerated the results.

A pharmacokinetic study was conducted that compared the plasma levels of 410 mg unformulated curcumin (500 mg total curcuminoids) with an equal amount of curcumin in a micronized powder and in liquid micelles mixed with 50 mL woodruff syrup.[30] The curcumin powder (micronisate) consisted of curcumin with tricetin and Panodan® sprayed on to silicon dioxide. The curcumin micelles were prepared using Tween-80. The micellar product clearly provided the highest blood levels of curcuminoids. As compared to most other enhanced absorption products, the micellar formulation provided enhanced absorption with peak plasma concentrations of total curcumin at 2 and 6 hours. However, the authors hydrolyzed their plasma samples prior to extraction and analysis, and therefore the actual benefit in terms of free, bioactive plasma levels of curcumin was not determined. In addition, the micelles were administered in woodruff syrup, and the relative benefits of the micellar formation of the product *vs.* the influence of the syrup cannot be differentiated, and direct comparisons with other orally administered products cannot be made.

A pharmacokinetic study was conducted on curcuminoids in combination with polyvinylpyrrolidone, cellulose and antioxidants known as CurcuWIN® as compared to Meriva® and BCM-95®.[27] Blood samples were collected over 12 hours following the oral ingestion of 376 mg curcuminoids in each of the three formulations. All plasma samples were enzymatically hydrolyzed prior to analysis. Peak blood levels of curcumin, demethoxycurcumin, bis-demethoxycurcumin and tetrahydrocurcumin occurred one hour following oral ingestion, and multiple peaks were not observed for any analyte. The blood levels of curcumin and tetrahydrocurcumin remained elevated over the 12 hours of the study. Curcuminoids in plasma were higher for CurcuWIN® as compared to Meriva® and BCM-95®. Tetrahydrocurcumin is not normally detected in non-hydrolyzed plasma samples due to its very rapid conjugation, primarily resulting in the formation of tetrahydrocurcumin glucuronide. Tetrahydrocurcumin is readily detected following enzymatic hydrolysis of plasma samples and is the most prominent metabolite detected in plasma.[38]

Theracurmin® is a colloidal nano-particle based curcumin formulation that contains glycerin, guar gum and water. The size of the particles is believed to be 100 times smaller than unformulated curcumin powder. Several studies have assessed the pharmacokinetic properties of this more bioavailable form of curcumin.[25,41] When blood samples were collected over a 24-hour time period, the Theracurmin® formulation containing 182 mg curcumin was shown to exhibit greater increases of curcuminoids in the blood as compared to a dose of 153 mg curcumin in Meriva® and 279 mg curcumin in BCM-95® by factors of approximately 5-fold and 11-fold, respectively.[25] Differences in material doses were not reported. The doses of curcumin were not comparable, and again, enzymatic hydrolysis of plasma samples was employed prior to analysis. Therefore, direct comparisons could not be made and the only provided information was regarding total curcuminoids but not free, bioactive curcumin.

The bioavailability of curcumin from a complex with cyclodextrin (Cavacurmin®) has been studied in comparison with Meriva®, BCM-95® and unformulated curcumin in a crossover designed human study.[19] Similar amounts of curcumin (*ca.* 350 mg) were given for the three formulated products while 1774 mg was given for the unformulated curcumin product. Blood samples were drawn over a 12-hour time period. All plasma samples were enzymatically hydrolyzed prior to analysis. An initial plasma peak was observed for curcumin at one-hour post-ingestion and a secondary peak occurred at the 6-hour time point. Smaller peaks were observed at these time points for demethoxyccurcumin and bis-demethoxycurcumin. In terms of total curcuminoids, the Cavacurmin® provided about 4.5-fold better bioavailability than the Meriva® and about 32-fold greater absorption than the BCM-95®.

The human pharmacokinetics of curcumin in the form of the product BCM-95® have been determined alone,[18] and as compared to other products[14,15] with doses of curcuminoids in the range of 279–1116 mg. This product is composed of curcuminoids in combination with small amounts of turmeric

volatile oils to enhance absorption. Wide variations have been reported in bioavailability of BCM-95® when data are expressed as AUC per mg administered curcumin.[14,15] As noted above, in comparison to various other products, the curcuminoids from BCM-95® are generally more poorly absorbed. However, the product has been reported to be approximately 7-fold better absorbed than unformulated curcumin.[18]

A human pharmacokinetic study has been reported on a micronized formulation of 25% curcumin in a matrix consisting of fatty acid glycerol esters, medium chain triglycerides, hydroxymethylcellulose and sodium alginate (MicroActive Curcumin™) relative to 95% unformulated curcumin.[29] Subjects received a 500 mg dose of unformulated or formulated curcumin in gelatin capsules, and blood samples were collected over a 12-hour time period. All plasma samples were hydrolyzed prior to analysis. A single plasma peak was observed for curcumin with at T_{max} at approximately 4 hours. The total curcumin (free plus conjugated) from the formulated product was approximately 10-fold more bioavailable than from the unformulated curcumin product.

C^3 Complex® is a combination of curcuminoids with small amounts of Bioperine™. Piperine is the primary active constituent in Bioperine™, which is believed to inhibit the efflux of absorbed curcumin, and was one of the first components reported to enhance curcumin blood levels.[40] Single doses of 10 grams and 12 grams of curcuminoids in the product were used in a pharmacokinetic study.[16] The very high doses of curcumin in the form of this product were used due to the undetectability of curcumin in the plasma at lower doses. Free curcumin was not detected, while curcumin glucuronide and curcumin sulfate were readily detected. Plasma samples were also enzymatically hydrolyzed and total curcumin was determined. Plasma levels of tetrahydrocurcumin were not determined.

6.5 Discussion

Diverse methods have been employed to enhance the solubility, absorbability and bioavailability of curcumin. Micellar and micronized formulations appear to be the most successful with respect to enhancing curcumin blood levels. Several formulations of curcumin have been reported to yield over 100-fold greater absorption relative to reference unformulated curcumin,[41] while the liquid droplet nano-micellar formulation (BioCurc®) was reported to provide over 400-fold greater blood levels of curcumin based on AUC per mg.[14]

It has been difficult to directly and indirectly compare the bioavailability of curcumin in various formulations between studies in the literature due to wide variations in both the actual doses of curcumin and the material doses of the products.[42] As a consequence, claims of a product being *X*-fold better absorbed than unformulated curcumin are difficult to interpret when different doses are employed and when plasma samples are enzymatically hydrolyzed, which results in the reporting of total but not free curcumin. The

normalization of results based on AUC per mg and C_{max} per mg of administered curcumin has provided a useful method of assessing and comparing the pharmacokinetic efficacy of various products.[14,15]

The analytical methods used in studying the pharmacokinetics of curcumin and other polyphenols have been reviewed.[40,43] Although sample preparation methods have been summarized,[40] this review did not discuss enzymatic hydrolysis and its misleading effects on the reporting of curcumin plasma levels. Enzymatic or acid hydrolysis of biological samples such as plasma for the analysis of drugs and polyphenols was widely employed until the mid-1990s, enabling total aglycones to be measured and reported. However, the subsequent development of more sensitive analytical methods using high-performance liquid chromatography coupled with mass spectrometric detection systems has greatly facilitated the routine direct extraction and determination of polyphenols and their metabolites including conjugates with much enhanced accuracy of compound identification and sensitivity of quantification.[44] It is therefore evident that studies involving curcumin pharmacokinetics that employ enzymatic hydrolysis of plasma samples are using outmoded and inappropriate techniques.

As previously noted, pharmacokinetic studies associated with almost all enhanced absorption formulations have employed enzymatic hydrolysis, freeing conjugated curcumin prior to analysis, and subsequently reporting the results as 'curcumin'. The unwary readers of these studies do not understand that the term 'curcumin' or 'total curcumin' refers almost entirely to inactive curcumin conjugates, and not to free, bioactive curcumin. What is not known with respect to these studies is to what extent the hydrolysis of plasma samples reflects the amount of free curcumin in plasma as compared to the total amount that is detected (free plus conjugated form). What should be stated is that the results of hydrolyzed plasma samples reflect free plus conjugated curcumin, not merely curcumin. Furthermore, many studies report curcuminoid and not curcumin levels.

In order to assess metabolic pathways and metabolism of polyphenolics as curcumin, hydrolysis with glucuronidase and sulfatase enzymes may be used.[44] When there is a lack of authentic reference standards of the conjugates, enzymatic hydrolysis can be a useful tool. However, with respect to curcumin, both curcumin glucuronide and curcumin sulfate reference standards are available. The use of enzymatic hydrolysis as a reliable analytical procedure in the quantification of glucuronidated and sulfated polyphenolic metabolites has been questioned, and may adversely and negatively impact recovery of free-form polyphenols that are being determined in plasma.[45] As a consequence, enzymatic hydrolysis of plasma samples containing curcumin and its metabolites may not provide reliable data regarding the actual levels, particularly of free, bioactive curcumin.

The pharmaceutical pharmacokinetics model and standard methods require the determination of blood plasma levels of the active form(s) of a drug. The amounts of the conjugated and pharmacologically inactive forms of the drug are of interest from a metabolic perspective only.[46,47] Tamoxifen,

which is widely used to treat breast cancer, is an excellent example of a drug that can be used to highlight drug pharmacokinetics.[48,49] The metabolism of tamoxifen is similar to curcumin, with both undergoing demethylation, hydroxylation and extensive glucuronidation.[14,48,49] The glucuronides of both tamoxifen and curcumin are excreted in the bile, urine and feces. Glucuronidation plays a major role in developing therapeutic resistance to tamoxifen and is responsible for inter-individual variability in responsiveness.[49] It is the plasma levels of free, pharmacologically active tamoxifen and several of its metabolites, and not the amount of the glucuronides or a combination of the free and conjugated forms that is predictive and indicative of efficacy and therapeutic potential.[49] Curcumin should be viewed in the same light.

6.6 Conclusions

Various formulations of curcumin have been shown to enhance its oral bioavailability, therefore, rendering it more therapeutically efficacious. However, it has been difficult to make comparisons between various enhanced absorption formulations due to factors such as differences in doses of curcumin and material doses, and differences in analytical methods including the use of enzymatic hydrolysis of plasma samples as well as wide inter-individual variability of its metabolism. Micellar and micronized formulations of curcumin appear to provide the greatest absorption. In general, the same principles that apply to drugs in terms of pharmacokinetics should be applied to all nutraceuticals.

Acknowledgements

Support from Boston Biopharm Inc., Southlake, TX, USA is acknowledged.

References

1. A. Amalraj, A. Pius, S. Gopi and S. Gopi, *J. Tradit. Complementary Med.*, 2017, **7**, 205.
2. A. H. Rahmani, M. A. Alsahli, S. M. Aly, M. A. Khan and Y. H. Aldebasi, *Adv. Biomed. Res.*, 2018, **7**, 38.
3. T. Ak and I. Gülçin, *Chem.-Biol. Interact.*, 2008, **174**, 27.
4. S. Prasad, A. K. Tyagi and B. B. Aggarwal, *Cancer Res. Treat.*, 2014, **46**, 2.
5. B. Kocaadam and N. Sanlier, *Crit. Rev. Food Sci. Nutr.*, 2015, 2889–2895.
6. A. B. Kunnumakkara, D. Bordoloi, G. Padmavathi, J. Monisha, N. K. Roy, S. Prasa and B. B. Aggarwal, *Br. J. Pharmacol.*, 2017, **174**, 1325.
7. M. Pulido-Moran, J. Moreno-Fernandez, C. Ramirez-Tortosa and C. M. Ramirez-Tortosa, *Molecules*, 2016, **21**, 264.
8. R. Kotecha, A. Takami and J. L. Espinoza, *Oncotarget*, 2016, **7**, 52517.
9. L. Huminiecki, J. Horbanczzuk and A. G. Alanasov, *Semin. Cancer Biol.*, 2017, 107–118.

10. X. Fan, C. Zhang, D. B. Liu, J. Yan and H. P. Liang, *Curr. Pharm. Des.*, 2013, **19**, 2011.
11. J. S. Jurenka, *Altern. Med. Rev.*, 2009, **14**, 141.
12. E. Bulku, S. J. Stohs, L. Cicero, T. Brooks, H. Halley and S. D. Ray, *Curr. Neurovasc. Res.*, 2012, **9**, 58.
13. B. J. Douglass and D. L. Clouatre, *J. Am. Coll. Nutr.*, 2015, **34**, 347.
14. S. J. Stohs, J. Ji, L. R. Bucci and H. G. Preuss, *J. Am. Coll. Nutr.*, 2018, **37**, 51.
15. S. Gopi, J. Jacob, K. Varma, S. Jude, A. Amalraj, C. A. Arundhathy, R. George, T. R. Sreeraj, C. Divya, A. B. Kunnumakkara and S. J. Stohs, *Phytother. Res.*, 2017, **31**, 1883.
16. S. K. Vareed, M. Kakarala, M. T. Ruffin, J. A. Crowell, D. P. Normolle, Z. Djuric and D. E. Brenner, *Cancer Epidemiol., Biomarkers Prev.*, 2008, **17**, 1411.
17. V. S. Gota, G. B. Maru, T. G. Soni, T. R. Gandhi, N. Kochar and M. G. Agarwal, *Agric. Food Chem.*, 2010, **58**, 2095.
18. B. Antony, B. Merina, V. S. Iyer, N. Judy, K. Lennartz and S. Joyal, *Indian J. Pharm. Sci.*, 2008, **70**, 445.
19. M. Purpura, R. P. Lowrey, J. M. Wilson, H. Mannan, G. Munch and V. Razmovski-Maumovski, *Eur. J. Nutr.*, 2018, **57**, 929.
20. D. Kumar, J. Della, P. S. Subash, A. Maliakkal, N. M. Johannah, K. Ramadassan, M. Balu, K. Veera and I. M. Krishnakumar, *J. Funct. Foods*, 2016, **22**, 478.
21. H. Sasaki, Y. Sunagawa, K. Takahashi and A. Imaizumi, *Biol. Pharm. Bull.*, 2011, **34**, 660.
22. M. Kanai, A. Imaizumi, Y. Otsuka, H. Sasaki, M. Hashiguchi, K. Tsujiko, S. Matsumoto, H. Ishiguro and T. Chiba, *Cancer Chemother. Pharmacol.*, 2012, **69**, 65.
23. M. Kanai, Y. Otsuka, K. Otsuka, M. Sato, T. Nishimura, Y. Mori, M. Kawaguchi, E. Hatano, Y. Kodama, S. Matsumoto, Y. Murakami, A. Imaizumi, T. Chiba, J. Nishira and H. Shibata, *Cancer Chemother. Pharmacol.*, 2013, **71**, 1521.
24. G. N. Asher, Y. Xie, R. Moaddel, M. Sanghvi, K. S. S. Sossou, A. D. M. Kashuba, R. S. Sandler and R. L. Hawke, *J. Clin. Pharmacol.*, 2017, **57**, 185.
25. Y. Sunagawa, S. Hirano, Y. Katanaska, Y. Miyazaki, M. Funamoto, N. Ksamura, Y. Hojo, J. Sukuki, O. Doi, T. Yokoji, E. Mirimoto, T. Takahashi, H. J. Ozawa, A. Imaizumi, M. Ueno, H. Kakeya, A. Shimatsu, H. Wada, K. Hasegawa and T. Morimoto, *J. Nutr. Sci. Vitaminol.*, 2015, **61**, 37.
26. T. Morimoto, Y. Sunagawa, Y. Katanassaka, S. Hiraon, M. Namiki, Y. Watanabe, H. Suzuki, O. Doi, K. Suzuki, M. Yamauchi, T. Yokoji, E. Miyoshi-Morimoto, Y. Otsuka, T. Hamada, A. Imaizumi, Y. Nonaka, T. Fuwa, T. Teramoto, H. Kakeya, H. Wad and K. Hasegawa, *Biol. Pharm. Bull.*, 2013, **36**, 1708.
27. R. Jager, R. P. Lowrey, A. V. Calvanese, I. M. Joy, M. Purpura and J. M. Wilson, *Nutr. J.*, 2014, **13**, 11.
28. J. Cuomo, G. Appendino, A. S. Dern, E. Schneider, T. P. McKinnon, M. J. Brown, S. Togni and B. M. Dixon, *J. Nat. Prod.*, 2011, **74**, 664.

29. D. Madhavi and D. Kagan, *Integr. Med.*, 2014, **13**, 24.
30. C. Schiborr, A. Kocher, D. Behnam, J. Jandasek, S. Toelstede and J. Frank, *Mol. Nutr. Food Res.*, 2014, **58**, 516.
31. D. Briskey, A. Sax, A. R. Mallard and A. Rao, *Eur. J. Nutr.*, 2018, **58**, 2087.
32. M. Gera, N. Sharma, M. ghosh, D. L. Huynh, S. J. Lee, T. Min, T. Kwon and D. K. Jeong, *Oncotarget*, 2017, **8**, 66680.
33. M. M. Yallapu, P. K. B. Nagesh, M. Jaggi and S. C. Chauhan, *AAPS J.*, 2015, **17**, 1341.
34. H. R. Rahimi, R. Nedaeinia, A. S. Shamloo, S. Nikdoust and R. K. Oskuee, *Avicenna J. Phytomed.*, 2016, **6**, 383.
35. J. M. Carbonell-Capella, M. Buniowska, F. J. Barba, M. J. Esteve and A. Frigola, *Compr. Rev. Food Sci. Food Saf.*, 2014, **13**, 155.
36. M. H. Pan, T. M. Huang and J. K. Lin, *Drug Metab. Dispos.*, 1999, **27**, 486.
37. J. Janjun, M. Wyganowska-Swiatkowska, K. Detttlaff, A. Jelinska, A. Surdacka, D. Watrobska-Sweitlikowska and E. Skrozypczak-Jankun, *Int. J. Mol. Med.*, 2016, **37**, 1151.
38. S. J. Stohs, C. Y. O. Chen, H. G. Preuss, S. D. Ray, L. R. Bucci, J. Ji and K. J. Ruff, *BMC Complementary Altern. Med.*, 2019, **19**, 293.
39. S. J. Stohs and S. D. Ray, *Biomed. J. Sci. Tech. Res.*, 2019, **19**, 293.
40. R. R. Kotha and D. L. Luthria, *Molecules*, 2019, **24**, 2930.
41. R. Jamwal, *J. Integr. Med.*, 2018, **16**, 367.
42. G. W. Small, P. Siddarth, Z. Li, K. J. Miller, L. Ercoli, N. D. Emerson, J. Martinez, K. P. Wong, J. Liu, D. A. Merrill, S. T. Chen, S. M. Henning, N. Sastyamurthy, S. C. Huang, D. Heber and J. R. Barrio, *Am. J. Geriatr. Psychiatry*, 2018, **26**, 266.
43. A. C. Santos, G. Costa, F. Veiga, V. Figueiredo, M. T. Batista and A. J. Ribeiro, *Curr. Drug Metab.*, 2014, **15**, 96.
44. Z. Mazerska, A. Miroz, M. Pawlowska and E. Augustin, *Pharmacol. Ther.*, 2016, **159**, 35.
45. N. Yang, R. Sun, X. Liao, J. Aa and G. Wang, *Pharmacol. Res.*, 2017, **121**, 169.
46. J. Le, 2018, https://www.merckmanuals.com/professional/clinical-pharmacology/pharmacokinetics/drug-bioabvailability.
47. M. Karaś, A. Jakubczyk, U. Szymanowska, U. Złotek and E. Zielińska, *Int. J. Food Sci. Tech.*, 2017, **52**, 291.
48. Y. Ding, M. Peng, T. Zhang, J. S. Tao, Z. Z. Cai and Y. Zhang, *Biomed. Chromatogr.*, 2013, **27**, 1280.
49. J. Johänning, P. Kröner, M. Thomas, U. M. Zanger, A. Nörenberg, M. Eichelbaum, M. Schwab, H. Brauch, W. Schroth and T. E. Mürdter, *Arch. Toxicol.*, 2018, **92**, 1099.

CHAPTER 7

Curcumin: A Potential Molecule for the Prevention and Treatment of Inflammatory Diseases

BANO SHABNAM[a], CHOUDHARY HARSHA[a], KRISHAN KUMAR THAKUR[a], ELINA KHATOON[a] AND AJAIKUMAR B. KUNNUMAKKARA*[a]

[a]Cancer Biology Laboratory, DBT-AIST International Center for Translational and Environmental Research (DAICENTER), Department of Biosciences & Bioengineering, Indian Institute of Technology Guwahati, Assam 781039, India
*E-mail: kunnumakkara@iitg.ac.in, ajai78@gmail.com

7.1 Introduction

The word 'inflammation' originates from the Latin word 'inflammare', which means "to set on fire".[1] Therefore, inflammation is called an immediate body response against pathogens or toxic stimuli inside the body. Inflammation is classified into two different types, *i.e.* acute inflammation and chronic inflammation. Acute inflammation is described as a short-term healing response by infiltration of leukocytes on the damaged area that leads to repair. On the other hand, chronic inflammation deals with prolonged, dysregulated tissue

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

destruction and attempts at tissue repair.[2] It is now well-established that chronic inflammation leads to several types of chronic diseases in human body including arthritis, cancer, cardiovascular diseases, diabetes, neurological diseases and obesity.[1] Based on recent studies it was shown that initiation and development of acute inflammation are well defined, but much less is known about the causes of chronic inflammation and the associated molecular and cellular pathways. Furthermore, chronic inflammation is linked with various types of inflammatory diseases including bronchial asthma (BA), Crohn's disease, gastritis, gingivitis, nephritis, oral mucositis (OM), osteoarthritis (OA), pancreatitis, peptic ulcer, periodontitis, plaque, prostatitis, pulmonary complication, rheumatoid arthritis (RA), ulcerative colitis (UC), ulcerative proctitis (UP), uveitis, *etc.*[3] Based on National Cancer Institute data, abnormal immune reactions to normal tissues, or conditions such as obesity are the major risk factor for the development of chronic inflammation (https://www.cancer.gov/). It was shown that pro-inflammatory transcription factors including nuclear factor kappa B (NF-κB) and signal transducer and activator of transcription-3 (STAT-3) are the crucial regulators of inflammation that further leads to inflammatory diseases. Previous studies have reported that interleukins (ILs), tumor necrosis factor-alpha (TNF-α), cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), C-reactive protein (CRP) and prostate specific antigen (PSA) are major pro-inflammatory molecules.[1] Research over the past several years have developed many drugs for the prevention and treatment of inflammatory diseases; however, their usage is not devoid of severe adverse side effects. Several reports have shown that natural products are highly safe and efficacious against various inflammatory diseases. One such agent is turmeric, also known as 'Indian saffron'. The present chapter describes the anti-inflammatory potential of curcumin, the active component of turmeric with more emphasis on clinical studies.

Turmeric is largely used in Southeast Asia as a principal spice. Besides, it is one of the main components used in religious rituals.[4] Not only this, turmeric has been used as an integral component of the traditional medicine used for the treatment of various chronic diseases including Alzheimer's disease,[5–8] arthritis,[9–12] arteriosclerosis,[13] diabetes mellitus,[14–18] multiple sclerosis,[19] non-alcoholic fatty liver disease (NAFLD),[20] psoriasis,[21] pulmonary fibrosis[22] and schizophrenia.[23] It is now well established that the yellow component (curcumin) of turmeric is responsible for its anti-inflammatory and prebiotic activities.[24]

Curcumin helps in the prevention of several inflammatory diseases including allergic rhinitis (AR), Behcet's disease, chronic gastritis (CG), chronic kidney disease (CKD), chronic prostatitis (CP), gingivitis, RA, inflammatory bowel disease (IBD), *etc.* (Figure 7.1).[25–32] Additionally, it has the ability to interact with various inflammatory proteins and molecules that further modifies their existence and expression levels.[33] Furthermore, curcumin has also shown to bind with several biological molecules including enzymes, growth factors, inflammatory cytokines receptors and transcription factors.

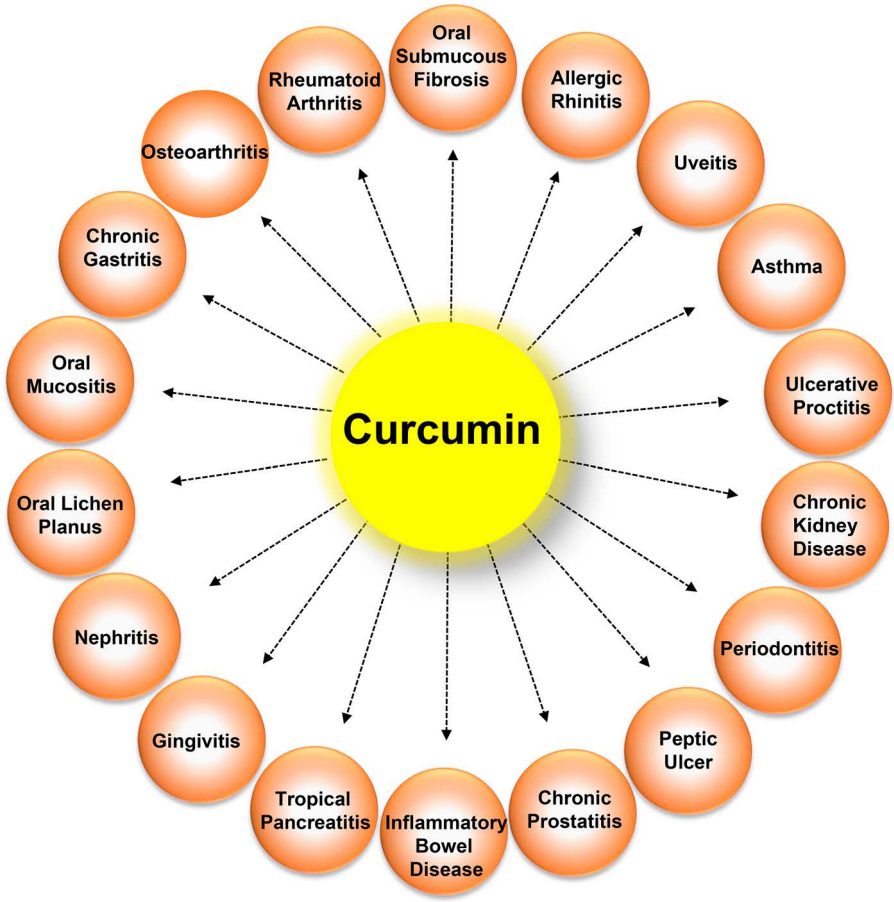


Figure 7.1 Potential of curcumin against various inflammatory diseases.

In addition, the compound was shown to suppress survival, proliferation, invasion and angiogenesis of cancer cells.[34] Numerous studies have proved that curcumin has high potential in the management of various inflammatory diseases by acting on various immune signaling pathways (Figure 7.2).[35] Curcumin effectively inhibits multiple signaling molecules associated with inflammation including NF-κB, Janus kinase (JAK)/STAT, mitogen-activated protein kinase (MAPK), β-catenin and Notch-1.[35] Moreover, it has the ability to modulate various inflammatory molecules including NF-κB, TNF-α, IL-1β, IL-8, monocyte chemoattractant protein-1 (MCP-1), monocyte inflammatory protein-1 (MIP-1α), inducible nitric oxide synthase (iNOS), prostaglandin E-2 (PGE-2) and nitric oxide (NO) along with generation of DNA oxidative damage (DOD).[35–43] Therefore, owing to its multi-targeting properties, curcumin plays a significant role in the treatment of several inflammatory diseases.

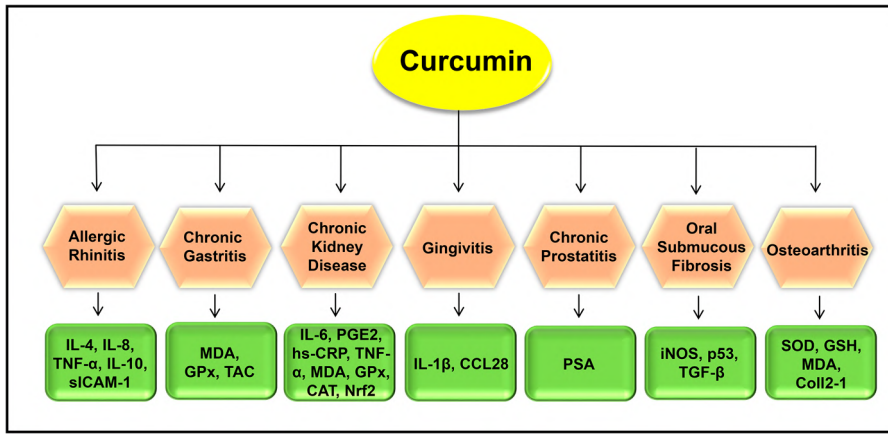


Figure 7.2 Molecular targets of curcumin in different inflammatory diseases (Abbreviations: CAT: catalase; CCL28: chemokine C–C motif ligand 28; Coll2-1: type II collagen peptide; GPx: glutathione peroxidase; GSH: glutathione; hs-CRP: high-sensitivity C-reactive protein; IL: interleukin; iNOS: inducible nitric oxide synthase; MDA: malondialdehyde; Nrf2: nuclear factor erythroid 2 related factor 2; PGE-2: prostaglandin E-2; PSA: prostate specific antigen; SOD: superoxide dismutase; sICAM: soluble intercellular adhesion molecule; TAC: total antioxidant capacity; TNF-α: tumor necrosis factor-α; TGF-β: transforming growth factor-beta.)

7.2 Molecular Targets of Curcumin in Inflammation

Various investigations have been performed to analyze the exact mechanism of action of curcumin regarding its anti-inflammatory activity. Curcumin has been found to reduce inflammation in various chronic diseases including OA, BA, IBD, uveitis, periodontitis, *etc.* It was suggested that curcumin inhibited various inflammatory and pro-inflammatory pathways that are linked with most of these chronic diseases. For instance, curcumin and its analogues have been reported to block TNF-α-mediated inflammatory pathways such as NF-κB pathway.[44,45] Additionally, curcumin has been found to regulate the expression of inflammatory ILs including IL-1, IL-1β, IL-6, IL-8, and IL-12.[38,40] Similarly, curcumin also stimulated apoptosis in colon cancer cell lines *via* inhibition of the inflammatory markers, PGE-2 and COX-2.[43] On the contrary, curcumin reduced the levels of pro-apoptotic proteins such as Bcl-2 associated X protein (Bax) and caspase-3, thereby inhibiting apoptosis in human tenocytes, however, the reduction in the levels of COX-2 and matrix metalloproteinases (MMPs) was similar to the anti-inflammatory activity of curcumin in other *in vitro* models.[41] Moreover, curcumin was also reported to reduce oxidative stress and inflammation in several chronic diseases by modulating the nuclear factor erythroid 2 related factor 2-kelch like ECH-associated protein 1

(Nrf2-keap1) pathway.[46] Furthermore, studies in multiple myeloma cells have suggested that curcumin exerted its anti-inflammatory activity by inhibiting IL-6 induced STAT3 phosphorylation that further led to the suppression of nuclear translocation of STAT3.[47] Curcumin has also been reported to inhibit the expression of IL-1β, IL-6, TNF-α and MCP-1 in lipopolysaccharide stimulated RAW264.7 cells.[48] In this study, curcumin was also found to exhibit renoprotection from cisplatin *via* suppression of macrophage-inducible C-type lectin (Mincle), which is known to influence the expression of several cytokines involved in inflammation. In case of AR patients, curcumin supplementation was found to effectively control inflammation by preventing high expression levels of serum IgE, IL-4 and NO in nasal lavage and that of eosinophil peroxidase in nasal homogenate.[49] It has also been reported that curcumin reduced nasal airflow resistance by suppressing the activity of IL-4, IL-8, TNF-α and inducing the production of IL-10 and soluble intercellular adhesion molecule in AR models. However, curcumin did not show any effects on the secretion of prostaglandin E2 and leukotriene C4 from polymorphonuclear neutrophils in these models.[25] Studies with *in vivo* models of AR suggested that curcumin inhibited components of the MAPK/NF-κB pathway such as phosphorylated extracellular-signal-regulated kinase (p-ERK), phosphorylated-p38 (p-p38), phosphorylated c-Jun N-terminal kinase (p-JNK), phosphorylated IκB kinase (p-IκBα) and NF-κB, thereby imparting anti-allergic response.[50] Another study has reported the involvement of ERK signaling in curcumin-mediated reduction of hypoxia-inducible factor 1-alpha (HIF-1α) induced apoptosis and inflammation in macrophages.[51] Curcumin was also found to prevent hyperlipidemia-induced inflammation through reduction of triglycerides and enhancement of high density lipoprotein (HDL) levels in a hyper-lipidemia model.[52] Additionally, curcumin was reported to modulate the expression level of inflammatory molecules, IL-1β and Chemokine C–C motif ligand 28 (CCL28) in gingivitis patients.[30] It also inhibited the proliferation of human mesangial cells and modified the extracellular matrix turnover in a glomerular mesangial cell line. In these cells, curcumin reduced IL-1β and MCP-1 expression induced by lipopolysaccharides, thereby further inhibiting the development of glomerulosclerosis.[53] Thus, curcumin is a multitargeted agent that has the potential to control the onset and development of inflammation in several chronic diseases thereby preventing them.

7.3 Curcumin for the Treatment of Inflammatory Diseases

As evidenced from the above-mentioned studies, curcumin can effectively control inflammation by targeting several signaling molecules and pathways. This multitargeted, anti-inflammatory molecule has been found to

be beneficial in the treatment of various inflammatory diseases. Numerous *in vitro* and *in vivo* models using rats,[54] mice[50,55] and guinea pigs[49] have proved the anti-inflammatory properties of curcumin. Many of these studies have demonstrated that curcumin holds potential therapeutic effects against several inflammatory diseases such as chronic interior uveitis, Crohn's disease, gastric ulcer, *Helicobacter pylori* infection, idiopathic orbital inflammatory pseudotumor, IBD, irritable bowel syndrome, RA, *etc.*[3]

7.4 Curcumin and Inflammatory Diseases: Clinical Trials

A large number of preclinical studies have demonstrated the anti-inflammatory activity of turmeric and its chief ingredient, curcumin, against several chronic diseases. This has led to the evaluation of the potential of this golden nutraceutical in the management of inflammatory chronic diseases in clinical settings. Studies have documented the effect of curcumin against different inflammatory diseases in humans, such as AR,[25,56] acute algesic episodes,[57] CKD,[26,48] CP,[27] gastritis,[29,55] gingivitis,[30,58] IBD,[32,60–64] refractory lupus nephritis,[65] oral lichen planus (OLP),[66,67] OM,[68,69] oral submucous fibrosis (OSMF),[70–75] OA,[76,77] RA[31,59,78–81] and tropical pancreatitis.[82] The potential of curcumin against such chronic inflammatory diseases, as evident in clinical settings, has been discussed below and listed in Table 7.1.[25–27,29–32,58–60,65–70,72–74,76,77,82–124]

7.4.1 Allergic Rhinitis

AR can be defined as IgE-mediated inflammation of the mucosal lining of the nasal area which is characterized by nasal congestion, sneezing, runny nose, and itching. This disease, also known as 'hay fever', is one of the most common inflammatory diseases of the respiratory system, and can also cause respiratory ailment like asthma. Curcumin being a potent anti-inflammatory molecule was tested against AR in clinical settings. A randomized, double-blind trial was performed on 241 AR patients.[25] These patients were treated with either curcumin or placebo for 2 months. It was reported that supplementation of oral curcumin improved the nasal symptoms in AR patients by improving the nasal airflow resistance and modulation of immune responses. Treatment with curcumin was found to reduce the release of IL-4, IL-8, and TNF-α, while it increased the synthesis of IL-10 and soluble intercellular adhesion molecule (sICAM-1). Thus, curcumin was found to provide health benefits in AR patients.

Table 7.1 Potential of curcumin in the prevention and treatment of inflammatory diseases: clinical trials.[a]

Disease	Pts.	Clinical outcomes	Molecular targets	Reference
Allergic rhinitis	241	Improved nasal airflow	↓IL-4, ↓IL-8, ↓TNFα, ↑IL-10 ↑sICAM-1	25
Asthma	77	Improved airway obstruction and hematological parameters	—	83
	30	—	—	84
CBP	143	Improvement in symptoms	—	85
CG	100	Reduced inflammation	↓MDA, ↑GPx, ↑TAC	29
	36	Improvement in symptoms	—	86
	25	Reduced inflammation and improvement in symptoms	—	87
CH	71	Improvement in symptoms	↓hs-CRP, ↓IL-6, ↓TNF-α	88
CKD	101	Reduced oxidative stress	↓MDA, ↑GPx, ↑CAT, ↑Nrf2	26
	16	Reduced inflammation	↓PGE-2	89
	16	Reduced inflammation	↓IL-6	90
CP	60	Reduced inflammation	—	27
	85	Reduced PSA	—	91
Gingivitis	60	Improvement in symptoms	↓IL-1β, ↓CCL28	30
	30	Improvement in symptoms	—	58
	30	↓PI, ↓GI, ↓ROM	—	92
	150	↓PI, ↓GI, ↓SBI	—	93
IBD	11	Well-tolerated	—	32
	10	Improvement in symptoms	—	60
	50	Effective with no adverse effects	—	94
	56	Improvement in symptoms	—	95
	89	Effective and safe	—	96
	45	Improvements in disease activity	—	97
	1	Effective	—	98
Nephritis	24	↓Hematuria, ↓Proteinuria, ↓BP	—	65
OLP	100	—	—	66
	20	↓Erythema, ↓Ulceration	—	67
	50	—	—	99
	75	Improvement in symptoms	—	100
	40	Improvement in symptoms	—	101
OM	60	Improvement in symptoms	—	68
	7	Safe and tolerable	—	69
	20	Safe and tolerable	—	102
	20	Improvement in symptoms	—	103
	32	Improvement in symptoms		104

OSMF	90	Improvement in symptoms		72
	28	Effective chemoprevention	↓p53, ↓TGF-β, ↓iNOS	73
	40	Improvement in symptoms		74
	60	Improvement in symptoms	—	70
	30	Improvement in symptoms	—	105
	30	Improvement in symptoms	—	106
	48	Improvement in symptoms	—	107
OA	201	Improvement in symptoms	—	76
	53	Improvement in symptoms	—	108
	40	Effective	↑SOD, ↑GSH, ↓MDA	77
	50	Improvement in symptoms	—	109
	40	Improvement in symptoms	—	110
	22	Improvement in symptoms	↓Coll2-1	111
	53	Safe and effective	—	112
	44	No significant effects	—	113
	100	Safe and tolerable	—	114
Peptic Ulcer	68	Safe and effective	—	115
	45	Effective	—	116
Periodontitis	30	Minimal side-effects	—	117
	10	Effective	—	118
	30	No adverse effect	—	119
	25	↑Clinical benefits	—	120
RA	36	Well tolerated	—	31
	45	Improvement in symptoms	—	59
	45	Effective	—	121
	65	No significant changes	—	122
TP	20	Reversed lipid peroxidation	—	82
UP	5	Effective	—	60
Uveitis	53	Safe and effective	—	123
	106	Safe and effective	—	124

[a]Abbreviations: BP: blood pressure; CAT: catalase; CBP: chronic bacterial prostatitis; CCL28: chemokine C–C motif ligand 28; CG: chronic gastritis; CH: chronic hemodialysis; CKD: chronic kidney disease; CP: chronic prostatitis; Coll2-1: type II collagen peptide; FAP: familial adenomatous polyposis; GI: gingival index; GPx: glutathione peroxidase; GSH: glutathione; hs-CRP: high-sensitivity C-reactive protein; IBD: inflammatory bowel disease; IL: interleukin; iNOS: inducible nitric oxide synthase; MDA: malondialdehyde; Nrf2: nuclear factor erythroid 2 related factor 2; OA: osteoarthritis; OLP: oral lichen planus; OM: oral mucositis; OSMF: oral submucous fibrosis; PGE-2: prostaglandin E-2; PI: plaque index; PSA: prostate specific antigen: RA: rheumatoid arthritis; ROM: reactive oxygen metabolites; SBI: sulcus bleeding index; SOD: superoxide dismutase; sICAM: soluble intercellular adhesion molecule; TAC: total antioxidant capacity; TNF-α: tumor necrosis factor-α; TGF-β: transforming growth factor; TP: tropical pancreatitis; UP: ulcerative proctitis. Pts: number of patient; ↓: down-regulation; ↑: upregulation.

7.4.2 Asthma

Asthma is another common chronic inflammatory disease of the respiratory tract which is characterized by hyper-responsiveness of the bronchia, restricted airflow, and remodelling of the airway. This disease is a serious health issue that causes remarkable morbidity worldwide and the commonly used asthma drugs like corticosteroids and short-acting beta-adrenergic agonists pose adverse side effects. Therefore, a number of studies have been performed with the natural phytochemical, curcumin for the treatment of this disease. In 2014, Abidi *et al.* reported amelioration in haematological parameters and airway obstruction in BA patients treated with curcumin.[83] Recently, a phase II clinical trial was performed where asthma patients, including both children and adolescents, were treated with root extract of *C. longa* for 6 months as supplement to standard treatment. Compared to placebo, the turmeric supplemented group exhibited better disease control.[125] The beneficial effects of curcumin in asthma patients has led to initiation of another phase II trial for evaluating the potential of curcumin in moderate to severe asthmatic patients (NCT04353310).[84]

7.4.3 Chronic Gastritis

CG involves prolonged inflammation of the protective lining of the stomach. If left untreated, it may lead to peptic ulcer or even gastric cancer. One of the major causes of this disease is *H. pylori* infection in the stomach that induces the release of several inflammatory cytokines. Studies have shown that curcumin helps to reduce chronic inflammation in CG patients. In a clinical trial, curcumin, in combination with the standard triple therapy regimen for *H. pylori* infection, was reported to cause remarkable reduction in the levels of malonyldialdehyde (MDA), glutathione peroxidase (GPx) and an increase in the total antioxidant capacity (TAC) of the gastric mucosa in gastritis patients.[29] However, curcumin alone did not show a significant reduction in *H.pylori* infection, though positive change was noted in the gastritis symptoms.[86] Similarly, a combination of curcumin with lactoferrin, *N*-acetylcysteine, and pantoprazole was administered to *H. pylori* infected patients. This combination also did not reduce the *H.pylori* infection, but it reduced inflammation and improved symptoms of gastritis.[87] Thus, curcumin alone or in combination can be used for the treatment of CG.

7.4.4 Chronic Kidney Disease

CKD is a type of inflammatory disorder that is characterized by increased inflammation, loss of kidney function and decrease in antioxidant capacity. It has been reported that proteinuria also induces the progression of this disease. It was shown that curcumin supplementation reduced oxidative

stress in both non-diabetic and diabetic proteinuric Mexican CKD patients.[26] In a set of studies, a herbal supplement containing curcumin along with *Boswellia serata* was administered to CKD patients. This combination was well-tolerated and was found to reduce inflammation in CKD patients by modulating the expression of inflammatory markers, IL-6 and PGE-2.[89,90] Moreover, a study was performed in hemodialysis patients where turmeric proved to be a potent anti-inflammatory supplement that reduced the levels of inflammatory mediators like hs-CRP, IL-6 and TNF-α in plasma.[88] Interestingly, CKD patients undergoing haemodialysis face adverse effects like malnutrition. The administration of curcumin in combination with resveratrol for twelve weeks demonstrated beneficial effects on protein oxidation, lipid peroxidation, bone and muscle mass recovery and iron overload in these patients.[126] However, curcumin treatment did not show any significant effect on contrast nephropathy post coronary angiography or angioplasty in CKD patients.[127]

7.4.5 Chronic Prostatitis

CP, the inflammation of the prostate gland, is one of the most commonly occurring diseases in males below 50 years. This disease has a detrimental effect on the quality of life of the patients and it can lead to more aggressive form *i.e.*, prostate cancer. The clinical efficacy of curcumin against CP has been evinced in a study that reported reduction of inflammatory cytokines and inflammatory cells upon treatment of CP patients with curcumin in combination with *Calendula* extract.[27] One of the prime characteristics of CP samples is high level of PSA, and curcumin, in combination with soy isoflavones, was found to reduce serum PSA levels in CP patients after 6 months of administration.[91] In another study, curcumin (FlogMEV) in association with *Serenoa repens, Urtica dioica* (ProstaMEV) and quercitin was found to enhance the effect of prulifloxacin in patients with chronic bacterial prostatitis (CBP).[85] Thus, curcumin seems to be a promising agent for the treatment of CP.

7.4.6 Gingivitis

Gingivitis is a type of periodontal inflammatory disease that involves the development of pathogenic biofilms around the teeth.[30] Chlorhexidine is considered as the gold standard in the treatment and prevention of gingivitis. However, due to adverse side effects of chlorhexidine, several clinical trials have been conducted to assess the efficacy of curcumin due to its anti-inflammatory and anti-oxidant properties. For example, a randomized, double-blinded, parallel clinical study was conducted with sixty healthy subjects in a human experimental gingivitis model. This study showed that application of curcumin two times a day for 10 minutes for 29 days helped to improve gingivitis by modulating IL-1β and CCL28 levels.[30]

Moreover, couple of clinical studies comprising of generalised chronic gingivitis patients showed the efficacy of curcumin as an adjunct therapy in the treatment of gingivitis and suggested curcumin as an alternative anti-gingivitis agent.[58,92,93]

7.4.7 Inflammatory Bowel Disease

IBD is a chronic inflammatory gastrointestinal disease that comprises both Crohn's disease and UC.[32] In 2013, a clinical study of IBD patients was conducted where the patients were treated with curcumin along with the standard IBD therapy for three weeks. It was found that curcumin was well tolerated without severe side effects.[32] Similarly, in another study involving five Crohn's disease patients, curcumin treatment was reported to show positive response with reduced Crohn's disease activity index (CDAI) score in four patients.[60] Emerging studies have also reported the potential role of curcumin in the treatment of UC. For example, randomized, placebo-controlled, double-blind trial was performed in 50 UC patients. Curcumin along with mesalamine was given to a group while other group was treated with placebo and it was observed that the combination of curcumin and mesalamine was superior to placebo or mesalamine alone, in UC patients with no significant adverse effects.[94] A similar trial was conducted in 56 UC patients who were administered with curcuminoids nanomicelles and mesalamine for four weeks. It was observed that curcuminoids nanomicelles and mesalamine significantly improved the symptoms in UC patients.[95] In another study, 89 UC patients were enrolled in a randomized, double-blind, multicenter, placebo-controlled trial. These patients were either treated with curcumin, in combination with sulfasalazine or mesalamine, or placebo, along with sulfasalazine or mesalamine, for six months. The results reported that the curcumin group showed promising effects with no adverse effects in UC patients.[96] In 2011, another study reported promising effects of oral curcumin in patients with UC resistant to mesalamine and steroids.[98] Additionally, a randomized, double-blinded, placebo-controlled trial was conducted on forty-five UC patients and it was observed that treatment with a curcumin formulation, NCB-02, resulted in significant improvements in symptoms as compared to placebo treatments groups.[97] Thus, curcumin alone or in combination with standard therapies may be useful for the treatment of IBD.

7.4.8 Nephritis

Nephritis is a chronic inflammatory disease of nephrons that adversely affects kidney function. Curcumin treatment is known to improve nephritis-associated kidney pathology. For instance, a placebo-controlled and randomized clinical trial was carried out with twenty-four refractory or relapsing lupus nephritis patients in which twelve patients were treated with 500 mg

turmeric for three months. This study reported that short-term supplementation of turmeric decreased hematuria, proteinuria and systolic blood pressure (BP) in lupus nephritis patients without any adverse effect.[65] Thus, curcumin supplementation can act as a safe adjuvant therapy against relapsing or refractory lupus nephritis.

7.4.9 Oral Lichen Planus

OLP is a chronic, inflammatory, and mucocutaneous disease causing pain, burning, and other discomfort inside the mouth.[66] Although corticosteroid treatment is usually successful for OLP, alternative treatment is needed due to the side effects caused. Curcuminoids are components of turmeric that are known to have anti-inflammatory activity. Many studies assessed the efficacy of systemic administration of curcuminoids in OLP patients.[66,67] A randomized, placebo-controlled, double-blind clinical study with twenty OLP patients showed that curcuminoids at a dose of 6000 mg day^{-1} in 3 divided doses were well tolerated and efficacious in controlling symptoms of OLP.[67] Kia and group, 2015 conducted a randomized, active controlled clinical trail with fifty OLP patients to assess the effectiveness of topical curcumin and triamcinolone and reported that the application of curcumin was safe and efficient for OLP treatment.[99] In addition, couple of clinical studies in OLP patients have demonstrated the efficacy of topical application of curcumin at addressing symptoms and lesions of OLP that resulted in reduction of lesion pain, sizes and burning sensation.[100,101]

7.4.10 Oral Mucositis

OM is a distinct and complex health complication that arises due to injuries in the mucosa, usually in cancer patients during the time of radiotherapy as well as chemotherapy.[68] Curcumin has immense therapeutic potential in OM treatment due to its radiosensibilizing and chemosensibilizing properties. A clinical study with sixty cancer patients showed the effectiveness of application of Indian turmeric and honey on treatment-induced OM.[68] Additionally, another study on seven pediatric and young-adult cancer patients demonstrated that curcumin acts as an efficient and well tolerated mouth wash for the prevention and treatment of OM.[69] A randomized clinical study with twenty participants demonstrated that mucoadhesive formulation consisting of curcuminoids and *Bidens pilosa* Linn extract (FITOPROT) are safe and well-tolerated for the treatment and prevention of OM without any genotoxicity, systemic and local side effects.[102] In couple of clinical trials, administration of curcumin mouth wash or curcumin in the form of nanomicelle was found to be beneficial in radio-chemotherapy induced OM patients in terms of better patient compliance and fast wound healing.[103,104]

7.4.11 Oral Submucous Fibrosis

OSMF is a pre-malignant condition due to betel quid or areca nut chewing that leads to restricted mouth opening and burning sensation.[70,74] Several clinical trials have demonstrated that curcumin is a promising effective treatment option for the management of patients with OSMF.[71] For instance, a randomized clinical trial comprising of sixty OSMF patients showed that both lycopene and curcumin were equally efficient in relieving burning sensation and improving mouth opening in OSMF patients.[70] Another randomized placebo-controlled parallel clinical study with ninety OSMF patients demonstrated that the therapeutic effects of both curcumin and lycopene were almost equal with overall amelioration in burning sensation, mouth opening, cheek flexibility and tongue protrusion.[72] Moreover, curcumin was shown to exert chemopreventive effects in OSMF tissue samples *via* modulation of the expression of iNOS, p53, and tumor growth factor-beta (TGF-β) proteins.[73] Additionally, a randomized, interventional study was conducted in forty OSMF patients and this study reported that oral administration of two curcumin tablets (Turmix 300 mg) daily for 3 months improved burning sensation in the OSMF patients.[74] Other clinical trials in OSMF patients also signified the effectiveness of curcumin in reducing clinical symptoms of OSMF.[105–107]

7.4.12 Osteoarthritis

OA is a type of chronic, degenerative and inflammatory disease of bone affecting aging populations all over the world. Compounds with anti-inflammatory properties *e.g.*, curcumin holds potential for treatment of OA. Many clinical trials were carried out in osteoarthritic patients to determine the effectiveness of curcumin. For example, a randomized, double-blind, placebo-controlled clinical trial in 201 osteoarthritic patients was conducted to assess the safety and efficacy of the combination of curcuminoid and boswellic acid and reported that this combination helped in reducing the pain of osteoarthritic patients.[76] Another randomized controlled trial in 40 knee osteoarthritic patients was conducted and were administered curcuminoid capsules at a dose of 1500 mg day^{-1} with piperine (15 mg day^{-1}) for a duration of 6 weeks while other group was treated with placebo. It was observed that in the curcuminoid group the serum superoxide dismutase (SOD) activities and glutathione (GSH) concentrations elevated significantly while the MDA concentration was found to be reduced compared to the placebo group. Thus, this study demonstrated that curcuminoids target OA *via* attenuating oxidative stress.[77] In another randomized, double-blind, placebo-controlled study, CartiJoint Forte, a commercially available combination of glucosamine hydrochloride, chondroitin sulfate and Bio-Curcumin BCM-95®, was found to ameliorate pain in knee osteoarthritic patients.[108] Several other clinical studies were conducted in osteoarthritic patients that

signified the potential beneficial effect of curcumin as an adjuvant therapy in the treatment of OA.[109–114]

7.4.13 Peptic Ulcer

H. pylori infection is one of the major causes of peptic ulcer disease. Studies have reported that curcumin has been used widely for the treatment of peptic ulcer.[115,116] A phase II clinical trial was carried out to evaluate the effect of curcumin on healing of peptic ulcer on forty-five patients (24 males and 21 females). It was observed that curcumin treatment resulted in the absence of ulcers, in 48 percent patients after 4 weeks, and 76 percent patients after 12 weeks of treatment.[116] In another double-blind, placebo-controlled trial, curcumin was used along with adjunctive therapy (clarithromycin, amoxicillin, and pantoprazole) in peptic ulcer patients. The combination of curcumin and adjunctive therapy was found to be safer and improved dyspepsia symptoms in peptic ulcer patients.[115]

7.4.14 Periodontitis

Periodontitis is an inflammatory disease that leads to the destruction of supporting structures of the teeth. Recently, studies have shown that curcumin can be effective in the treatment of periodontitis and it also minimizes the side effects along with other therapeutic agents. A randomized controlled clinical trial on 30 periodontitis patients in the age group 20–50 years was performed to determine the efficacy of curcumin compared to chlorhexidine. It was observed that curcumin was as effective as chlorhexidine with minimal side effects, thus it might be a potential alternative for the treatment of periodontitis.[117] Another randomized controlled clinical trial was conducted in 10 periodontitis patients to evaluate the efficacy of curcumin against periodontitis. This study reported that curcumin showed significant improvement in clinical symptoms and thus can be used as an adjunct therapy.[118] Similarly, 30 periodontitis patients were enrolled in a randomized double blind clinical trial and it was observed that curcumin was effective in the treatment of periodontitis without any adverse effects.[119] Recently, a randomized controlled trial was conducted on 25 patients to evaluate the efficacy of curcumin (100 mg L^{-1}), antimicrobial photodynamic therapy and LED irradiation (465–485 nm). The combination treatment was found to be effective and yielded short-term clinical benefits against the disease.[120]

7.4.15 Rheumatoid Arthritis

RA is an autoimmune disorder, which is characterized by chronic inflammation.[31] Several studies have reported the anti-inflammatory effects of curcumin against RA. A randomized, double-blind, placebo-controlled trial was conducted on RA patients in order to evaluate the suitable dose of curcumin.

Two different doses of curcumin (250 and 500 mg) and placebo were administered twice in a day for ninety days to each group comprising 12 patients. It was observed that low dose of curcumin (250 mg twice daily) has shown significant improvement compared to placebo group and it was well tolerated without any adverse effects.[31] A similar clinical trial was performed on 45 RA patients with a dose of 500 gm curcumin and has confirmed the safety of curcumin without any adverse effects in RA patients.[59] Another triple-blinded controlled trial was performed on 45 RA patients (female) having chronic periodontitis by using curcumin and essential oils combination. The combination was very effective and reduced the severity of RA.[121] Additionally, the effect of curcumin nanomicelle was examined in a randomized, double-blind, controlled trial by including 65 RA patients; however no significant changes were observed in curcumin nanomicelle group as compared to placebo groups.[122]

7.4.16 Tropical Pancreatitis

Tropical pancreatitis is a special type of chronic pancreatitis that is seen mainly in tropical countries. A randomized controlled trial was conducted with twenty tropical pancreatitis patients. Patients were treated with curcumin (500 mg) and piperine (5 mg) or placebo for six weeks. It was observed that combination of curcumin and piperine was effective and reversed lipid peroxidation by reducing the malonyldialdehyde levels and increasing GSH levels, as compared to placebo in tropical pancreatitis patients.[82]

7.4.17 Ulcerative Proctitis

UP is a type of mild UC that mainly occurs due to inflammation of the rectum.[128] A pilot study was conducted on five (three female and two male) UP patients. Patients were given 550 mg curcumin orally twice a day for one month and then thrice a day for the next month, along with their existing treatments. Treatment of UP patients with curcumin resulted in reduction of concomitant medications, which warrants further studies.[60]

7.4.18 Uveitis

Uveitis is a common ocular inflammatory disease. A clinical trial was conducted on fifty-three chronic anterior uveitis patients to evaluate the efficacy of curcumin in the treatment of uveitis. Oral administration of curcumin (375 mg thrice a day for 12 weeks) to uveitis patients resulted in improvement of symptoms with no side effects compared to corticosteroid treated groups.[123] In another trial, curcumin-phosphatidylcholine complex (Norflo tablets) was given to 106 uveitis patients and it was observed that curcumin was well tolerated and reduced eye discomfort sign and symptoms in majority of the patients (80%) after a few weeks of treatment.[124]

7.5 Conclusion

Extensive research within the past half-century has indicated that curcumin, a yellow pigment commonly used in curry powder, works as an effective anti-oxidant, anti-inflammatory and proapoptotic drug. Curcumin contributes to the prevention of multiple inflammatory diseases by targeting various biological molecules and proteins including growth factors, receptors, transcription factors, inflammatory cytokines, transcription factors *etc.* Curcumin is a natural, non-toxic, multi-targeted mediator that has substantial overwhelming activities in drug resistance in malignant cells and also helps to sensitize cancer cells to various chemotherapeutic medicines. Moreover, curcumin has proven itself as 'Curecumin' for the prevention of numerous chronic diseases. Curcumin exhibits anti-inflammatory activities *via* targeting several signaling molecules such as JAK/STAT, NF-κB, Notch, and TNF *etc.* The anti-oxidant and anti-inflammatory effects of curcuminoids may account for their cardioprotective effects. Furthermore, curcumin also helps to manage various inflammatory diseases including intestinal inflammatory diseases, such as UC, Crohn's disease, and necrotizing enterocolitis. Also, curcumin, as an adjuvant therapy, may play a potential role in the treatment of intestinal inflammatory diseases. Therefore, it can be concluded that curcumin has the ability to manage inflammatory diseases in humans. Evidently, curcumin holds promise in the future for its potential use in the treatment of human diseases.

Conflict of Interest

The authors express no conflict of interest.

References

1. S. C. Gupta, A. B. Kunnumakkara, S. Aggarwal and B. B. Aggarwal, *Front. Immunol.*, 2018, **9**, 2160.
2. U. Weiss, *Nature*, 2008, **454**, 427.
3. A. B. Kunnumakkara, D. Bordoloi, G. Padmavathi, J. Monisha, N. K. Roy, S. Prasad and B. B. Aggarwal, *Br. J. Pharmacol.*, 2017, **174**, 1325.
4. S. Prasad and B. B. Aggarwal, Turmeric, the Golden Spice: From Traditional Medicine to Modern Medicine, in *Herbal Medicine: Biomolecular and Clinical Aspects*, ed. I. F. F. Benzie and S. Wachtel-Galor, CRC Press/Taylor & Francis, USA, Boca Raton (FL), 2nd edn, 2011, ch. 13.
5. T. Farkhondeh, S. Samarghandian, A. M. Pourbagher-Shahri and M. Sedaghat, *J. Cell. Physiol.*, 2019, **234**, 16953.
6. T. K. Das, S. K. Chakrabarti, I. N. Zulkipli and M. R. W. Abdul Hamid, *J. Alzheimer's Dis. Res.*, 2019, **3**, 59.
7. S. Gagliardi, V. Franco, S. Sorrentino, S. Zucca, C. Pandini, P. Rota, S. Bernuzzi, A. Costa, E. Sinforiani, O. Pansarasa, J. R. Cashman and C. Cereda, *Front. Pharmacol.*, 2018, **9**, 1404.

8. H. Y. Liu, X. Fu, Y. F. Li, X. L. Li, Z. Y. Ma, Y. Zhang and Q. C. Gao, *Neural Regener. Res.*, 2019, **14**, 1603.
9. J. L. G. da Silva, D. F. Passos, V. M. Bernardes, F. L. Cabral, P. G. Schimites, A. G. Manzoni, E. G. de Oliveira, C. de Bona da Silva, R. C. R. Beck, M. H. Jantsch, R. M. Maciel and D. B. R. Leal, *Inflammation*, 2019, **42**, 1595.
10. Q. Wang, C. Ye, S. Sun, R. Li, X. Shi, S. Wang, X. Zeng, N. Kuang, Y. Liu, Q. Shi and R. Liu, *Int. Immunopharmacol.*, 2019, **72**, 292.
11. X. Li, D. Q. Xu, D. Y. Sun, T. Zhang, X. He and D. M. Xiao, *J. Cell. Biochem.*, 2019, **120**, 6718.
12. Q. Dai, D. Zhou, L. Xu and X. Song, *Drug Des., Dev. Ther.*, 2018, **12**, 4095.
13. Y. Li, L. Tian, D. Sun and D. Yin, *J. Cell. Physiol.*, 2019, **234**, 21049.
14. D. F. Roxo, C. A. Arcaro, V. O. Gutierres, M. C. Costa, J. O. Oliveira, T. F. O. Lima, R. P. Assis, I. L. Brunetti and A. M. Baviera, *Diabetol. Metab. Syndr.*, 2019, **11**, 33.
15. N. Poolsup, N. Suksomboon, P. D. M. Kurnianta and K. Deawjaroen, *PLoS One*, 2019, **14**, e0215840.
16. S. Asadi, M. S. Gholami, F. Siassi, M. Qorbani, K. Khamoshian and G. Sotoudeh, *Complement. Ther. Med.*, 2019, **43**, 253.
17. M. Adibian, H. Hodaei, O. Nikpayam, G. Sohrab, A. Hekmatdoost and M. Hedayati, *Phytother. Res.*, 2019, **33**, 1374.
18. A. E. Bulboacă, A. S. Porfire, L. R. Tefas, P. M. Boarescu, S. D. Bolboacă, I. C. Stănescu, A. C. Bulboacă and G. Dogaru, *Molecules*, 2019, **24**, 846.
19. E. Esmaeilzadeh, M. Soleimani, D. Zare-Abdollahi, B. Jameie and H. R. Khorram Kohrshid, *Drug Dev. Res.*, 2019, **80**, 629.
20. S. Gheibi, H. E. Gouvarchin Ghaleh, B. M. Motlagh, A. F. Azarbayjani and L. Zarei, *Biomed. Pharmacother.*, 2019, **115**, 108938.
21. Y. Zhang, Q. Xia, Y. Li, Z. He, Z. Li, T. Guo, Z. Wu and N. Feng, *Theranostics*, 2019, **9**, 48.
22. H. Chen, R. Yang, Y. Tang and X. Fu, *Exp. Ther. Med.*, 2019, **17**, 3664.
23. C. Miodownik, V. Lerner, N. Kudkaeva, P. P. Lerner, A. Pashinian, Y. Bersudsky, R. Eliyahu, A. Kreinin and J. Bergman, *Clin. Neuropharmacol.*, 2019, **42**, 117.
24. F. Ghiamati Yazdi, S. Soleimanian-Zad, E. van den Worm and G. Folkerts, *Plant Foods Hum. Nutr.*, 2019, **74**, 293.
25. S. Wu and D. Xiao, *Ann. Allergy, Asthma, Immunol.*, 2016, **117**, 697.
26. A. S. Jiménez-Osorio, W. R. García-Niño, S. González-Reyes, A. E. Álvarez-Mejía, S. Guerra-León, J. Salazar-Segovia, I. Falcón, H. Montes de Oca-Solano, M. Madero and J. Pedraza-Chaverri, *J. Renal Nutr.*, 2016, **26**, 237.
27. G. Morgia, G. I. Russo, D. Urzì, S. Privitera, T. Castelli, V. Favilla and S. Cimino, *Arch. Ital. Urol. Androl.*, 2017, **89**, 110.
28. M. T. Palizgir, M. Akhtari, M. Mahmoudi, S. Mostafaei, A. Rezaiemanesh and F. Shahram, *Immunopharmacol. Immunotoxicol.*, 2018, **40**, 297.
29. A. Judaki, A. Rahmani, J. Feizi, K. Asadollahi and M. R. Hafezi Ahmadi, *Arq. Gastroenterol.*, 2017, **54**, 177.
30. S. J. Pulikkotil and S. Nath, *Aust. Dent. J.*, 2015, **60**, 317.

31. A. Amalraj, K. Varma, J. Jacob, C. Divya, A. B. Kunnumakkara, S. J. Stohs and S. Gopi, *J. Med. Food*, 2017, **20**, 1022.
32. D. L. Suskind, G. Wahbeh, T. Burpee, M. Cohen, D. Christie and W. Weber, *J. Pediatr. Gastroenterol. Nutr.*, 2013, **56**, 277.
33. A. B. Kunnumakkara, P. Anand and B. B. Aggarwal, *Cancer Lett.*, 2008, **269**, 199.
34. A. B. Kunnumakkara, D. Bordoloi, C. Harsha, K. Banik, S. C. Gupta and B. B. Aggarwal, *Clin. Sci.*, 2017, **131**, 1781.
35. K. R. Kahkhaie, A. Mirhosseini, A. Aliabadi, A. Mohammadi, M. J. Mosavi, S. M. Haftcheshmeh, T. Sathyapalan and A. Sahebkar, *Inflammopharmacology*, 2019, **27**, 885.
36. N. S. Chang, N. Joki, J. Mattison, T. Dinh and S. John, *Cell Death Differ.*, 1997, **4**, 779.
37. Y. G. Lin, A. B. Kunnumakkara, A. Nair, W. M. Merritt, L. Y. Han, G. N. Armaiz-Pena, A. A. Kamat, W. A. Spannuth, D. M. Gershenson, S. K. Lutgendorf, B. B. Aggarwal and A. K. Sood, *Clin. Cancer Res.*, 2007, **13**, 3423.
38. B. B. Aggarwal, S. C. Gupta and B. Sung, *Br. J. Pharmacol.*, 2013, **169**, 1672.
39. C. Buhrmann, P. Shayan, B. B. Aggarwal and M. Shakibaei, *Arthritis Res. Ther.*, 2013, **15**, R202.
40. Y. Abe, S. Hashimoto and T. Horie, *Pharmacol. Res.*, 1999, **39**, 41.
41. C. Buhrmann, A. Mobasheri, F. Busch, C. Aldinger, R. Stahlmann, A. Montaseri and M. Shakibaei, *J. Biol. Chem.*, 2011, **286**, 28556.
42. V. P. Menon and A. R. Sudheer, *Adv. Exp. Med. Biol.*, 2007, **595**, 105.
43. S. Lev-Ari, Y. Maimon, L. Strier, D. Kazanov and N. Arber, *J. Soc. Integr. Oncol.*, 2006, **4**, 21.
44. A. Anthwal, B. K. Thakur, M. S. Rawat, D. S. Rawat, A. K. Tyagi and B. B. Aggarwal, *BioMed Res. Int.*, 2014, **2014**, 524161.
45. S. C. Gupta, A. K. Tyagi, P. Deshmukh-Taskar, M. Hinojosa, S. Prasad and B. B. Aggarwal, *Arch. Biochem. Biophys.*, 2014, **559**, 91.
46. Y. He, Y. Yue, X. Zheng, K. Zhang, S. Chen and Z. Du, *Molecules*, 2015, **20**, 9183.
47. A. C. Bharti, N. Donato and B. B. Aggarwal, *J. Immunol.*, 2003, **171**, 3863.
48. R. Z. Tan, J. Liu, Y. Y. Zhang, H. L. Wang, J. C. Li, Y. H. Liu, X. Zhong, Y. W. Zhang, Y. Yan, H. Y. Lan and L. Wang, *Phytomedicine*, 2019, **52**, 284.
49. V. N. Thakare, M. M. Osama and S. R. Naik, *Int. Immunopharmacol.*, 2013, **17**, 18.
50. N. Zhang, H. Li, J. Jia and M. He, *Cell. Immunol.*, 2015, **298**, 88.
51. S. Ouyang, Y. H. Yao, Z. M. Zhang, J. S. Liu and H. Xiang, *Eur. Rev. Med. Pharmacol. Sci.*, 2019, **23**, 1816.
52. A. G. Manzoni, D. F. Passos, J. L. G. da Silva, V. M. Bernardes, J. M. Bremm, M. H. Jantsch, J. S. de Oliveira, T. R. Mann, C. M. de Andrade and D. B. R. Leal, *Blood Cells, Mol., Dis.*, 2019, **76**, 13.
53. H. Y. Bao, R. H. Chen, S. M. Huang, X. Q. Pan and L. Fei, *Zhonghua Erke Zazhi*, 2003, **41**, 822.

54. M. Acar, N. B. Muluk, S. Yigitaslan, B. P. Cengiz, P. Shojaolsadati, H. Karimkhani, S. Ada, M. Berkoz and C. Cingi, *J. Laryngol. Otol.*, 2016, **130**, 1103.
55. A. M. Santos, T. Lopes, M. Oleastro, I. V. Gato, P. Floch, L. Benejat, P. Chaves, T. Pereira, E. Seixas, J. Machado and A. S. Guerreiro, *Nutrients*, 2015, **7**, 306.
56. N. Altıntoprak, M. Kar, M. Acar, M. Berkoz, N. B. Muluk and C. Cingi, *Eur. Arch. Otorhinolaryngol.*, 2016, **273**, 3765.
57. F. Di Pierro, G. Rapacioli, E. A. Di Maio, G. Appendino, F. Franceschi and S. Togni, *J. Pain Res.*, 2013, **6**, 201.
58. S. Muglikar, K. C. Patil, S. Shivswami and R. Hegde, *Oral Health Prev. Dent.*, 2013, **11**, 81.
59. B. Chandran and A. Goel, *Phytother. Res.*, 2012, **26**, 1719.
60. P. R. Holt, S. Katz and R. Kirshoff, *Dig. Dis. Sci.*, 2005, **50**, 2191.
61. Y. T. Jian, J. D. Wang, G. F. Mai, Y. L. Zhang and Z. S. Lai, *Diyi Junyi Daxue Xuebao*, 2004, **24**, 1353.
62. K. Nones, Y. E. Dommels, S. Martell, C. Butts, W. C. McNabb, Z. A. Park, S. Zhu, D. Hedderley, M. P. Barnett and N. C. Roy, *Br. J. Nutr.*, 2009, **101**, 169.
63. V. R. Yadav, S. Suresh, K. Devi and S. Yadav, *J. Pharm. Pharmacol.*, 2009, **61**, 311.
64. J. Epstein, G. Docena, T. T. MacDonald and I. R. Sanderson, *Br. J. Nutr.*, 2010, **103**, 824.
65. P. Khajehdehi, B. Zanjaninejad, E. Aflaki, M. Nazarinia, F. Azad, L. Malekmakan and G. R. Dehghanzadeh, *J. Renal Nutr.*, 2012, **22**, 50.
66. N. Chainani-Wu, S. Silverman Jr, A. Reingold, A. Bostrom, C. Mc Culloch, F. Lozada-Nur and J. Weintraub, *Phytomedicine*, 2007, **14**, 437.
67. N. Chainani-Wu, E. Madden, F. Lozada-Nur and S. Silverman Jr, *J. Am. Acad. Dermatol.*, 2012, **66**, 752.
68. M. Francis and S. Williams, *Nurs. J. India*, 2014, **105**, 258.
69. S. Elad, I. Meidan, G. Sellam, S. Simaan, I. Zeevi, E. Waldman, M. Weintraub and S. Revel-Vilk, *Altern. Ther. Health Med.*, 2013, **19**, 21.
70. G. Saran, D. Umapathy, N. Misra, S. G. Channaiah, P. Singh, S. Srivastava and S. Shivakumar, *Indian J. Dent. Res.*, 2018, **29**, 303.
71. S. A. Al-Maweri, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.*, 2019, **127**, 300.
72. P. Piyush, A. Mahajan, K. Singh, S. Ghosh and S. Gupta, *Oral Dis.*, 2019, **25**, 73.
73. S. Gupta, S. Ghosh, S. Gupta and P. Sakhuja, *J. Invest. Clin. Dent.*, 2017, **8**, e12252.
74. M. Yadav, K. Aravinda, V. S. Saxena, K. Srinivas, P. Ratnakar, J. Gupta, A. S. Sachdev and P. Shivhare, *J. Oral Biol. Craniofacial Res.*, 2014, **4**, 169.
75. S. S. Zhang, Z. J. Gong, W. H. Li, X. Wang and T. Y. Ling, *Asian Pac. J. Cancer Prev.*, 2012, **13**, 289.
76. A. Haroyan, V. Mukuchyan, N. Mkrtchyan, N. Minasyan, S. Gasparyan, A. Sargsyan, M. Narimanyan and A. Hovhannisyan, *BMC Complementary Altern. Med.*, 2018, **18**, 7.

77. Y. Panahi, G. H. Alishiri, S. Parvin and A. Sahebkar, *J. Diet. Suppl.*, 2016, **13**, 209.
78. Z. Fan, J. Li, J. Liu, H. Jiao and B. Liu, *ACS Appl. Mater. Interfaces*, 2018, **10**, 23595.
79. M. Fu, L. Chen, L. Zhang, X. Yu and Q. Yang, *Int. J. Mol. Med.*, 2017, **39**, 1164.
80. W. Shang, L. J. Zhao, X. L. Dong, Z. M. Zhao, J. Li, B. B. Zhang and H. Cai, *Mol. Med. Rep.*, 2016, **14**, 3620.
81. J. K. Ahn, S. Kim, J. Hwang, J. Kim, Y. S. Lee, E. M. Koh, K. H. Kim and H. S. Cha, *PLoS One*, 2015, **10**, e0145539.
82. S. Durgaprasad, C. G. Pai, Vasanthkumar, J. F. Alvres and S. Namitha, *Indian J. Med. Res.*, 2005, **122**, 315.
83. A. Abidi, S. Gupta, M. Agarwal, H. L. Bhalla and M. Saluja, *J. Clin. Diagn. Res.*, 2014, **8**, HC19.
84. www.clinicaltrials.gov, as accessed on 10th July 2020.
85. T. Cai, S. Mazzoli, A. Bechi, P. Addonisio, N. Mondaini, R. C. Pagliai and R. Bartoletti, *Int. J. Antimicrob. Agents*, 2009, **33**, 549.
86. C. Koosirirat, S. Linpisarn, D. Changsom, K. Chawansuntati and J. Wipasa, *Int. Immunopharmacol.*, 2010, **10**, 815.
87. F. Di Mario, L. G. Cavallaro, A. Nouvenne, N. Stefani, G. M. Cavestro, V. Iori, M. Maino, G. Comparato, L. Faniglіulo, E. Morana, A. Pilotto, L. Martelli, M. Martelli, G. Leandro and A. Franzè, *Helicobacter*, 2007, **12**, 238.
88. F. Samadian, N. Dalili, F. Poor-Reza Gholi, M. Fattah, N. Malih, M. Nafar, A. Firoozan, P. Ahmadpoor, S. Samavat and S. Ziaie, *Clin. Nutr. ESPEN*, 2017, **22**, 19.
89. B. D. Shelmadine, R. G. Bowden, J. J. Moreillon, M. B. Cooke, P. Yang, E. Deike, J. O. Griggs and R. L. Wilson, *J. Altern. Complementary Med.*, 2017, **23**, 632.
90. J. J. Moreillon, R. G. Bowden, E. Deike, J. Griggs, R. Wilson, B. Shelmadine, M. Cooke and A. Beaujean, *J. Complementary Integr. Med.*, 2013, **10**, 143.
91. H. Ide, S. Tokiwa, K. Sakamaki, K. Nishio, S. Isotani, S. Muto, T. Hama, H. Masuda and S. Horie, *Prostate*, 2010, **70**, 1127.
92. L. T. Arunachalam, U. Sudhakar, J. Vasanth, S. Khumukchum and V. V. Selvam, *J. Indian Soc. Periodontol.*, 2017, **21**, 478.
93. A. Chatterjee, K. Debnath and N. K. H. Rao, *J. Indian Soc. Periodontol.*, 2017, **21**, 132.
94. A. Lang, N. Salomon, J. C. Wu, U. Kopylov, A. Lahat, O. Har-Noy, J. Y. Ching, P. K. Cheong, B. Avidan, D. Gamus, I. Kaimakliotis, R. Eliakim, S. C. Ng and S. Ben-Horin, *Clin. Gastroenterol. Hepatol.*, 2015, **13**, 1444 e1.
95. M. Masoodi, M. A. Mahdiabadi, M. Mokhtare, S. Agah, A. H. F. Kashani, A. M. Rezadoost, M. Sabzikarian, A. Talebi and A. Sahebkar, *J. Cell. Biochem.*, 2018, **119**, 9552.
96. H. Hanai, T. Iida, K. Takeuchi, F. Watanabe, Y. Maruyama, A. Andoh, T. Tsujikawa, Y. Fujiyama, K. Mitsuyama, M. Sata, M. Yamada, Y. Iwaoka,

K. Kanke, H. Hiraishi, K. Hirayama, H. Arai, S. Yoshii, M. Uchijima, T. Nagata and Y. Koide, *Clin. Gastroenterol. Hepatol.*, 2006, **4**, 1502.
97. V. Singla, V. Pratap Mouli, S. K. Garg, T. Rai, B. N. Choudhury, P. Verma, R. Deb, V. Tiwari, S. Rohatgi, R. Dhingra, S. Kedia, P. K. Sharma, G. Makharia and V. Ahuja, *J. Crohn's Colitis*, 2014, **8**, 208.
98. C. Lahiff and A. C. Moss, *Inflammatory Bowel Dis.*, 2011, **17**, E66.
99. S. J. Kia, S. Shirazian, A. Mansourian, L. Khodadadi Fard and S. Ashnagar, *J. Dent.*, 2015, **12**, 789.
100. A. E. Thomas, B. Varma, S. Kurup, R. Jose, M. L. Chandy, S. P. Kumar, M. S. Aravind and A. A. Ramadas, *J. Clin. Diagn. Res.*, 2017, **11**, ZC89.
101. T. Nosratzehi, F. Arbabi-Kalati, H. Hamishehkar and S. Bagheri, *J. Natl. Med. Assoc.*, 2018, **110**, 92.
102. E. X. D. Santos Filho, D. A. C. Arantes, A. F. Oton Leite, A. C. Batista, E. F. Mendonça, R. N. Marreto, L. N. Naves, E. M. Lima and M. C. Valadares, *Chem.-Biol. Interact.*, 2018, **291**, 228.
103. K. Patil, M. V. Guledgud, P. K. Kulkarni, D. Keshari and S. Tayal, *J. Clin. Diagn. Res.*, 2015, **9**, ZC59.
104. Z. Delavarian, A. Pakfetrat, A. Ghazi, M. R. Jaafari, F. Homaei Shandiz, Z. Dalirsani, A. H. Mohammadpour and H. R. Rahimi, *Spec. Care Dent.*, 2019, **39**, 166.
105. R. K. C. Kopuri, C. Chakravarthy, S. Sunder, R. S. Patil, W. Shivaraj and G. Arakeri, *J. Int. Oral Health*, 2016, **8**, 687.
106. V. K. Hazarey, A. R. Sakrikar and S. M. Ganvir, *J. Oral Maxillofac. Pathol.*, 2015, **19**, 145.
107. A. D. Deepa, A. Balan and K. Sreelatha, *J. Indian Acad. Oral Med. Radiol.*, 2010, **22**, 88.
108. S. Sterzi, L. Giordani, M. Morrone, E. Lena, G. Magrone, C. Scarpini, S. Milighetti, L. Pellicciari, M. Bravi, I. Panni, C. Ljoka, F. Bressi and C. Foti, *Eur. J. Phys. Rehabil. Med.*, 2016, **52**, 321.
109. Y. Nakagawa, S. Mukai, S. Yamada, M. Matsuoka, E. Tarumi, T. Hashimoto, C. Tamura, A. Imaizumi, J. Nishihira and T. Nakamura, *J. Orthop. Sci.*, 2014, **19**, 933.
110. A. R. Rahimnia, Y. Panahi, G. Alishiri, M. Sharafi and A. Sahebkar, *Drug Res.*, 2015, **65**, 521.
111. Y. Henrotin, M. Gharbi, Y. Dierckxsens, F. Priem, M. Marty, L. Seidel, A. Albert, E. Heuse, V. Bonnet and C. Castermans, *BMC Complementary Altern. Med.*, 2014, **14**, 159.
112. Y. Panahi, A. R. Rahimnia, M. Sharafi, G. Alishiri, A. Saburi and A. Sahebkar, *Phytother. Res.*, 2014, **28**, 1625.
113. P. Pinsornsak and S. Niempoog, *J. Med. Assoc. Thailand*, 2012, **95**, S51.
114. G. Belcaro, M. R. Cesarone, M. Dugall, L. Pellegrini, A. Ledda, M. G. Grossi, S. Togni and G. Appendino, *Altern. Med. Rev.*, 2010, **15**, 337.
115. A. Khonche, O. Biglarian, Y. Panahi, G. Valizadegan, S. S. Soflaei, M. E. Ghamarchehreh, M. Majeed and A. Sahebkar, *Drug Res.*, 2016, **66**, 444.
116. C. Prucksunand, B. Indrasukhsri, M. Leethochawalit and K. Hungspreugs, *Southeast Asian J. Trop. Med. Public Health*, 2001, **32**, 208.

117. V. Anitha, P. Rajesh, M. Shanmugam, B. M. Priya, S. Prabhu and V. Shivakumar, *Indian J. Dent. Res.*, 2015, **26**, 53.
118. K. V. Raghava, K. P. Sistla, S. J. Narayan, U. Yadalam, A. Bose and K. Mitra, *J. Contemp. Dent. Pract.*, 2019, **20**, 42.
119. H. Kaur, V. Grover, R. Malhotra and M. Gupta, *Infect. Disord.: Drug Targets*, 2019, **19**, 171.
120. C. A. Ivanaga, D. M. J. Miessi, M. A. A. Nuernberg, M. M. Claudio, V. G. Garcia and L. H. Theodoro, *Photodiagn. Photodyn. Ther.*, 2019, **27**, 388.
121. D. Anusha, P. E. Chaly, M. Junaid, J. E. Nijesh, K. Shivashankar and S. Sivasamy, *Indian J. Dent. Res.*, 2019, **30**, 506.
122. M. Javadi, H. Khadem Haghighian, S. Goodarzy, M. Abbasi and M. Nassiri-Asl, *Int. J. Rheum. Dis.*, 2019, **22**, 1857.
123. B. Lal, A. K. Kapoor, O. P. Asthana, P. K. Agrawal, R. Prasad, P. Kumar and R. C. Srimal, *Phytother. Res.*, 1999, **13**, 318.
124. P. Allegri, A. Mastromarino and P. Neri, *Clin. Ophthalmol.*, 2010, **4**, 1201.
125. G. Manarin, D. Anderson, J. Silva, J. Coppede, P. Roxo-Junior, A. Pereira and F. Carmona, *J. Ethnopharmacol.*, 2019, **238**, 111882.
126. B. O. Murillo Ortiz, A. R. Fuentes Preciado, J. Ramírez Emiliano, S. Martínez Garza, E. Ramos Rodríguez and L. A. de Alba Macías, *Clin. Interv. Aging*, 2019, **14**, 2055.
127. M. Hami, A. Bigdeli, R. Khameneh Bagheri, O. Rajabi, M. Salehi and F. Zahedi Avval, *Iran. J. Kidney Dis.*, 2019, **13**, 304.
128. X. R. Wu, X. L. Liu, S. Katz and B. Shen, *Inflammatory Bowel Dis.*, 2015, **21**, 703.

CHAPTER 8

Biological Activities of Curcuminoids

RITU MISHRA[a,b] AND ANIL K. GUPTA*[a,b]

[a]Division of Genetics and Plant Breeding, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow–226015, UP, India; [b]Academy of Scientific and Innovative Research (AcSIR), CSIR-Human Resource Development Centre Campus, Sector-19, Kamla Nehru Nagar, Gaziabad–201002, UP, India
*E-mail: ak.gupta@cimap.res.in

8.1 Introduction

Turmeric (*Curcuma longa* L.), a perennial plant in the zingiberaceae family, comprises more than 80 species. It is widely distributed throughout the tropical and subtropical regions that may be either in the wild or in cultivated forms. Because of its medicinal and aromatic importance turmeric is also known as the 'spice of life'. In India, turmeric cultivation is done in 150 000 hectares, which accounts for about 80 percent of world turmeric production and 60 percent of world exports.[1] It was also estimated that consumption of turmeric powder by Indians (culinary) is approximately 80 to 200 mg per day.[2] It was calculated that about 90% of turmeric that arrives in Andhra Pradesh is exported to other states as turmeric rhizomes and the other 10% is used for local consumption.[3] Turmeric is an erect, perennial plant up to 1 metre long. Generally, harvesting is done from the beginning of February to the end of April. The leaves are oblong, large and narrowed to the base. They are light

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

green or dark green, have a length around 35–50 cm and width up to 13 cm.[4] The inflorescence is cone shaped, 10–15 cm long and generally attached to stem. The flower has 5–6 cm long two pale green bracts and whitish covering bracts. In *Curcuma longa* L., $2n = 63$ numbers of chromosomes have been reported.[5] Curcumin, demethoxycurcumin and bis-demethoxycurcumin are the major curcuminoids present in turmeric.[6–8] Curcuminoids show virtually no side effects, and thus are a potential source of new drugs for a number of diseases.[9,10] The different varieties of Indian turmeric having curcuminoids composition were found to be in the range of curcumin 51–64%, demethoxycurcumin 20–28% and bis-demethoxycurcumin 19–29%.[11] Curcumin, the bioactive pigment, is the major component of turmeric having an orange-yellow crystalline structure; it is used as a food colorant and is practically insoluble in water.[12–14] In 1913, Lampe and Milobedeska described the structure of curcumin ($C_{21}H_{20}O_6$).[15] Since curcuminoids show a wide spectrum of biological activities, turmeric has gained importance all over the world. Curcuminoids have anti-inflammatory,[16] antidiabetic,[15,17] hypocholesterolemic, choleratic, anti-oxidant,[18] antibacterial,[19] antiparasitic, antispasmodic,[20] antimicrobial,[21] antifungal,[22] anti-allergic,[23] antirheumatic,[24] antifibrotic, antiviral, anticancerous, antihepatotoxic properties as well as being used for anorexia, coughs and as an insect repellent.[25–28] The essential oils of *C. longa* have shown anti-inflammatory,[29] antibacterial[30] and antifungal activities.[31] Recently, an investigation reported that the largest number of diabetic patients found in the world are Indian, which is about 40.9 million in 2007 and will probably be 69.9 million by the year 2025.[32] Curcumin shows hypolipidimic and antidepressive activity.[33] Curcumin is a highly safe, non-toxic and easily available natural compound inhibiting pro-inflammatory markers.[34] Curcumin, a keto–enol tautomeric compound also acts as chelator of major ions. According to the keto or enol forms of curcumin, different activities have been directly associated with it.[35] Although curcumin has insoluble properties in water, it shows solubility in alkaline or acidic solvents.[36] The aim of this chapter is to provide exhaustive information regarding the biological activities of curcuminoids. Chemical constituents of turmeric have achieved wide recognition among researchers to cure various diseases such as cancer, cardiovascular diseases, neurological disorders and so on, with minimal side effects.

8.2 Composition of Turmeric

8.2.1 Chemical Composition of Turmeric and Their Natural Analogues

The chemical compounds and essential oil obtained from the rhizomes, flowers and leaves of *Curcuma longa* are very important because of their potential as a source of novel drugs for various diseases.[37–39] The plants of turmeric, flowers, rhizomes, oil and powder have been shown as a diagrammatic representation in Figure 8.1. In turmeric 69.3% of carbohydrates, 14.4% moisture,

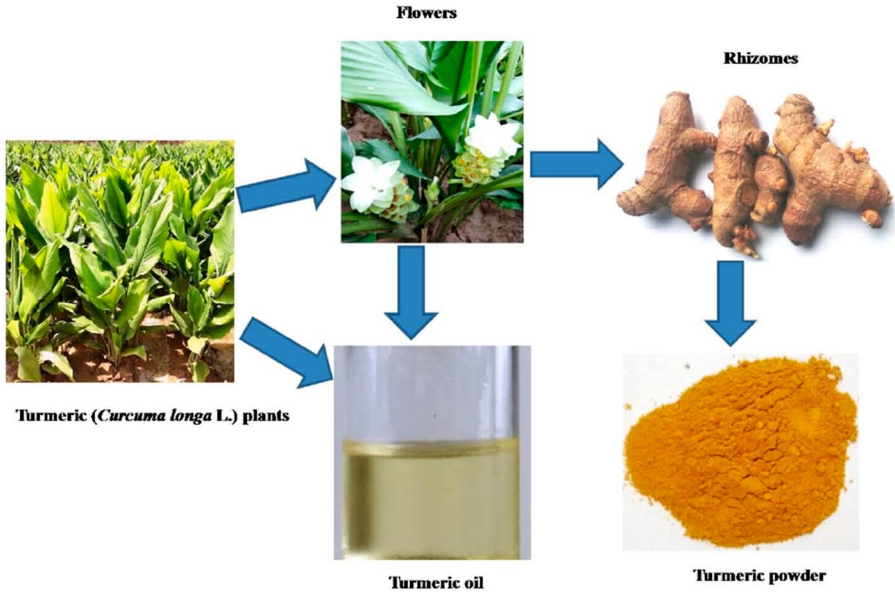


Figure 8.1 The plants of turmeric, flowers, rhizomes, oil and powder as a diagrammatic representation.

6.4% protein, 5.4% fat and 4.5% of minerals were found. The essential oil present in turmeric rhizomes has α-phellandrene (1%), zingiberene (24%), sesquiterpines (54%), cineol (1%), sabinene (0.6%) and borneol (0.5%). In 1815, curcumin was first isolated and named 1, 6-heptadiene-3, 5-dione-1, 7-bis (4-hydroxy-3-methoxyphenyl)-(1*E*, 6*E*) or diferuloylmethane.[40] The isolation of curcumin from turmeric was a very difficult procedure because of its insoluble nature so a technique for curcumin isolation has been developed.[41] In this process the powdered form of turmeric was kept in dichloromethane, magnetically stirred and reflux heated for one hour. After that the filtrate was kept in a hot water bath at 50 °C. A reddish-yellow coloured oily residue was obtained, which was titrated with hexane and the solid residue collected through suction filtration was analysed by TLC in the ratio of 97 : 3 (97% dichloromethane and 3% methanol) proving the presence of all three major curcuminoids. An experiment on curcumin extraction from turmeric powder was done using a solvent mixture of ethanol and acetone.[42] Since turmeric has specific flavour and colour, it maintains the nutritional value and freshness of food items.[43] $C_{21}H_{20}O_6$ is the molecular formula of curcumin keeping a molecular weight of 368.37 g mole^{-1} and a melting point of 183 °C. The three major compounds of turmeric *viz.* curcumin, demethoxycurcumin and bisdemethoxycurcumin, differ in methoxy substitution on the aromatic ring.[44] Cyclocurcumin, another curcuminoid of turmeric that is lesser known compared to curcumin, demethoxycurcumin and bisdemethoxycurcumin, was first isolated and characterized in 1993.[45] The structure of cyclocurcumin

differs from curcumin by the β-diketone link, where a replacement was done by an α, β-unsaturated dihydropyranone moiety. In 1998, a number of reports regarding cyclocurcumin were published, which stated that cyclocurcumin was not effective during the inhibition of MCF-7 tumour cell proliferation and also during the progression of cell cycle arrest.[46] The chemical profile of curcuminoids of turmeric has been shown in Table 8.1. According to the European Colour Directive, various kinds of food products required in the food industry need a valid and reliable technique for curcuminoids' determination.[47] In some areas, the bitterness of curcumin limits its use, but it can be removed by addition of glycine such as α-glycine, β-glycine, γ-glycine *etc.* In 1996, the removal of bitterness from curcumin was studied.[48] The dried pulverized turmeric was mixed with cereal husk and sugars, then this mixture was heat dried and inoculated with lactic acid bacteria, which resulted in curcumin with reduced bitterness. The potential of chemical constituents involved in various biological activities with multiple mechanisms is given in Table 8.2.

8.2.2 Essential Oil Composition in Turmeric

A number of aromatic volatiles/essential oils are found in the leaves and rhizomes of turmeric, which have a wide range of pharmacological properties due to which they are extensively used in pharmaceutical, flavouring and perfumery industries. These essential oils obtained from turmeric plants have been studied and reported by a number of researchers and they found that they contain α-phellandrene, terpinolone, β-sesquiphellandrene, *ar*-curcumene, 1, 8-cineole, turmerone, turmerol and zingiberene as the major constituents.[68] Ar-turmerone, turmerone, curdione, *ar*-curcumene, turmeronol A, turmeronol B, 1, 8-cineole and β-pinene (Figure 8.2) are found in both leaves and rhizomes of this herb. These essential oils show antimicrobial,

Table 8.1 Chemical profile of curcuminoids of turmeric (*Curcuma longa* L.).

Compounds	R_1	R_2	Chemical formula	MW	IUPAC name
Curcumin-I (curcumin)	OCH_3	OCH_3	$C_{21}H_{20}O_6$	368	1,7-Bis (4-hydroxy-3-methoxyphenyl) 1,6-hepatadiene-3, 5-dione
Curcumin-II (demethoxy-curcumin)	H	OCH_3	$C_{20}H_{18}O_5$	338	1-(4-Hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) hepta-1,6-diene-3,5-dione
Curcumin-III (bisde methoxy curcumin)	H	H	$C_{19}H_{16}O_4$	308	1,7-Bis(4-hydroxyphenyl)-1,6-heptadiene3,5-dione

Table 8.2 The various biological activities from chemical constituents of *Curcuma longa* L. involving multiple mechanisms.

Biological activity	Model used and study design	Dose/frequency	Effects of treatment	Reference
Neuroprotective activity	Sprague-Dawley male rats	CUR – (300 mg kg^{-1})	Curcuma oil prevented post-ischaemic brain neutrophil infiltration and NO metabolites and reduced the production of ROS.	49
Oxidative stress	Wistar male rats	L-thyroxine (0.0012%) þ 200 mg vitamin E þ 30 mg curcumin	CUR was efficient in protecting testis from oxidative stress generated by T4 mainly by restoring antioxidant enzymes	50
Alzheimer's disease	Sprague-Dawley male rats	Curcuminoids and its individual components (CUR, DMC and BDMC) – (3–10 mg kg^{-1})	Curcuminoids and its individual components showed dependent inhibition in frontal cortex and hippocampus with *ex vivo* AChE assay.	51
Hepatoprotective activity	Adult Swiss albino mice	CUR (5 M)	The release of lactate dehydrogenase was significantly reduced along with lipid peroxidation.	52
Anti-arthritic activity	Male Sprague-Dawley rats	CUR (30 μg kg^{-1})	CUR exhibited superior anti-arthritic effect through augmenting SOM secretion from the endocrine cells in small intestines	53
Anti-angiogenic and antiproliferative activities	Human colon cancer cells and endothelial cells	CUR and Arturmerone (0.4–7.0 μg mL^{-1})	Turmeric extract provided better anti-angiogenic and antiproliferative activities than the CUR alone.	54
Antiviral activity	Madin–Darby canine kidney (MDCK) cells	CUR (30 μM)	CUR interrupts virus–cell attachment, which led to inhibition of influenza virus propagation.	55
Antifungal activity	Pathogens of *Candida albicans*	CUR and DMC (20–200 μg mL^{-1})	The antifungal effect of CUR was stronger than that of DMC due the existence of methoxy group	56
Anticancer activity	MDA-MB-231 cells	CUR, DMC, and BDMC (20 μM)	Curcuminoids-PDT significantly inhibited cell viability in breast cancer cell lines. DMC-PDT has the highest antiproliferative effect.	57

Antioxidant activity	Wistar strain rats	CUR (200 mg kg^{-1})	Anti-inflammatory, hepatoprotective and cardioprotective effects of CUR correlated with its antioxidant activity.	58
Antioxidant activity	Spraguee-Dawley rats: 6 males and 6 females	Curcuminoids (150 mg kg^{-1})	Significant reduction in the amount of urinary biomarkers of oxidative stress such as allantoin, m-tyrosine, 8-hydroxy-2′-deoxyguanosine and 3-nitrotyrosine.	8
Neuroprotective activity	New born Spraguee-Dawley rats	Curcuminoids (1–10 μM)	CUR, DMC and BDMC are significantly suppressed nitric oxide production by LPS-activated microglia and the relative potency was DMC > BDMC > CUR.	59
Oxidative stress	Swiss albino inbred mice	CUR (50, 100 and 200 mg kg^{-1})	CUR administration significantly reduced the progression of kindling and attenuated the oxidative stress in mice. Control both development of seizure and oxidative stress during epilepsy.	60
Mitochondrial dysfunction in the brain	Male mice-SAMP8 and SAMR1	CUR (500 mg kg^{-1})	Significant decreases were observed in mitochondrial function and energy production in brain cells from SAMP8	61
Neuroprotective and antioxidant activity	Ketamine-induced model of mania in female adult Wistar rats	CUR (20 and 50 mg kg^{-1})	Pretreatment of rats with CUR prevented behavioural and pro-oxidant effects induced by ketamine.	62
Premenstrual syndrome (PM)	70 women with PM	CUR (100 mg kg^{-1})	CUR reduced severity of PMS symptoms can be through increasing serum BDNF levels.	63
Radioprotective effect	Female Sprague Dawley rats	CUR (50 mg kg^{-1})	Less cell necrosis. Lycopene and CUR reduced the structural damage to the salivary glands.	64
Sexually transmitted infections	Adult male Wistar rats	Turmeric (4% w/w)	Improvement of antioxidant status in the epididymides and testes of L-NAME induced hypertensive rats. Restoration of systolic blood pressure, sperm motility, testosterone level.	65
Antiviral activity	Madin–Darby canine kidney (MDCK) cells	CUR (30 μM)	CUR interrupts virus-cell attachment, which led to inhibition of influenza virus propagation.	66
Anti-acidogenic activity	Streptococcus mutans	Turmeric extracts (100 μL)	Separated fraction from turmeric and curcuminoids had inhibitory effects on the sucrose-dependant adherence of *S. mutans* to saliva-coated hydroxyapatite discs.	67

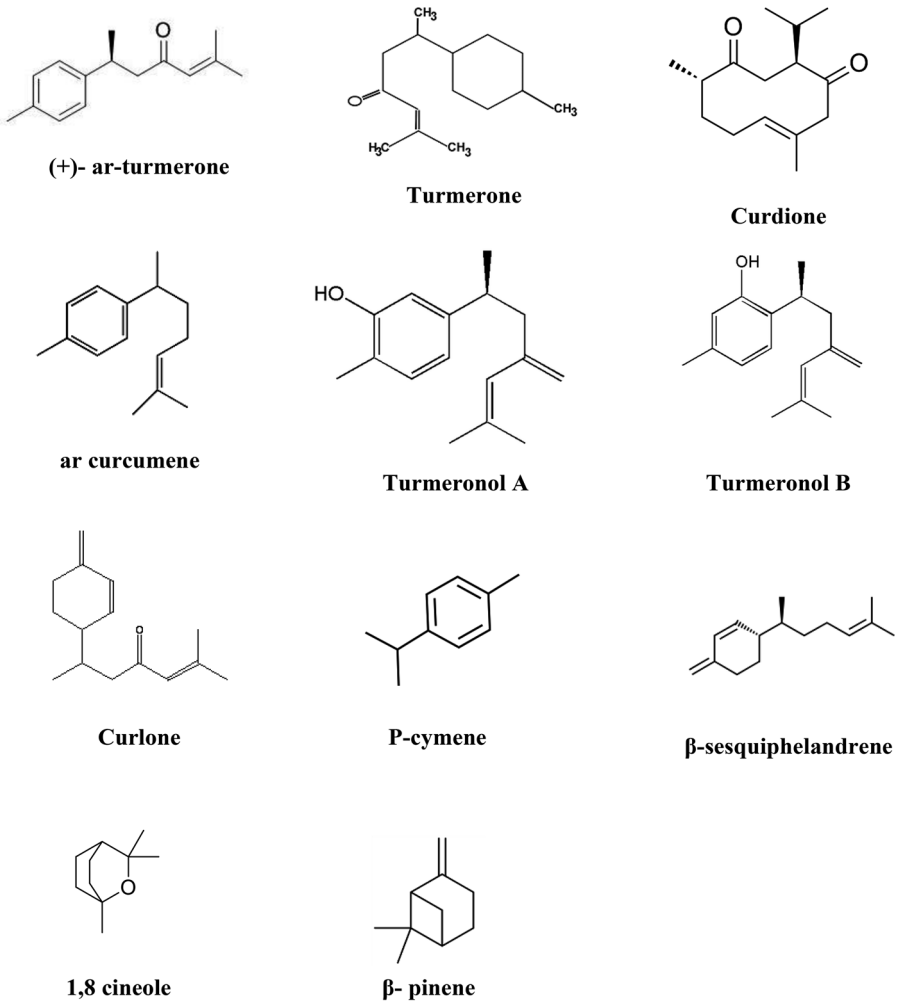


Figure 8.2 Major chemical compounds present in turmeric oils.

anti-inflammatory, cytotoxic and antioxidant properties.[69–72] The antimicrobial activity has always been calculated in terms of the diameter of the zone of inhibition. The essential oils obtained from different genotypes of *Curcuma* exhibit antibacterial activity against *E. coli* bacteria, *B. subtilis* and *S. aureus*. The chemical constituents of the essential oil were analysed through GC and GC-MS techniques.[73] The individual oil content (%) has been expressed as peak percent area by electronic integration.[74] In 1990, α-phellandrene and terpinolene were identified as the predominant constituents of leaf oil of *C. longa*.[75]

8.2.3 Curcuminoids

Curcumin, a crystalline, bright orange-yellow food colorant[14] and the essential oil that it contains is responsible for turmeric's aroma and taste (Figure 8.3).[76] It maintains the nutritional value and freshness of food items due to its specific flavour and colour. Because of its specific characteristics, it is treated as a 'supernatural' compound in the fields of pharmacology and molecular biology, and is also known as a 'model compound'.[77] Since curcumin has low bioavailability there is a need to enhance its bioavailability. Several researchers have worked on animals and reported the application of turmeric oil in animals which has been infected with various pathogens. A reduction of these lesions was observed when infected animals were treated with turmeric oil.[78] Curcuminoids show a number of biological activities such as anti-oxidant, anti-tumour, anti-inflammatory, anti-acidogenic, neuroprotective, radioprotective and anti-arthritic.[79] Various clinical trials suggested that curcuminoids have a potential therapeutic role in numerous chronic diseases such as colon cancer, lung cancer, breast cancer and inflammatory bowel diseases (Tables 8.3 and 8.4). Curcuminoids have the potential to cure immunity related metabolic diseases due to a vast number of biological targets with no side effects.[91] Curcumin acts as an antidiabetical agent by increasing the β glutathione level.[92] The name 'curcuminoids' refers both to the natural curcuminoids such as curcumin, demethoxycurcumin, bisdemethoxycurcumin and synthetic curcumin such as tetrahydrocurcumin (THC), bis-o-hydroxy-cinnamoylmethane, bis-demethoxycurcumin analogues and two new curcumin derivatives (2*E*,6*E*)-2,6-bis(2-(trifluoromethyl) benzylidene)cyclohexanone [C66] and (1*E*,4*E*)-1,5-bis(2-bromophenyl)penta-1,4-dien-3-one [B06].[93] These synthetic curcuminoids have better solubility and bioavailability as compared to natural curcuminoids. A number of preclinical studies on turmeric and its chemical constituents have been done.

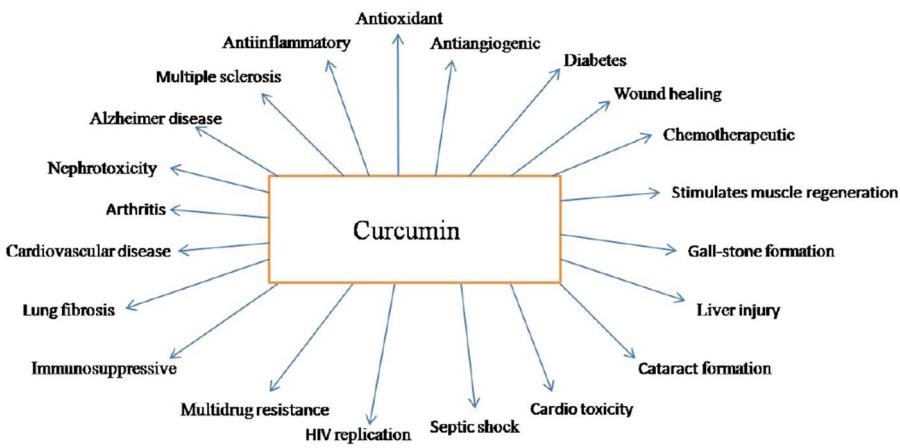


Figure 8.3 Curcumin as a most active biological constituent in *Curcuma longa* L.

Table 8.3 Animal studies investigating the anti-inflammatory effects of curcumin in cancer models.

Animal model	Route of curcumin administration	Dose	Reference
Murine (liver) iNOS production	Oral by gavage, intravenous	0.5 mL of 10 μM solution 0.5 μg g^{-1} body weight	80
Rat colonic aberrant crypt foci	Oral (diet), subcutaneous	50–2000 ppm 15 mg kg^{-1} body weight	81
Rat colon cancer	Oral (diet)	2000 ppm	82
Murine familial adenomatous polyposis	Oral (diet), intraperitoneal	0.1, 0.2, 0.5% diet 100 mg kg^{-1} body weight	83
Rat colonic aberrant crypt foci	Oral (diet)	0.6% diet	84
Rat colonic apoptosis	Oral	0.6% diet	85
Murine xenograft tumour	Intraperitoneal	200 μL of 0.2–1.0 μg mL^{-1} curcumin suspension	86
Murine liver, lung tumour initiation	Oral (diet)	0.01 or 0.0% diet	87
Rat colonic apoptosis	Oral	0.2 or 0.6% diet	88
Murine breast cancer with lung metastasis	Oral (diet)	2% diet	89
Murine T-cell leukaemia	Oral (gavage)	300 mg kg^{-1} body weight	90

Being antioxidant in nature, curcumin protects DNA against single strand breaks.[94] Curcumin has the capability to suppress the mutagenicity of several mutagens including benzopyrene, DMBA, cigarette smoke, *etc.*[95] Curcumin also inhibits NF-κB activation. It inhibits platelet aggregation by inhibition of thromboxanes' formation and enhancement of prostacylin.[96] Curcumin shows chemopreventive actions by inhibiting phase 1 cytochrome p450 enzymatic activity of pro-carcinogens and enhanced phase 2 detoxification activities.[97,98] Orally dosed turmeric and curcumin interferes with intestinal cholesterol uptake, increases the conversion of cholesterol into bile acids by increasing the activity of hepatic cholesterol-7-alpha-hydroxylase, and increases bile acid secretion.[94,99] Curcumin induces apoptosis by suppressing NF-κB and AP-1 in cancer cells and decreases the frequency and size of tumours.[100] Curcumin also possesses hepatoprotective and choleretic properties. The relative potency of curcumin and its analogues are shown in Table 8.5. In animal models, curcumin shows a potent choleretic nature by increasing bile output by almost 100%.[134,135]

8.2.4 Antioxidant Activity of Curcuminoids

In several *in vitro* and *in vivo* experiments, curcuminoids exhibited differential antioxidant activity. Curcuminoids inhibited lipid peroxidation (LPO) by inducing Fenton reagent and metals such as H_2O_2 and 2-amidinopropane.[101,110,111,136,137] The antioxidant activity of curcumin helps in the protection of haemoglobin from oxidation.[138,139] The curcumin also acts as an oil preservative. The

Table 8.4 Ongoing clinical trials investigation to explore the benefits of curcumin in inflammatory conditions (curcumin clinical trials, 2009).

Clinic trial identifier	Condition	Trial site	Intervention	Trial phase	Completion date
NCT00752154	Rheumatoid arthritis	University of California, Los Angeles	Curcumin, 4–12 g daily	Pilot study	September 2009
NCT00792818	Knee osteoarthritis	Mahidol University, National Research Council of Thailand	*Curcuma longa* extracts, Ibuprofen	Phase III	November 2009
NCT00793130	Ulcerative colitis	Tel-Aviv Sourasky Medical Center	Coltect-(curcumin 1 g daily, green tea, selenium)	Unknown	November 2009
NCT00779493	Irritable bowel syndrome	Kaiser Permanente	Curcumin, 900 mg twice daily	Phase IV	November 2009
NCT00528151	Leber's hereditary optical neuropathy	Mahidol University	Curcumin, 250 mg twice daily	Phase III	Unknown
NCT00595582	Mild cognitive impairment	Louisiana State University	Curcumin + Bioperine, 5.4 g daily	Unknown	January 2009
NCT00365209	Colon cancer prevention	Chao Family Comprehensive Cancer Center	Curcumin	Phase II	Unknown
NCT00118989	Colon cancer prevention	University of Pennsylvania	Curcuminoid complex, 4 g daily	Phase II	June 2009
NCT00641147	Familial adenomatous polyposis	Johns Hopkins University	Curcumin, 700 mg twice daily	Phase II	March 2013
NCT00745134	Rectal cancer	MD Anderson Cancer Center	Curcumin, 4 g daily, Capecitabine	Phase II	July 2010
NCT00486460	Pancreatic cancer	Tel-Aviv Sourasky Medical Center	Gemcitabine, Curcumin, Celebrex (doses unknown)	Phase III	Unknown
NCT00094445	Pancreatic cancer	MD Anderson Cancer Center	Curcumin, 8 g daily	Phase II	December 2009
NCT00113841	Multiple myeloma	MD Anderson Cancer Center	Curcumin + Bioperine, 2 g twice daily	Pilot Study	December 2008
NCT00689195	Osteosarcoma	Tata Memorial Hospital	Curcumin and Ashwagandha (doses unknown)	Phase I and II	May 2012
NCT00475683	Oral mucositis-Children on chemotherapy	Hadassah Medical Organization	Curcumin liquid extract, 10–30 drops 3 times daily	Phase III	December 2009

Table 8.5 Relative potency of curcumin and its analogues.

Curcumin	It is more active than Bisdemethoxycurcumin as an antioxidant and as an oxidative DNA cleaving agent.[101]
	It is more active than Bisdemethoxycurcumin as an inhibitor of peroxynitrite scavenger.[102]
	It is more active than demethoxycurcumin and Bisdemethoxycurcumin in inhibiting singlet oxygen-induced DNA damage.[103]
	It is more active than demethoxycurcumin and Bisdemethoxycurcumin in binding and inhibiting Pgp and sensitizing cells to vinblastin.[104]
	It helps in protecting rats from lead-induced neurotoxicity.[105]
	Curcumin is more active than THC in preventing PMA-induced skin tumour promotion.[106]
	Curcumin is more active than DMC, BDMC and THC in suppressing NF-κB activation.[107]
	Curcumin is more active than tetrahydrocurcumin, hexahydrocurcumin, octahydrocurcumin are less active than curcumin in suppressing NF-κB activation.[108]
Demethoxycurcumin	It is more active than curcumin and BDMC in inducing p38 MAPK mediated induction of heme oxygenase-1.[109]
	It helps in inhibiting H2O2-induced lipid peroxidation and haemolysis of erythrocytes.[110]
	It helps in inhibiting the liposomal peroxidation; and of COX1 and COX2 activity.[111]
	It is more potent than curcumin, BDMC and cyclocurcumin in inhibiting proliferation of breast cancer cells.[46]
	It is more potent than curcumin and BDMC in inducing nematocidal activity.[45]
Bisdemethoxycurcumin	It is more active than curcumin and demethoxycurcumin for cytotoxicity against ovarian cancer cells.[112]
	It was most active when compared with curcumin or DMC for antimutagenic and anticarcinogenic activity.[113]
	BDMC was more active than curcumin for reducing nicotine-induced oxidative stress.[114]
	It is more active than curcumin for modulation of MDR1 gene.[115]
	It was more active than curcumin or DMC in protecting nerve and endothelial cells from beta amyloid-induced oxidative stress.[116]
	It is more active than curcumin in preventing alcohol and PUFA-induced oxidative stress.[117]
	It prevents DMH induced colon carcinogenesis.[118]
	BDMC is more active than curcumin in preventing CCL4-induced hepatotoxicity in rats.[119]
	BDMC is more active than curcumin in preventing alcohol and PUFA-induced cholesterol, TGs, PLs and FFA.[120]
	BDMC is more active than DMC or curcumin in inducing NRF2-mediated induction of heme oxygenase-1.[121]

Tetrahydrocurcumin	THC is more active than curcumin in suppressing nitrilotriacetate-induced oxidative renal damage.[122]
	THC is more active than curcumin in protecting from chloroquine-induced hepatotoxicity in rats.[123]
	THC is more active than curcumin in preventing brain lipid peroxidation in diabetic rats.[124]
	THC is more potent than curcumin for antioxidant and antidiabetic effects in rats.[125]
	THC is more potent than curcumin for modulation of renal and hepatic functional markers in diabetic rats.[126]
	THC is more potent than curcumin for modulation of blood glucose, plasma insulin and erythrocyte TBARS in diabetic rats.[127]
	THC is more potent than curcumin in decreasing blood glucose and increasing plasma insulin in diabetic rats.[128]
	THC was more potent than curcumin in suppressing LDL oxidation.[129]
	THC is less potent than curcumin in inhibiting the activity of 5-LOX; but more potent than curcumin in inhibiting COX-dependent arachidonic acid metabolism.[130]
	THC is more active than curcumin in preventing DMH-induced ACF formation in mice.[131]
	Does not induce ROS production and membrane mobility coefficient but curcumin does.[132]
	THC is less active under aerated condition than curcumin but under N2O purged conditions, THC is more active than curcumin in suppressing radiation-induced lipid peroxidation.[133]

antioxidant activity of turmeric has been applied in cosmetics,[140] nutraceuticals and phytomedicines.[141] By the activation of macrophages *in vitro* curcumin significantly inhibits the generation of reactive oxygen species.[142,143] Curcumin exerts a powerful inhibitory effect against H_2O_2-induced damage in human keratinocytes and fibroblasts[144] and in NG 108–15 cells.[145] The antioxidant activity of curcumin prevents the rancidity of oils and fat.[146,147] Being a good antioxidant, it helps in the inhibition of lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates.[148,149] Curcumin prevents inflammation by suppressing LPO. It was reported that vitamin E shows ten times less antioxidant activity compared to curcumin.[150] Moreover, it was observed that curcumin with the phenolic group having the methoxy group at ortho position shows accelerated antioxidant activity.[151] Curcuminoids prevent cyclo-oxygenase (COX)-1 and (COX)-2 enzymes[136] and during linoleic acid oxidation they reduce AAPH-induced conjugated diene formation.[111] In most of these actions, curcumin was the most potent among all curcuminoids whereas bisdemethoxycurcumin was less active than the other curcuminoids. Enzymes such as superoxide

dismutase, catalase and glutathione peroxidase act as mediators for curcumin during antioxidant activity. These agents, when reacting with curcumin, reduce intracellular GSH in the cells.

8.3 Pharmacological Activities of Curcuminoids

During many *in vivo* experiments curcumin was found to be more effective than demethoxycurcumin and bisdemethoxycurcumin.[152] Curcuminoids could also act as pro-oxidants. Through the production of ROS A, pro-oxidant activities of curcuminoids were compared for the enhancement of Cu(II)-induced cleavage of plasmid pBR322 DNA.[101] For the induction of DNA cleavage, curcumin was found to be more effective compared to demethoxycurcumin and bisdemethoxycurcumin.

The curcuminoids exhibit cardioprotective, antidiabetic and nematocidal activity by inhibiting proliferation of BVSM cells.[153] Among them, curcumin was the most efficient cardioprotective agent. Curcuminoids have the ability to lower the level of blood glucose in type 2 diabetic KK-A$^{\gamma}$ mice.[154]

Curcuminoids act as inhibitors of lead acetate induced neurotoxicity by decreasing LPO and improving glutathione levels in rat brains.[105] Immunostimulatory effects were exhibited by bisdemethoxycurcumin, which helps in the enhancement of β-amyloid phagocytosis and regulation of transcription of β-1, 4-mannosyl-glycoprotein to correct the immune defects of Alzheimer's disease patients.[155]

A number of *in vitro* and *in vivo* experiments on anti-inflammatory and anti-tumour activities of curcuminoids have been studied. During the study of cancers such as leukaemia, lung cancer, pancreatic cancer and breast cancer, it was found that the curcuminoids were helpful in the inhibition of cell proliferation.[46,107,113] The main aim of the present study is to investigate how these curcuminoids show various kinds of biological activities such as antioxidant, anti-inflammatory and antiproliferative responses. During the study of p38 MAPK-mediated heme oxygenase-1 expression induction and their activity in human endothelial cells, it was found that curcumin and demethoxycurcumin were more effective than bisdemethoxycurcumin.[109]

It was reported that curcumin, demethoxycurcumin and bisdemethoxycurcumin exhibited potential for the suppression of tumour necrosis factor in the order of curcumin > demethoxycurcumin > bisdemethoxycurcumin.[107] Maintaining the same experimental conditions, it was found that used individually, these curcuminoids show lesser activity in comparison to a mixture of curcuminoids. It was found that curcumin and demethoxycurcumin showed almost similar potential for inducing glutathione levels, whereas bisdemethoxycurcumin showed less effectiveness during glutathione induction, which indicates that curcuminoids' activities for anti-inflammation and antiproliferation are independent of their redox-modulatory property.[107]

8.3.1 Anti-inflammatory

Inflammation is a process by which tissues show harmful stimuli against biological responses such as injury, exogenous and endogenous antigens. Inflammation is a short term response that has the capability to eliminate the infective stimulus and repair the injured tissue, which results in regeneration and maintaining homeostasis.[156] Generally, inflammation shows a positive defence mechanism but sometimes unregulated and prolonged inflammatory response may cause several disorders, such as diabetes, allergies, atherosclerosis, obesity, cancer, pain and chronic diseases.[157,158] During the inflammatory response many nonsteroidal anti-inflammatory drugs (NSAIDs), steroids and immunosuppressant drugs may be helpful[159] to react with adverse conditions like ulceration, perforation, gastric irritation,[160] angioedema, hepatic failure, headache, haemolytic anaemia, hyperglycaemia, osteoporosis and other immunodeficiency-related problems.[161,162] Among the three major curcuminoids, curcumin has made a major contribution for the suppression of NF-κB and activation of COX-2.[163,164] Many researchers have studied the other bioactive components of turmeric such as volatile oils and turmerones and reported immunomodulatory and anti-inflammatory activities.[54,165] An investigation was done for anti-inflammatory potential using xylene induced ear oedema and cotton pellet granuloma inflammation models.[166,167] During the study of these two models using turmeric essential oils, turmerones show an inhibitory effect against inflammation by releasing histamine, bradykinin, 5-hydroxytryptamine and prostaglandins as the mode of action by signifying the presence of bioactive components, capable of producing an anti-inflammatory effect.

8.3.2 Neuroprotective

Since curcumin has multiple biological functions, it finds various therapeutic applications in neurological disorders.[168] Analyses on the pharmacological effects against central nervous disorders are minimal, therefore more efforts are needed to find out how curcumin acts during the whole process.[169] A report proved that curcumin degradation products play a substantial role in exhibiting diverse biological activities.[170] Curcumin and its analogues have protective effects against αβ toxicity and have the capability to bind αβ peptides by promoting disaggregation of existing amyloid deposits.[116,171] When loss of dopaminergic neurons occurs in the nigrostriatal pathway, a neurodegenerative disorder occurs known as Parkinson's disease.[172] This disease causes autonomic dysfunction, protein aggregation and proteasome inhibition anxiety, depression, sleep disturbance and cognitive dysfunction.[173,174] In Parkinson's disease patients, due to degeneration of substantia nigra, the level of ROS generation increases.[175,176] During mental stress, hypochondriac pain and mania, curcumin treatment is very helpful because of its valuable therapeutic nature and fewer side effects.[177,178] A study was done on the brains of stressed rats and it was reported that curcumin produces a

widespread increase in brain-derived neurotrophic factors.[179] Pre-treatment of curcumin activates the downregulated expression of phosphorylated-TrκB induced by glutamate in the primary cultured cortical neurons and hence promotes neuronal survival.[180,181]

8.3.3 Antidiabetic

An Ayurvedic mixture of turmeric rhizome powder with amla juice and honey may be very useful in diabetes mellitus (madhumeha).[182] An experimental report explains that the ingestion of 6 g of turmeric increases insulin levels without affecting plasma glucose levels.[183] Curcuminoids lower lipid peroxidation.[184] A scientific study revealed that when freeze-dried rhizome powder of turmeric is dissolved in milk it may be used as an effective and safe antidiabetic dietary supplement.[185] Besides curcuminoids, a number of glycosides and flavonoids were obtained from turmeric using isopropanol and acetone extract. This may cause inhibitory action on human pancreatic amylase (HPA) by reducing starch hydrolysis and enhancing lowered glucose levels.[186] Curcumin suppresses the development of diabetic cataracts in rat models.[187–190] In Alzheimer's disease curcumin-mediated suppression of β-amyloid oligomers induces phosphorylation and degradation of insulin receptors in the cerebral cortices of diabetic rats.[191–194]

8.3.4 Anticancer

Curcumin acts as a chemopreventive agent against various cancers such as lung, breast and prostate cancer.[195] Curcumin inhibits bile acid induction of COX-2 gene expression by reversing bile acid suppression of gene expression of SOD-1.[196,197] Curcumin shows an antiproliferative effect through different biological pathways involved in mutagenesis, apoptosis, tumorigenesis and metastasis. Curcumin acts as an inhibitor of the transcription factor NF-κB and downstream gene products involved in tumour growth, angiogenesis and metastasis.[198] It exhibits anti-tumour activity by altering the unregulated cell cycle through cyclin-dependent, p53-dependent and p53-independent cancer cell pathways.[199] Curcumin acts as a natural phytochemical to communicate with these novel targets and show synergism with chemotherapy.[200]

8.3.5 Cardioprotective

Cholesterol damage can also be prevented by the antioxidants in turmeric to protect against atherosclerosis. The ability of antioxidants present in turmeric to decrease free radicals shows a similarity with vitamins C and E. Since the antioxidant activities of turmeric show a nondegradable nature against heat it becomes very beneficial for human health. A number of animal studies show that curcumin lowers cholesterol and triglycerides. Recently, a study of atherosclerosis reported an American diet fed to a number of mice,

having rich refined carbohydrates and saturated fat, but low in fibre. Among them, some mice also received the same diet mixed with turmeric. After four months of receiving these diets, the results showed that the mice that consumed the turmeric with their food had 20 percent less blockage of the arteries than the mice fed the diet without turmeric. Curcumin has beneficial effects on different forms of cardiovascular diseases like atherosclerosis, cardiac hypertrophy, hypertension and ischaemia/reperfusion.[201] Turmeric significantly decreased the biochemical markers of myocardial infarction, preserved myocardial antioxidant status and corrected the altered haemodynamic variables that were established by histopathological findings.[202] A number of investigations have been done on the cardioprotective effects of turmeric extracts against doxorubicin induced cardiotoxicity in rats. It was found that in turmeric pre-treated cardiotoxic rats, turmeric extract significantly decreases the serum CK-MB level.[203]

8.4 Conclusions

Turmeric has numerous active compounds, which possess various biological properties with anti-inflammatory, antioxidant, neuroprotective, antidiabetic, anticancer and cardioprotective activities. This chapter provides comprehensive information regarding various biological activities of curcuminoids. The chemical constituents of turmeric are of great interest among scientists and industrialists as a cure for various diseases due to the vast number of biological targets and few side effects.

Acknowledgements

The authors are very thankful to the Director, CSIR–CIMAP, Luckno, for providing the necessary facilities and infrastructure to carry out the research work. The first author (RM) is also thankful to AcSIR and CSIR, New Delhi, for providing PhD registration and DST, New Delhi, for providing a WOS-A fellowship.

References

1. B. Sasikumar, *Plant Genet. Resour.*, 2005, **3**, 230.
2. N. Deb, P. Majumdar and A. K. Ghosh, *J. Pharm. Sci. Technol.*, 2013, **2**, 81.
3. R. U. Kanthe and V. Y. Badave, Marketing of turmeric in the Sangli District, Maharashtra India, *Int. J. Econ. Manag. Sci.*, 2016, **5**, 374.
4. I. A. Ross, *Medicinal Plants of the World,* Humana Press, New Jersey, 2001, vol. 1, p. 139.
5. R. R. Nair and B. Sasikumar, *Curcuma longa* L, *Cytologia*, 2009, **74**, 153.
6. W. Tiyaboonchai, W. Tungpradit and P. Plianbangchang, *Int. J. Pharm.*, 2007, **337**, 299.
7. D. W. Zhang, M. Fu, S. H. Gao and J. L. Liu, *J. Evidence-Based Complementary Altern. Med.*, 2013, 636053.

8. S. Dall'Acqua, M. Stocchero, I. Boschiero, M. Schiavon, S. Golob, J. Uddin and E. Schievano, *Fitoterapia*, 2016, **109**, 125.
9. E. A. Corcolon and M. L. Dionisio-Sese, *J. Biol. Environ. Sci.*, 2014, **5**, 593.
10. K. Mahmood, K. M. Zia, M. Zuber, M. Salman and M. N. Anjum, *Int. J. Biol. Macromol.*, 2015, 877–890.
11. A. Siviero, E. Gallo, V. Maggini, L. Gori, A. Mugelli, F. Firenzuoli and A. Vannacci, *J. Ayurveda Holist. Med.*, 2015, **5**, 57.
12. V. P. Menon and A. R. Sudheer, *Adv. Exp. Med. Biol.*, 2007, **595**, 105.
13. H. Hatcher, R. Planalp, J. Cho, F. M. Torti and S. V. Torti, *Cell. Mol. Life Sci.*, 2008, **65**, 1631.
14. M. L. A. D. Lestari and G. Indrayanto, *Subst., Excipients, Relat. Methodol.*, 2014, **39**, 113.
15. B. B. Aggarwal, C. Sundaram, N. Malani and H. Ichikawa, *Adv. Exp. Med. Biol.*, 2007, **595**, 1.
16. S. C. Gupta, B. Sung, J. H. Kim, S. Prasad, S. Li and B. B. Aggarwal, *Mol. Nutr. Food Res.*, 2013, **57**, 1510.
17. H. P. Ammon, T. Mack, G. B. Singh and H. Safayhi, *Planta Med.*, 1991, **57**, 203.
18. Y. Panahi, M. S. Hosseini, N. Khalili, E. Naimi, M. Majeed and A. Sahebkar, *Clin. Nutr.*, 2015, **34**, 1101.
19. F. Di Mario, L. G. Cavallaro, A. Nouvenne, N. Stefani, G. M. Cavestro, V. Iori and A. Franzè, *Helicobacter*, 2007, **123**, 238.
20. A. Niranjan and D. Prakash, *J. Food Sci. Technol.*, 2008, **45**, 109.
21. M. A. Morshed, A. Uddin, R. Saifur, A. Barua and A. Haque, *Int. J. Biosci.*, 2011, **1**, 7.
22. I. Chattopadhyay, K. Biswas, U. Bandyopadhyay and R. K. Banerjee, *Curr. Sci.*, 2004, **87**, 44.
23. M. Suzuki, T. Nakamura, S. Iyoki, A. Fujiwara, Y. Watanabe and K. Mohri, *Biol. Pharm. Bull.*, 2005, **28**, 1438.
24. S. D. Deodhar, R. Sethi and R. C. Srimal, *Indian J. Med. Res.*, 1980, **71**, 632.
25. M. M. LoTempio, M. S. Veena, H. L. Steele, B. Ramamurthy, T. S. Ramalingam, A. N. Cohen and M. B. Wang, *Clin. Cancer Res.*, 2005, **11**, 6994.
26. T. Hamaguchi, K. Ono and M. Yamada, *CNS Neurosci. Ther.*, 2010, **16**, 285.
27. S. Horie, S. Hu, P. Maiti, Q. Ma, X. Zuo, M. R. Jones, G. M. Cole and S. A. Frautschy, *Expert Rev. Neurother.*, 2015, **15**, 629.
28. P. N. Ravindran, K. N. Babu and K. Sivaraman, *Med. Aromat. Plants*, 2007, **45**, 150.
29. D. Chandra and S. S. Gupta, *Indian J. Med. Res.*, 1972, **60**, 138.
30. D. Rai, J. K. Singh, N. Roy and D. Panda, *Biochem. J.*, 2008, **410**, 147.
31. A. Banerjee and S. S. Nigam, *Indian J. Med. Res.*, 1978, **68**, 864.
32. J. Santosh kumar, D. D. Mariguddi and S. Manjunath, *Int. J. Pharmacol. Clin. Sci.*, 2016, **5**, 5.
33. M. K. Bhutani, M. Bishnoi and S. K. Kulkarni, *Pharmacol., Biochem. Behav.*, 2009, **92**, 39.

34. P. Anand, A. B. Kunnumakkara, R. A. Newman and B. B. Aggarwal, *Mol. Pharmaceutics*, 2007, **4**, 807.
35. Y. Manolova, V. Deneva, L. Antonov, E. Drakalska, D. Momekova and N. Lambov, *Spectrochim. Acta, Part A*, 2014, **132**, 815.
36. E. V. Rao and P. Sudheer, *Indian J. Pharm. Sci.*, 2011, **73**, 262.
37. P. C. Lekshmi, R. Arimboor, V. M. Nisha, A. N. Menon and K. G. Raghu, *Food Sci. Technol.*, 2014, **51**, 3910.
38. A. K. Dyab, D. A. Yones, Z. Z. Ibraheim and T. M. Hassan, *Parasitol. Res.*, 2016, **115**, 2637.
39. Y. Q. Zhou, C. M. Wang, R. B. Wang, L. G. Lin, Z. Q. Yin, H. Hu, Q. Yang and Q. W. Zhang, *J. Pharm.*, 1815, **2**, 50.
40. A. Vogel and J. Pelletier, *J. Pharm.*, 1815, **2**, 50.
41. A. M. Anderson, M. S. Mitchell and R. S. Mohan, *J. Chem. Educ.*, 2000, **77**, 359.
42. A. Bagchi, *IOSR J. Environ. Sci., Toxicol. Food Technol.*, 2012, **1**, 1.
43. A. Joe, M. Vijaykumar and B. R. Lokesh, *Crit. Rev. Food Sci. Nutr.*, 2004, **44**, 97.
44. J. L. Funk, J. B. Frye, J. N. Oyarzo, H. Zhang and B. N. Timmermann, *J. Agric. Food Chem.*, 2010, **58**(2), 842.
45. F. Kiuchi, G. Yoshihisa, N. Sugimoto, N. Akao, K. Kondo and T. Yoshisuke, *Chem. Pharm. Bull.*, 1993, **41**, 1640.
46. J. P. Simon, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 11181.
47. M. J. Scotter, *LWT--Food Sci. Technol.*, 2009, **42**, 1345.
48. M. Inafuku, Ukon (rhizome of *Curcuma longa*) for preparation of food, *Japanese Pat.* Jpn. Kokai Tokkyo Koho JP 08,214,825 (96,214,825), 27 August 1996, (CA Vol 125/1996, 274339a).
49. P. Dohare, P. Garga, V. Jain, C. Natha and M. Ray, *Behav. Brain Res.*, 2008, **193**, 289.
50. D. K. Sahoo, A. Roy and G. B. N. Chainy, *Chem.-Biol. Interact.*, 2008, **176**, 121.
51. T. Ahmed and A. Gilani, *Pharmacol., Biochem. Behav.*, 2009, **91**, 554.
52. R. S. Naik, A. M. Mujumdar and S. Ghaskadbi, *J. Ethnopharmacol.*, 2004, **95**, 31.
53. Y. Yang, X. Wu and Z. Wei, *Pharmacol. Res.*, 2015, **95**, 71.
54. G. G. Yue, L. Jiang and H. Kwok, *J. Funct. Foods*, 2016, **22**, 565.
55. D. Chen, J. Shien and L. Tiley, *Food Chem.*, 2010, **119**, 1346.
56. D. Zhang, J. Luo, D. Yan, C. Jin, X. Dong and X. Xiao, *Chin. Herb. Med.*, 2012, **4**, 205.
57. M. Li, J. Cui, M. O. Ngadi and Y. Ma, *Food Chem.*, 2015, **180**, 48.
58. S. R. Naik, V. N. Thakare and S. R. Patil, *Exp. Toxicol. Pathol.*, 2011, **63**, 419.
59. L. J. Zhang, C. F. Wu and H. L. Meng, *Neurosci. Lett.*, 2008, **447**, 48.
60. N. B. Agarwal, S. Jain, N. K. Agarwal, P. K. Mediratta and K. K. Sharma, *Phytomedicine*, 2011, **18**, 756.
61. G. P. Eckert, C. Schiborr and S. Hagl, *Neurochem. Int.*, 2013, **62**, 595.

62. M. Gazal, M. R. Valente and B. A. Acosta, *Eur. J. Pharmacol.*, 2014, **724**, 132.
63. H. Fanaei, S. Khayat, A. Kasaeian and M. Javadimehr, *Neuropeptides*, 2016, **56**, 25.
64. P. Lopez-Jornet, F. Gómez-García, N. G. Carrillo, E. Valle-Rodríguez, A. Xerafinc and V. Vicente-Ortega, *Br. J. Oral Maxillofac. Surg.*, 2016, **54**, 275.
65. A. J. Akinyemi, I. A. Adedara and G. R. Thome, *Toxicol. Rep.*, 2015, **2**, 1357.
66. D. Chen D, J. Shien and L. Tiley, *Food Chem.*, 2010, **119**, 1346.
67. S. Pandit, H. Kim, J. Kim and J. Jeon, *Food Chem.*, 2011, **126**, 1565.
68. R. Mishra, A. K. Gupta, A. Kumar, R. K. Lal, D. Saikia and C. Chanotiya, *J. Appl. Res. Med. Aromat. Plants*, 2018, **10**, 75.
69. G. Singh, O. P. Singh and S. Maurya, *Prog. Cryst. Growth Charact.*, 2002, **45**, 75.
70. A. K. Tripathi, V. Prajapati, N. Verma, J. R. Bahl, R. P. Bansal, S. P. S. Khanuja and S. Kumar, *J. Econ. Entomol.*, 2002, **95**, 183.
71. J. L. Mau, E. Y. Lai, N. P. Wang, C. C. Chen, C. H. Chang and C. C. Chyau, *Food Chem.*, 2003, **82**, 583.
72. G. Sacchetti, S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice and R. Bruni, *Food Chem.*, 2005, **91**, 621.
73. W. Rödel, *Food Nahrung*, 1982, **26**, 830.
74. R. C. Padalia, R. S. Verma, A. Chauhan, P. Goswami, V. R. Singh, S. K. Verma and C. S. Chanotiya, *Rec. Nat. Prod.*, 2017, **11**, 193.
75. B. O. Oguntimein, P. Weyerstahl and H. Marschall-Weyerstahl, *Flavour Fragrance J.*, 1990, **5**, 89.
76. G. K. Jayaprakasha, L. Jaganmohan Rao and K. K. Sakariah, *Food Chem.*, 2006, **98**, 720.
77. S. Yuan, S. Cao, R. Jiang, R. Liu, J. Bai and Q. Hou, *Int. Immunopharmacol.*, 2014, **21**, 128.
78. A. Apisariyakul, N. Vanittanakom and D. Buddhasukh, *J. Ethnopharmacol.*, 1995, **49**, 163.
79. A. Amalraj and S. Gopi, *J. Tradit., Complementary Med.*, 2017, **7**, 65.
80. M. M. Chan, H. I. Huang, M. R. Fenton and D. Fong, *Biochem. Pharmacol.*, 1998, **55**, 1955.
81. C. V. Rao, T. Kawamori, R. Hamid and B. S. Reddy, *Carcinogenesis*, 1999, **20**, 641.
82. C. V. Rao, A. Rivenson, B. Simi and B. S. Reddy, *Cancer Res.*, 1995, **55**, 259.
83. S. Perkins, R. D. Verschoyle and K. Hill, *Cancer Epidemiol., Biomarkers Prev.*, 2002, **11**, 535.
84. B. Shpitz, N. Giladi and E. Sagiv, *Digestion*, 2006, **74**, 140.
85. Y. Kwon and B. A. Magnuson, *Food Chem. Toxicol.*, 2009, **47**, 377.
86. J. Dujic, S. Kippenberger and A. Ramirez-Bosca, *Int. J. Cancer*, 2009, **124**, 1422.

87. R. Garg, S. Gupta and G. B. Maru, *Carcinogenesis*, 2008, **29**, 1022.
88. T. Kawamori, R. Lubet and V. E. Steele, *Cancer Res.*, 1999, **59**, 597.
89. B. B. Aggarwal, S. Shishodia and Y. Takada, *Clin. Cancer Res.*, 2005, **11**, 7490.
90. M. Tomita, H. Kawakami and J. Uchihara, *Int. J. Cancer*, 2006, **118**, 765.
91. A. Siviero, E. Gallo, V. Maggini, L. Gori, A. Mugelli, F. Firenzuoli and A. Vannacci, *J. Ayurveda Holist. Med.*, 2015, **5**, 57.
92. A. N. Balamurugan, L. Akhov, G. Selvaraj and S. Pugazhenthi, *Pancreas*, 2009, **38**, 454.
93. D. W. Zhang, M. Fu, S. H. Gao and J. L. Liu, *J. Evidence-Based Complementary Altern. Med.*, 2013, 1–16.
94. K. Srinivasan and K. Sambaiah, *Int. J. Vitam. Nutr. Res.*, 1991, **61**, 364.
95. M. Nagabhushan and S. V. Bhide, *Nutr. Cancer*, 1986, **8**, 201.
96. R. Srivastava, M. Dikshit, R. C. Srimal and B. N. Dhawan, *Thromb. Res.*, 1985, **40**, 413.
97. H. Itokawa, Q. Shi, T. Akiyama, S. L. Morris-Natschke and K. H. Lee, *Chin. Med.*, 2008, **3**, 11.
98. A. Lubbad, M. A. Oriowo and I. Khan, *Mol. Cell. Biochem.*, 2009, **322**, 127.
99. K. B. Soni and R. Kuttan, *Indian J. Physiol. Pharmacol.*, 1992, **36**, 273.
100. T. Kawamori, R. Lubet, V. E. Steele, G. J. Kelloff, R. B. Kaskey, C. V. Rao and B. S. Reddy, *Cancer Res.*, 1999, **59**, 597.
101. A. H. Ahsan, N. Praveen, N. U. Khan and S. M. Hadi, *Chem.-Biol. Interact.*, 1999, **121**, 161.
102. J. E. Kim, A. R. Kim, H. Y. Chung, S. Y. Han, B. S. Kim and J. S. Choi, *Phytother. Res.*, 2003, **17**, 481.
103. S. B. Subramanian, A. P. Francis and T. Devasena, *Carbohydr. Polym.*, 2014, **114**, 170.
104. W. Chearwae, S. Anuchapreeda, K. Nandigama, S. V. Ambudkar and P. Limtrakul, *Biochem. Pharmacol.*, 2004, **68**, 2043.
105. A. Dairam, J. L. Limson, G. M. Watkins, E. Antunes and S. Daya, *J. Agric. Food Chem.*, 2007, **55**, 1039.
106. M. T. Huang, W. Ma, Y. P. Lu, R. L. Chang, C. Fisher and P. S. Manchand, *Carcinogenesis*, 1995, **16**, 2493.
107. S. K. Sandur, M. K. Pandey, B. Sung, K. S. Ahn, A. Murakami and G. Sethi, *Carcinogenesis*, 2007, **28**, 1765.
108. M. H. Pan, S. Y. Lin-Shiau and J. K. Lin, *Biochem. Pharmacol.*, 2000, **60**, 1665.
109. G. S. Jeong, G. S. Oh, H. O. Pae, S. O. Jeong, Y. C. Kim and M. K. Shin, *Exp. Mol. Med.*, 2006, **38**, 393.
110. S. Toda, M. Ohnish, M. Kimura and K. Nakashima, *J. Ethnopharmacol.*, 1988, **23**, 105.
111. R. S. Ramsewak, D. L. DeWitt and M. G. Nair, *Phytomedicine*, 2000, **7**, 303.
112. W. J. Syu, C. C. Shen, M. J. Don, J. C. Ou, G. H. Lee and C. M. Sun, *J. Nat. Prod.*, 1998, **61**, 1531.

113. R. J. Anto, J. George, K. V. Babu, K. N. Rajasekharan and P. Kuttan, *Mutat. Res.*, 1996, **370**, 127.
114. C. Kalpana, A. R. Sudheer, K. N. Rajasekharan and V. P. Menon, *Singapore Med. J.*, 2007, **48**, 124.
115. P. Limtrakul, W. Chearwae, S. Shukla, C. Phisalphona and S. V. Ambudkar, *Mol. Cell. Biochem.*, 2007, **296**, 85.
116. D. S. Kim, S. Y. Park and J. K. Kim, *Neurosci. Lett.*, 2001, **303**, 57.
117. R. Rukkumani, K. Aruna, P. S. Varma, K. N. Rajasekaran and V. P. Menon, *J. Pharm. Pharm. Sci.*, 2004, **7**, 274.
118. A. T. Dinkova-Kostova and P. Talalay, *Carcinogenesis*, 1999, **20**, 911.
119. N. Kamalakkannan, R. Rukkumani, P. S. Varma, P. Viswanathan, K. N. Rajasekharan and V. P. Menon, *Basic Clin. Pharmacol. Toxicol.*, 2005, **97**, 15.
120. Z. Sui, R. Salto, J. Li, C. Craik and P. R. Ortiz de Montellano, *Bioorg. Med. Chem.*, 1993, **1**, 415.
121. T. Devasena, K. N. Rajasekaran, G. Gunasekaran, P. Viswanathan and V. P. Menon, *Pharmacol. Res.*, 2003, **47**, 133.
122. K. Okada, C. Wangpoengtrakul, T. Tanaka, S. Toyokun, i K. Uchida and T. Osawa, *J. Nutr.*, 2001, **131**, 2090.
123. L. Pari and D. R. Amali, *J. Pharm. Pharm. Sci.*, 2005, **8**, 115.
124. L. Pari and P. Murugan, Antihyperlipidemic effect of curcumin and tetrahydrocurcumin in experimental type 2 diabetic rats, *Renal Failure*, 2007, **29**, 881.
125. P. Murugan and L. Pari, *J. Basic Clin. Physiol. Pharmacol.*, 2006, **17**, 231.
126. P. Murugan and L. Pari, *Basic Clin. Pharmacol. Toxicol.*, 2007, **101**, 241.
127. P. Murugan and L. Pari, *J. Ethnopharmacol.*, 2007, **113**, 479.
128. P. Murugan and L. Pari, *Life Sci.*, 2006, **79**, 172.
129. M. Naito, X. Wu, H. Nomura and M. Kodama, *J. Atheroscler. Thromb.*, 2002, **9**, 243.
130. J. Hong, M. Bose, J. Ju, J. H. Ryu, X. Chen and S. Sang, *Carcinogenesis*, 2004, **25**, 1671.
131. J. M. Kim, S. Araki, D. J. Kim, C. B. Park, N. Takasuka and H. Baba-Toriyama, *Carcinogenesis*, 1998, **19**, 81.
132. T. Atsumi, S. Fujisawa and K. Tonosaki, *Oral Dis.*, 2005, **4**, 236.
133. S. Khopde, K. I. Priyadarsini, P. Venkatesan and M. N. A. Rao, *Biophys. Chem.*, 1999, **80**(2), 83.
134. H. P. T Ammon and M. A. Wahl, *Planta Med.*, 1991, **57**, 1.
135. M. M. Chan, *Biochem. Pharmacol.*, 1995, **49**, 1551.
136. P. Somparn, C. Phisalaphong, S. Nakornchai, S. Unchern and N. P. Morales, *Biol. Pharm. Bull.*, 2007, **30**, 74.
137. N. Sreejayan and M. N. Rao, *J. Pharm. Pharmacol.*, 1994, **46**, 1013.
138. M. Subramanian, N. Sreejayan, M. N. Rao, T. P. Devasagayam and B. B. Singh, *Mutat. Res.*, 1994, **311**, 249.
139. M. K. Unnikrishnan and M. N. A. Rao, *Pharmazie*, 1995, **50**, 490.
140. C. R. Thornfeldt, *Skinmed*, 2005, **4**, 214.

141. B. B. Aggarwal and K. B. Harikumar, *Int. J. Biochem. Cell Biol.*, 2009, **41**, 40.
142. A. Joe, M. Vijaykumar and B. R. Lokesh, *Crit. Rev. Food Sci. Nutr.*, 2004, **44**, 97.
143. S. C. Gupta, B. Sung, J. H. Kim, S. Prasad, S. Li and B. B. Aggarwal, *Mol. Nutr. Food Res.*, 2013, **57**, 1510.
144. T. T. Phan, P. See, S. T. Lee and S. Y. Chan, *J. Trauma*, 2001, **51**(5), 927.
145. P. Mahakunakorn, M. Tohda, Y. Murakami, K. Matsumoto, H. Watanabe and O. Vajaragupta, *Biol. Pharm. Bull.*, 2003, **26**, 725.
146. O. P. Sharma, *Biochem. Pharmacol.*, 1976, **25**, 1811.
147. N. M. Khanna, *Curr. Sci.*, 1999, **76**, 1351.
148. M. K. Unnikrishnan and M. N. A. Rao, *FEBS Lett.*, 1992, **301**, 195.
149. M. E. M. Braga, P. F. Leal, J. E. Carvalho and M. A. A. Meireles, *J. Agric. Food Chem.*, 2003, **51**, 6604.
150. S. Khopde, K. I. Priyadarsini, P. Venkatesan and M. N. A. Rao, *Biophys. Chem.*, 1999, **80**, 83.
151. R. Motterlini, R. Foresti, R. Bassi and C. J. Green, *Free Radicals Biol. Med.*, 2000, **28**, 1303.
152. A. J. Ruby, G. Kuttan, K. D. Babu, K. N. Rajasekharan and R. Kuttan, *Cancer Lett.*, 1995, **94**, 79.
153. Y. Liu and X. Q. Hong, *Zhongguo Zhongyao Zazhi*, 2006, **31**, 500.
154. T. Nishiyama, H. Kishida, M. Tsukagawa, Y. Kuroda, Y. Sashida, K. Takahashi, T. Kawada, K. Nakagawa and M. Kitahara, *J. Agric. Food Chem.*, 2005, **23**(53), 959–963.
155. M. Fiala, P. T. Liu, A. Espinosa-Jeffrey, M. J. Rosenthal, G. Bernard and J. M. Ringman, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 12849.
156. G. Egger, *Prev. Chronic Dis.*, 2012, **9**, 1.
157. Y. Mizuno, R. F. Jacob and R. Preston Mason, Inflammation and the development of atherosclerosis-effects of lipid lowering therapy, *J. Atheroscler. Thromb.*, 2011, **18**, 351.
158. CIHR Canadian Institutes of Health Research, 2012, http://www.cihr-irsc.gc.ca/e/44070.html.
159. S. Su, T. Wang and J. A. Duan, *J. Ethnopharmacol.*, 2011, **134**, 251.
160. L. Laine, R. Smith, K. Min, C. Chen and R. W. Dubois, *Aliment. Pharmacol. Ther.*, 2006, **24**(5), 751–767.
161. L. Rodrigo, R. De Francisco and J. M. Perez-Pariente, *Scand. J. Gastroenterol.*, 2002, **37**, 1341.
162. H. Tag, A. K. Das and H. Loyi, *Nat. Prod. Rad.*, 2007, **6**, 334.
163. J. S. Jurenka, *Altern. Med. Rev.*, 2009, **14**, 141.
164. M. B. Patil, S. V. Taralkar, V. S. Sakpal, S. P. Shewale and R. S. Sakpal, *Int. J. Chem. Sci. Appl.*, 2011, **2**, 172.
165. V. B. Liju, K. Jeena and R. Kuttan, *Indian J. Pharmacol.*, 2011, **43**, 526.
166. I. Igbe, F. P. Ching and A. Eromon, *Acta Pol. Pharm.*, 2010, **67**, 81.
167. H. Hosseinzadeh and H. M. Younesi, *BMC Pharmacol.*, 2002, **2**, 7.
168. H. F. Ji and L. Shen, *CNS Neurol. Disord.: Drug Targets*, 2014, **13**, 369.

169. L. Shen and H. F. Ji, *Trends Mol. Med.*, 2012, **18**, 138.
170. L. Shen, C. C. Leu, C. Y. An and H. F. Ji, *Sci. Rep.*, 2016, **18**(6), 20872.
171. M. Garcia-Alloza, L. A. Borrelli, A. Rozkalne, B. T. Hyman and B. J. Backskai, *J. Neurochem.*, 2007, **102**, 1095.
172. P. Jenner and C. W. Olanow, *Neurology*, 1996, **47**, 161.
173. A. Samii, J. G. Nutt and B. R. Ransom, *Lancet*, 2004, **363**, 1783.
174. R. Katzenschlager, A. Evans, A. Manson, P. N. Patsalos, N. Ratnaraj and H. Watt, *J. Neurol., Neurosurg. Psychiatry*, 2004, **75**, 1672.
175. D. Blum, S. Torch, N. Lambeng, M. Nissou, A. L. Benabid and R. Sadoul, *Prog. Neurobiol.*, 2001, **65**, 135.
176. L. Xie, X. K. Li, N. Funeshima-Fuji, H. Kimura, Y. Matsumoto and Y. Isakal, *Int. Immunopharmacol.*, 2009, **9**, 575.
177. P. Gold and G. P. Chrousos, *Mol. Psychiatry*, 2002, **7**, 254.
178. Z. Ying, A. Wu and F. Gomez-Pinilla, *Exp. Neurol.*, 2006, **197**, 309.
179. Y. Xu, B. S. Ku, H. Y. Yao, Y. H. Lin, X. Ma, Y. H. Zhang and X. J. Li, *Eur. J. Pharmacol.*, 2005, **518**, 40.
180. Q. Wang, A. Y. Sun, A. Simonyi, M. Jensen, P. B. Shelat and G. E. Rottinghaus, *J. Neurosci. Res.*, 2005, **82**, 138.
181. Y. Xua, B. Kub, L. Cuic, X. Lib, P. A. Barisha, T. C. Fosterc and W. O. Oglea, *Brain Res.*, 2007, **116**, 9.
182. Y. T. Acharya, *Chaukambha Sanskrit Samstha*, Varanasi, India, 1994, p. 447.
183. J. Wickenberg, S. Ingemansson, J. Hlebowicz, K. B. Soni and R. Kuttan, *Indian J. Physiol. Pharmacol.*, 1992, **36**, 273.
184. P. Faizal, S. Suresh, R. S. Kumar and K. T. Augusti, *Indian J. Clin. Biochem.*, 2009, **24**, 82.
185. D. Rai, J. K. Singh, N. Roy and D. Panda, *Biochem. J.*, 2008, **410**, 147.
186. S. Ponnusamy, R. Ravindran, S. Zinjarde, S. Bhargava and R. Ameeta, *J. Evidence-Based Complementary Altern. Med.*, 2011, 10.
187. P. A. Kumar, A. Haseeb, P. Suryanarayana, N. Z. Ehtesham and G. B. Reddy, *Arch. Biochem. Biophys.*, 2005, **444**, 77.
188. A. Kuhad and K. Chopra, *Eur. J. Pharmacol.*, 2007, **576**, 34.
189. K. T. Peeyush, G. Gireesh, M. Jobin and C. S. Paulose, *Life Sci.*, 2009, **85**(19–20), 704.
190. T. P. Kumar, S. Antony, S. Soman, K. P. Kuruvilla, N. George and C. S. Paulose, *Mol. Cell. Endocrinol.*, 2011, **331**, 1.
191. L. Ma, L. A. Johns and M. J. Allen, *BMC Genet.*, 2009, **10**, 77.
192. T. P. Kumar, S. Antony, G. Gireesh, N. George and C. S. Paulose, *J. Biomed. Sci.*, 2010, **17**, 43.
193. P. T. Kumar, N. George, S. Antony and C. Skaria Paulose, *Eur. J. Pharmacol.*, 2013, **702**, 323.
194. S. Jayanarayanan, S. Smijin, K. T. Peeyush, T. R. Anju and C. S. Paulose, *Chem.-Biol. Interact.*, 2013, **201**, 39.
195. S. Horie, *Korean J. Urol.*, 2012, **53**, 665.
196. M. Bower, H. Aiyer, Y. Li and R. Martin, *World J. Gastroenterol.*, 2010, **16**, 4152.

197. J. Park and C. Conteas, *World J. Gastrointest. Oncol.*, 2010, **2**, 169.
198. R. Wilken, *Mol. Cancer*, 2011, **10**, 12.
199. G. Sa and T. Das, *Cell Div.*, 2008, **3**, 14.
200. M. Ye, Y. Li, H. Yin and J. Zhang, *Int. J. Mol. Sci.*, 2012, **13**, 3959.
201. G. Kapakos, V. Youreva and A. K. Srivastava, *Indian J. Biochem. Biophys.*, 2012, **49**, 306.
202. I. Mohanty, D. S. Arya, A. Dinda, S. Joshi, K. K. Talwar and S. K. Gupta, *Life Sci.*, 2004, **75**, 1701.
203. E. M. El-Sayed, A. S. El-Azeem, A. A. Afify, M. H. Shabana and H. H. Ahmed, *J. Med. Plants Res.*, 2010, **5**, 4049.

CHAPTER 9

Biosynthesis of Curcumin and Molecular Targets and the Biological Mechanism of Curcumin

Y. BASPINAR*[a] AND H. AKBABA[a]

[a]Ege University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, 35100 Bornova, Izmir, Turkey
*E-mail: yucel.baspinar@ege.edu.tr, yucelbaspinar77@gmail.com

9.1 Introduction

The biosynthesis of curcumin is generally divided into two, a natural pathway where curcumin is synthesized directly from turmeric and an artificial pathway where it is synthesized from *Oryza sativa* and rice bran using *E. coli*.[1] The natural pathway is further divided into an upstream and downstream section, both finally resulting in the biosynthesis of curcumin. In 1997, it was proposed that type III polyketide synthases (PKSs) and condensing enzymes are crucial for the biosynthesis of curcuminoids like curcumin. These PKSs, for example, curcumin synthase 1 (CURS1), use different starter molecules such as feruloyl-diketideCoA, resulting in a different final length of the polyketide, thus molecular diversity is generated. Approximately ten years later, in 2006, it was proposed that either a single enzyme or multiple

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

enzymes with similar properties are crucial for the biosynthesis of curcumin.[1] Three isoforms of the curcumin synthase (CURS), CURS1, CURS2 AND CURS3, and one diketide CoA synthase (DCS), all type III PKSs from turmeric, were identified and characterized.[2] In addition, it was suggested that DCS synthesized feruloyldiketide-CoA/p-coumaroyldiketide CoA and CURS1-3 converted diketide-CoA esters into a curcumin scaffold. An artificial pathway for curcumin biosynthesis was exploited using *E. coli*.[2–5] As discussed earlier, the biosynthesis pathway of curcumin can be divided into an upstream and downstream section. The upstream section involves the general phenylpropanoid pathway genes, leading to the formation of aromatic CoA esters, *p*-coumaroyl CoA and feruloyl CoA, namely phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate-coenzyme A ligase (4CL), hydroxycinnamoyl transferase (HCT), cinnamate 3-hydroxylase C3H, caffeoyl CoA *O*-methyltransferase (COMT) and *O*-methyltransferase (OMT). The downstream genes include DCS, CURS1, CURS2 and CURS3. The main part of the phenylpropanoid genes comprise multigene families.[6] A recent study described the characterization, cloning of novel PKS (ClPKS11) from *C. longa*.[7]

The molecular targets of curcumin are closely related to its biological mechanism. The transcription factor NF-κB governs several cellular signaling pathways associated with cancer, and various targets such as cytokines, pro-inflammatory molecules, growth factors, cell adhesion molecules, oncogenes and pro/anti-apoptotic proteins during carcinogenesis. Curcumin targets transcription factors, PKs, angiogenesis, cell cycle regulators, sirtuins, NF-κB signaling pathway, E-cadherin, VEGF, STAT-3 signaling pathway, apoptosis, Bcl-2, p53, DNA, miRNA and autophagy.

For the biological mechanism of curcumin, different activities such as antioxidant, anticancer, antiviral, antifungal, antiproliferative, antiimmunomodulatory and anti-inflammatory were described. However, the aim of this chapter is to provide information on the biosynthesis of curcumin, molecular targets of curcumin and its biological mechanism and mainly focus on the anticancer activities of curcumin.

In recent years, studies have unravelled the molecular mechanisms of curcumin's anticancer effects.[8,9] These activities involve various mechanisms, such as carcinogen-detoxifying enzymes/antioxidants, apoptosis, and transcription factors such as nuclear factor-κB (NF-κB), associated with tumor progression and inflammatory diseases.[10] The intrinsic (activated oncogenes) and extrinsic (inflammation, infections) pathways connected cancer and inflammation, leading to the activation of the three transcription factors: NF-κB, STAT3 and hypoxia-induced factor 1α (HIF1 α).[11] The modulation of NF-κB by curcumin was studied where p65, a subunit of NF-κB, is phosphorylated and translocates into the nucleus, and activates transcription of inflammation- and survival-related genes.[12,13] Curcumin inhibits STAT3 [14,15] and HIF1α directly, and indirectly through the aryl hydrocarbon receptor nuclear translocator.[16,17] Curcumin reduced the activity of NF-κB and STAT3, leading to a diminished expression of inflammatory cytokines,[8,18] which reduces the attraction between inflammatory cells and the tumor site. Key players

in inflammation are NF-κB, tumor necrosis factor (TNF)-α, and its downstream target cyclooxygenase (COX)-2, which promotes tumor growth and metastasis by triggering proliferation and angiogenesis and inhibits apoptosis.[19] Curcumin inhibits NF-κB expression and activity and decreases COX-2 levels, crucial for inflammation and cancer progression.[12,18,20,21] Curcumin regulates the inflammatory cytokines, mediating tumorigenesis, through the suppression of NF-κB, and inhibits the expression of interleukins (IL-1, -2, -5, -8, -12, -18),[22–25] which are part of matrix metalloproteinases (MMPs), adhesion molecules and signaling pathways related to invasion and angiogenesis. Inhibition of CXCL-1 and -2 expression by curcumin in breast cancer cells is mediated through NF-κB by gene silencing of the NF-κB subunit p65, requiring intact IκBα expression upstream of NF-κB.[18] Furthermore, curcumin inhibits translocation of NF-κB to the nucleus through the inhibition of the IκB-kinase (IKKβ), leading to stabilization of IκBα, an NF-κB inhibitor, in prostate cancer. The assumption that curcumin and the synthetic IKKβ inhibitor SC-541 could share the target of prostate cancer cells was proven by the combined application of them, showing no additive or synergistic effects.[8] The chemopreventive properties of curcumin are caused by an inactivation of NF-κB in cancer and inflammatory cells. However, downregulating the cytokine CXCL-1 resulted in reduced expression of CXCR-4, which could be used as a target for drug development.[26]

Transcription factors are crucial proteins in controlling the transcription rate of genetic information from DNA to messenger RNA (mRNA), by binding to specific DNA sequences. Transcription factors regulate genes in order to ensure that they are expressed in the right cell at the right time throughout the life of the cell. Some of these functions coordinate the cell division, cell growth and cell death. Transcription factors can regulate alone or in combination with other proteins in a complex, by promoting (activator), or blocking (suppressor) the recruitment of RNA polymerase to specific genes. Under normal physiological conditions, most of them are inactive, but deregulated expression was identified in numerous cancer tissues. Cancer development, cell survival, proliferation and tumor angiogenesis can be promoted by activation and inactivation of transcription factors.

9.2 Biosynthesis of Curcumin

9.2.1 Natural Pathway of Curcumin Biosynthesis in *C. longa*

The hydrophobic polyphenol curcumin, bisdemethoxycurcumin (BDMC) and demethoxycurcumin (DMC) are chemically unsaturated β-diketones, called curcuminoids. One of the first reports about the possible biosynthesis of curcumin is dated from 1973,[27] based on ^{14}C labeled compounds. In 1997 it was stated that curcuminoids synthesis was based on the phenylpropanoid pathway and that at least one PKS was crucial for its scaffold.[28] In 2008, ^{13}C labeled investigations revealed the natural pathway of curcuminoids in *Curcuma longa* (turmeric)[29] and a curcuminoid synthase (CUS) from rice (*Oryza sativa*).[2]

Two possible natural pathways for the biosynthesis of curcumin are described by Kita *et al.* and Katsuyama *et al.*, with some differences.[2,29] Here, some similarities and differences are pointed out: Both pathways start with the transformation of phenylalanine to cinnamic acid with the help of PAL and result in the biosynthesis of BDMC, DMC and curcumin, respectively. However, different intermediates are stated.

After the formation of cinnamic acid, in 2008 Kita *et al.* described one major and one minor pathway. For the major pathway, two equivalents of cinnamic acid condensates with malonic acid to an intermediate, which is hydroxylated with C4H to BDMC. For the minor pathway, cinnamic acid is transformed to *p*-coumaric acid. Afterwards, two equivalents of *p*-coumaric acid condensates with malonic acid to BDMC. CURS transforms BDMC to DMC and finally to curcumin.

The other pathway[2] described by Katsuyama *et al.* is also divided into two sections. Just like that described by Kita *et al.*, it starts with the transformation of phenylalanine to cinnamic acid with PAL. Cinnamic acid is transformed to cinnamoyl-CoA with 4CL, and further to *p*-coumaroyl-CoA with C4H. After that, one section involves a condensation of malonyl CoA and DCS to *p*-coumaroyl diketide CoA, which is transformed to BDMC with *p*-coumaroyl CoA and CURS3, or to DMC with help from feruloyl CoA and CURS1,2,3.

The other section involves transformation of *p*-coumaroyl CoA to feruloyl CoA with the help of HCT, C3H and COMT. Feruloyl CoA condensates with malonyl CoA and DCS to feruloyldiketide CoA, which is transformed to DMC with the help of *p*-coumaroyl CoA and CURS3, or to curcumin with the help of feruloyl CoA and CURS1,2,3.

Although one section of this pathway resulted in BDMC and DMC, respectively, they can be easily transformed to curcumin with the help of OMT. In Figure 9.1, both pathways described have been summarized excluding some minor intermediates.

9.2.2 Artificial Pathways of Curcuminoid Biosynthesis in Recombinant *E. coli*

9.2.2.1 Biosynthesis of Curcuminoids from O. sativa

Approximately ten years after the prediction that a type III PKS could be involved in curcuminoid synthesis, a screening for type III PKSs revealed its catalytic properties in organisms. One outcome of this screening was os07g17010, catalyzing the synthesis of curcumin from feruloyl-CoA as a starter and malonyl-CoA as an extender.[3] The reaction by the enzyme CUS begins with the thioesterification of the thiol group by feruloyl-CoA. After a decarboxylative condensation of malonyl-CoA to the thioester of ferulic acid, an intermediate as diketide-CoA is formed. Hydrolysis of this diketide-CoA leads to a β-keto acid joining the feruloyl-CoA by condensation to form curcumin.

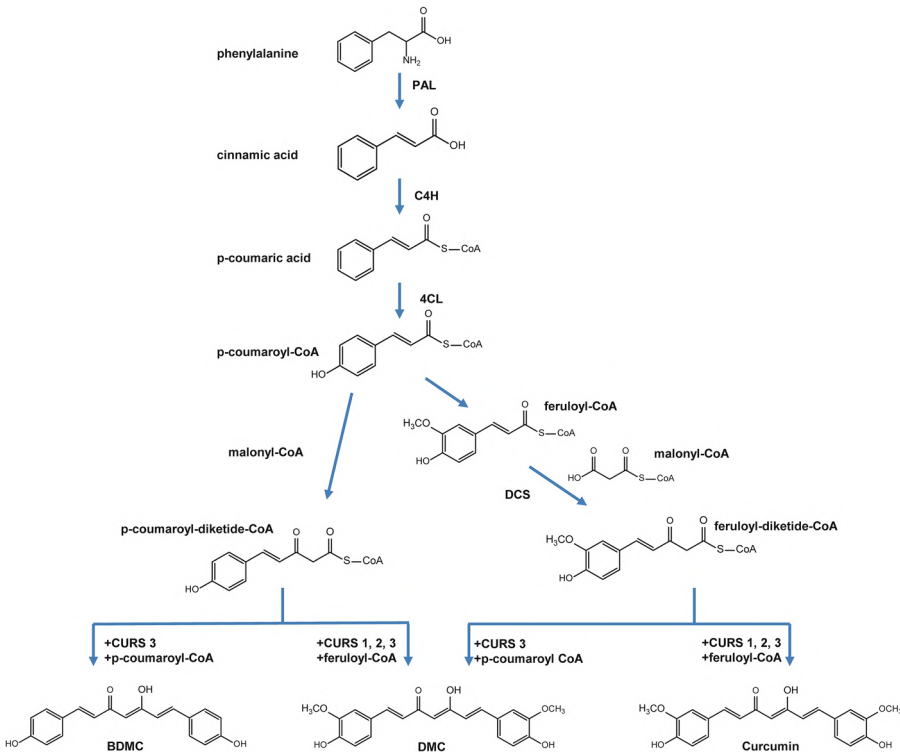


Figure 9.1 Biosynthetic pathways of curcumin.

9.2.2.2 Production of Curcuminoids by Recombinant E. coli

The production of curcuminoids by recombinant *E. coli* is based on the catalytic properties of CUS and consists of two steps: First, a substrate synthesis for CoA esters synthesis from tyrosine and phenylalanine and second, a polyketide synthesis for conversion of the CoA esters into curcuminoids.[4] Recombinant *E. coli* cells harboring pCDF-PAL/LE4CL-1 carrying the enzymes PAL and 4CL were used for CoA esters synthesis and plasmid pET-CUS carrying CUS was a vector for the conversion into curcuminoids. CUS condensed two molecules of the CoA ester and one molecule of malonyl-CoA to produce curcuminoids. Here, *E. coli* is like a bag with a set of enzymes for curcuminoid synthesis.

9.2.2.3 Production of Curcumin from Rice Bran Pitch by Recombinant E. coli

Besides the use of turmeric and rice, the question arises if any waste of rice production can be used for curcumin production/biosynthesis. The amount of production for brown rice is about 10 million tons every year, but 1 million tons of rice are lost, because it is polished away before eating. One side of the

product of rice is rice bran pitch, a dark and viscous oil. It was reported that rice bran contains bioactive compounds such as ferulic acid. Ferulic acid can be used as a substrate for curcumin biosynthesis, with the help of recombinant *E. coli*. After incubation at 26 °C for 60 hours, 60 mg curcumin were produced from 1 g of rice bran pitch.[30]

9.3 Biological Mechanism of Curcumin and Molecular Targets

The biological mechanism of curcumin is related to its molecular targets such as COX-2, cell cycle regulators, sirtuins, NF-κB signaling pathway, E-cadherin, vascular endothelial growth factor (VEGF) *etc.*[8–10] and is described in Figure 9.2 and Tables 9.1–9.5.

9.3.1 Pro-angiogenic Factors

Angiogenesis is the physiological process of forming new blood vessels from pre-existing vasculature and is important in growth, survival and metastasis of cancer cells. One key factor of angiogenesis is the production of pro-angiogenic factors such as VEGF. Basic fibroblast growth factor (bFGF), VEGF, MMPs, FGF and hepatocyte growth factor (HGF) are some important pro-angiogenic and anti-angiogenic factors. VEGF and its receptors are key factors of vasculogenesis and angiogenesis.[31,32] VEGF consists of mitogens such

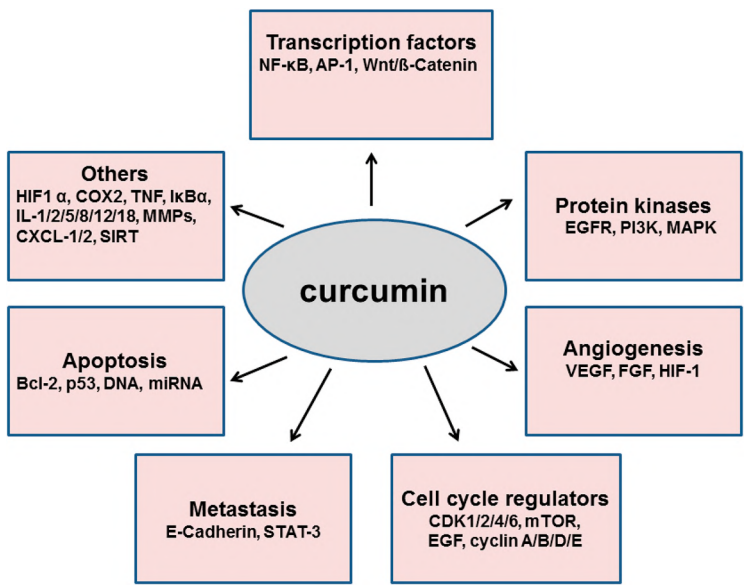


Figure 9.2 Biological mechanism of curcumin.

as VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PIGF), regulating VEGF receptors tyrosine kinases (VEGFR-1, VEGFR-2 and VEGFR-3), promoting endothelium and blood vessel regeneration and increasing vascular permeability. VEGF-A is the central member of this family and most of the angiogenic effects are attributed to the interaction of VEGF-A with VEGFR-2.[33,34] Curcumin mediated abrogation of angiogenic response, followed by downregulation of angiogenic proteins like VEGF, angiopoietin 1 and 2, PDGF, COX-2, HIF-1α, transforming growth factor (TGF)-β and bFGF.[16,35–39] More examples of curcumin's targets for angiogenesis are given in Table 9.1.[40–43]

9.3.2 Cell Cycle Regulators

The cell cycle consists of the four phases: G1- (first growth), S- (synthesis), G2- (sub phase) and M-phase (mitosis) (Figure 9.3).[44] Cyclin-dependent kinases (CDKs) are a family of PKs that play an important role in regulating the cell cycle. CDK represents a target for curcumin, leading to inhibition of tumor progression. There is a relation between the nuclear protein, early growth response protein 1 (Egr-1) and transcriptional regulation, tumor suppression, cell differentiation, and mitogenesis.[45,46] Another nuclear protein, cyclin E, plays an important role in the G1/S progression by interacting with CDK2.[47] Curcumin can downregulate cyclin E and upregulate the CDK inhibitors p53, p21 and p27. This may contribute to its antiproliferative effects.[48] The fact behind curcumin only targeting CDK2 overexpressed cancer cells and not healthy cells suggests that curcumin induces the p53-dependent apoptosis and causes G2 phase arrest in mammary carcinoma, while this is different in normal mammary cells. A relation between the tumor suppressor protein inhibitor of growth protein 4 (ING4) and cell cycle arrest and apoptosis induction of DNA damage was observed.[49] An overexpression of p53, p21 and ING4 was observed after curcumin treatment.

Table 9.1 The effects of curcumin on pro-angiogenic factors.[a]

Target	Cancer/cell line	Effects	Reference
Pro-angiogenic factor	Breast	VEGF suppression leads to inhibition of breast tumor growth	40
	Human umbilical vein endothelial cells	İnhibiting the expression of VEGF, VEGFR II, KDR, angiopoietin 1 and 2 genes	41
	Skin (B16-F10)	Enhanced expression of anti-metastatic TIMP-2, non-metastatic gene 23 and E-cadherin proteins	42
	Lung (A549)	Inhibition of VEGF	43

[a]↓downregulation/suppression/inhibition; ↑upregulation/expression/induction.

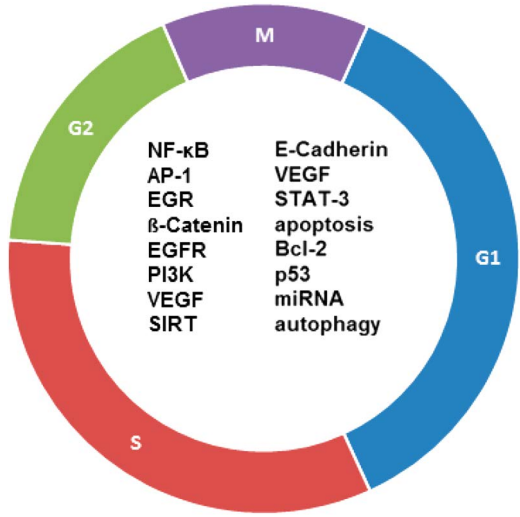


Figure 9.3 Molecular targets of curcumin.

Cyclin D1 is overexpressed in many cancers.[50] In prostate cancer cells curcumin downregulated p21WAF1/CIP1 expression.[51] An upregulation of p21 and p27, which are cyclin dependent kinase inhibitors, was observed in multiple tumor cell lines.[52] More examples of curcumin's targets for cell cycle regulators are given in Table 9.2.[53–68]

9.3.3 Metastasis and Transcription Factors

The mortality rate based on metastasis amounts is approximately 90%.[69] Due to that fact, cellular pathways, related to metastasis, are considered as therapeutic targets of cancer.[70,71] The spread and growth of cancers from the origin neoplasm to distant organs through mechanisms such as angiogenesis, invasion and proliferation are described as metastasis.[72] Metastasis depends on genetic, epigenetic and biochemical factors in the original cells and is associated with the novel environment. Curcumin can modulate pathways through transcription factors, proteins and growth factors.[73] Since, the precise mechanism of curcumin activity is not clear, the inhibitory effect for molecular targets and signaling pathways related to metastasis was investigated.[74,75] The transmembrane glycoprotein epithelial cadherin (E-cadherin) mediates cell–cell adhesion through binding between two E-cadherin molecules.[76] There is a clear relation between E-cadherin and the catenin protein family.[77] The loss of E-cadherin function results in the proliferation of various cancers.[78–83] Furthermore, the anti-metastatic effect of curcumin against colorectal cancer cells was demonstrated.[84] Curcumin upregulated the expression of E-cadherin, inhibiting mesenchymal transition (EMT). There is an association between EMT-related genes and

Table 9.2 Cell cycle regulators as targets of curcumin for various cancer types.[a]

Target	Cancer/cell line	Effects	Reference
Cyclin B1 STAT-3	SCLC (small cell lung) (NCIH446, NCI-1688)	Downregulation of protein and mRNA levels, cell cycle arrest in G2/M phase	53
Cyclin-dependent kinase 2 (CDK2)	Colon	Inhibition of CDK2 activity and decrease of cell proliferation	54
Cyclin E	Breast (MCF-7)	Reduced cell proliferation and arrested growth at the G1 phase	55
Cyclin B	Osteosarcoma	Cell cycle arrest in G1/S and G2/M phase	56
CDK (p21, p27, p53)	Umbilical vein endothelial cells (ECV304)	G0/G1 and/or G2/M phase cell cycle arrest, downregulation of cyclin B1 and CDC2	57
Inhibitor of growth protein 4 (ING4)	Brain; glioma (U251)	Suppressed expression of ING4; reduced cell proliferation and G2/M phase of cell cycle arrest	58 and 59
Cyclin A, B1, D2, E, CDK2, p21 and cdc2	Promyelocytic leukemia (HL-60); bladder (T24), endothelial-like cells (ECV304)	Inhibition of cell proliferation and cell cycle arrest in G0/G1 and/or G2/M phase	60 and 20
Cyclin B1 and CDK1	Pancreas	Cell cycle arrest in G2/M phase	61
Cyclin D, E, CDK2,4,6	Breast (MCF-7)	Cell cycle arrest in G1 phase	62
Akt and NF-κB ↓	Mantle cell lymphoma	Cell cycle arrest in G2/S phase	63
NF-κB and STAT-3	Hodgkin's lymphoma	Decreased expression of proteins involved in cell proliferation	15
mTOR	Breast (MCF-7), cervical (HeLa), prostate	Cell cycle arrest in G1/G0 phase	64
Aurora A, histone H3	Bladder	Cell cycle arrest in G2/M phase	65
Cyclin B, D, E	Colon (HCT-116)	Inhibition of cell proliferation and cell cycle arrest in G2/Mphase	66
Cyclin D1 and cdc 4	Adenocarcinoma	Suppression of cell proliferation and cell cycle arrest in G2/Mphase	67
Cyclin D1	Lung	Inhibited cell proliferation	68

[a] ↓downregulation/suppression/inhibition; ↑upregulation/expression/induction.

cancer progression and metastasis.[85] Some of the curcumin activity results are an overexpression of E-cadherin, suppression of Sp-1 transcriptional activity and inhibition of focal adhesion kinase (FAK) phosphorylation.[86] The transcription factor STAT-3, with the members STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6, plays a role as signaling proteins (cytoplasma) and as transcription factors (nucleus).[87] The membrane receptor-associated Janus kinase 2 (JAK2) is crucial STAT-3 phosphorylation and activation, entering into the nucleus and activating target genes, related to metastasis like an invasion, cell survival, angiogenesis, proliferation and apoptosis.[88] The importance of STAT-3 in the metastasis process of various cancers has been pointed out.[89]

The transcription factor NF-κB regulates the expression of more than 200 genes, crucial for cellular transformation, proliferation, anti-apoptosis, angiogenesis, invasion and metastasis.[90] The transcription factor activator protein-1 (AP-1) regulates the gene expression in response to various stimuli like cytokines, growth factors, stress as well as infections, and controlling cellular processes such as differentiation, proliferation and apoptosis. The growth and progression of various cancer types are associated with the dysfunctions in the AP-1 levels.[91] Hyperactivation and inappropriate regulation of Wnt/β-catenin signaling were associated with malignancies. An important key factor in this pathway is β-Catenin, a signal transducer for various Wnt targeted genes. An overexpression of β-catenin leads to the activation of cell proliferation.[92] In contrast, the tumor suppressor protein glycogen synthase kinase 3β (GSK3β) controls the activity of β-catenin, with a decreased expression in cancer cells.[93] More examples of curcumin's targets for metastasis and transcription factors are given in Table 9.3.[94–131]

9.3.4 Proteins Kinases

PKs are a group of tyrosine or serine/threonine kinase enzymes, modifying other proteins by adding phosphate moieties. In particular, tyrosine phosphorylation is important for cellular pathways of human diseases.[132] PKs control metabolism, transcription, mRNA processing, cell division, apoptosis, differentiation and mediate intracellular signal-transduction pathways. Within the cell, PKs are crucial for communication between neighboring cells, for motility of cells and transport of molecules.[133] Cancer, diabetes, cardiovascular disorders, inflammatory diseases and immune deficiencies are associated with deregulation in tyrosine phosphorylation.[134,135] The epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptors, also known as human epidermal growth factor receptor (HER1). The EGFR has an extracellular ligand-binding module, a transmembrane region, and an intracellular domain, including the tyrosine kinase domain.[136] The EGFR pathway plays an important role in cancer proliferation, differentiation and angiogenesis.

Table 9.3 The effects of curcumin on metastasis and transcription factors as targets for various cancer types.[a]

Target	Cancer/cell line	Effects	Reference
E-cadherin ↑	Lung	HLJ1 was modulated by enhancing the JNK/JunD expression	96
E-cadherin ↑	Papillary thyroid cancer	Migration and invasion was blocked by increasing E-cadherin expression and inhibiting of MMP-9 activity	97–99
E-cadherin ↑	Colon	Reduced progression and metastasis	100
STAT-3 ↓	Leukemia	Reduced mRNA of Jak-2 and cyclin D1	101
STAT-3 ↓ (Jak-1/2)	Lymphoma, Hodgkin's lymphoma, T-cell lymphoma, melanoma	Apoptosis induction, Bcl-2 reduction, caspase-3 and PARP cleavage activation, cell-cycle arrest	15, 102–104
	HNSCC breast	Induced apoptosis	105 and 106
STAT-3 ↓	Lymphocytic leukemia B, multiple myeloma (U266), pancreas, ovarian, endometrial, melanoma, T-lymphoma, glioma, lung, hepatocellular carcinoma	Inhibited cellular proliferation	107–118
NF-κB ↓	Melanoma	Downregulation of NF-κB and decreasing cell viability	119
	Myeloid leukemia	Inhibition of I-κB kinase complex and protein kinase B (Akt leading to inhibition of tumour cell invasion)	120
	Astroglioma	Inhibition of matrix metalloproteinase-9 leading to inhibition of astroglioma cells invasion and metastasis	121
	Breast	Reduced urokinase-type plasminogen activator (uPA) expression, leading to inhibition of metastatic progression of breast cancer cells	122
	Melanoma	Apoptosis induction by caspase-3 activation, downstream of target genes such as COX-2 and cyclin D1	123
	Breast (MCF-7, MDA-MB-231, BT-483)	Downregulation of proliferation and invasion	124 and 125
	T-cell lymphoma	Degradation of IKK *via* downregulation of Hsp90	126
AP-1 ↓	PC-3	>50% of cells arrested in G2/M phase, reduction to 25% of cells in S phase	127
	Colon (HCT-116)	PKC activation was inhibited	128 and 129
	Prostate (LnCap)	Proliferation of cells was abrogated	130
Wnt/β-catenin ↓	Meduloblastoma	Cell growth was suppressed by inhibiting Wnt/β-catenin, activation of GSK-3β and of its target cyclin D1 was promoted; cell cycle arrest in G2/M phase	131 and 132
	Breast (MCF-7, MDA-MB-231)	Cyclin D1 expression was suppressed and apoptosis was induced	133

[a]↓downregulation/suppression/inhibition; ↑upregulation/expression/induction.

Curcumin decreases EGFR expression and its mRNA levels in bladder cancer.[137] After treatment of curcumin in epithelial cancer cells (A431) an autophosphorylation activity of the EGFR tyrosine kinase was determined. Curcumin inhibited EGFR tyrosine kinase, without any detailed information about the mechanism.[136] Phosphatidylinositol 3-kinases (PI3Ks)/Akt are a family of enzymes involved in cellular functions of cancer such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. The Akt mediates PI3K signaling and affects apoptosis by targeting proteins.[138] HIF-1α accumulation and increase of VEGF expression, leading to angiogenesis, are associated with PI3K and mitogen activated protein kinases (MAPK).[139] It provides information about the inhibition of the PI3K pathway, exhibiting a relationship with decreased activity in glycolysis and is probably responsible for cell cycle arrest in G0/G1 phase.[140]

9.3.4.1 p53

One of the most important tumor suppressor proteins, p53, regulates cellular processes such as cell proliferation, DNA damage and apoptosis. Its effect is based on activating repair proteins or inducing apoptosis.[141] p53, from intrinsic and extrinsic pathways, reacts on mitochondrial membrane potential changes.[141] More examples of curcumin's targets for tumor suppressor p53 are given in Table 9.4.

9.3.4.2 SIRT

SIRTs are a class of proteins composed of seven types: ribosyltransferase, deacylase, deacetylase, desuccinylase, demalonylase, demyristoylase and depalmitoylase, respectively. SIRT-1 is important for deacetylation of transcriptional factors, DNA repair proteins and signaling factors and regulating biological activities such as cell survival, gene expression, metabolism and senescence.[142] The anticancer effects of curcumin were shown by upregulation of SIRT1 in head and neck squamous cell carcinoma.[143] More examples of curcumin's targets for PKs are given in Table 9.4.[136,144–164]

9.3.5 miRNA

Small, single stranded and non-coding miRNAs are endogenous molecules of 18–25 nucleotides, related to development, differentiation, proliferation and apoptosis. The involvement of miRNA in cancer, with up- and downregulations was reported.[165–170] miRNA controls the protein expression post-transcriptionally by regulating the expression of proteins *via* binding to 30 UTR of the respective mRNA, leading to degraded mRNA or inhibited mRNA translation.[171] miRNAs can operate as tumor promoters (oncogenes) or suppressors.[165,166] miRNAs regulate each single step of tumor progression such

Table 9.4 Protein kinases and tumour suppressor p53, as targets of curcumin for various cancer types.[a]

Target	Cancer/cell line	Effects	Reference
EGFR ↓	Epithelial (A431)	Autophosphorylation activity of EGFR	139
	Bladder	Decreased mRNA levels	140
	Colorectal (Caco-2, HT-29)	Cell growth was inhibited	141
	Hepatoma (Hca-F)	Modulation of migration and invasiveness	142
	Breast	Functional interaction between integrin α6β4 and EGFR was inhibited	143
	Ovarian (CaOV3)	Aquaporin water channels were attenuated leading to inhibition of cell migration and metastatic potential	144
PI3K ↓	Breast	Akt and GSK3β phosphorylation was induced	145
	T-ALL malignant cells and upper aero-digestive tract	De-phosphorylation/inactivation of Akt, Forkhead box O (FOXO) and GSK3 was promoted	146 and 147
	Pancreas	Increased FOXO1 expression, correlated with PI3K and Akt inhibition	148
	Uterine leiomyosarcoma	Inhibition of the Akt upstream modulator mTOR, decreased m-TOR and S6 phosphorylation, leading to reduction in tumor size	149 and 150
	Breast	Blocking of PI3K and overcoming the oncogenic expression of Bcl-2 protein	151
p53↑	Basal cell, mammary epithelial carcinoma, lung, ovarian	Induced apoptosis	152–155
	Melanoma, colon (HT-29), colorectal, ovarian, prostate (LnCap)	Upregulated p53	156–161
	Neuroblastoma	Upregulated p53 and Bax expression, induction of p21	162
	Nasopharyngeal	Induced expression of FOXO3a and p53	163
	Prostate	Downregulated MDM2	164
	Glioma	Induced apoptosis, p21 WAF-1/CIP-1 and ING4	165

[a]↓downregulation/suppression/inhibition; ↑upregulation/expression/induction.

as proliferation, growth, migration and invasion. miR181b downregulates the expression of matrix-degrading enzymes MMPs, resulting in decreased tumor cell invasion. Furthermore, miR181b overexpression in breast cancer cells suppresses gene expression.[172] To sum up, the anticancer activity of curcumin is induced in part by miRNAs, regulating complex tumor progression-associated signaling pathways, such as Akt, phosphatase and tensin homolog (PTEN), Bcl-2, p53, Notch and ErbB.

9.3.6 DNA

Intact DNA is a prerequisite to ensure correct relay of genetic information from mother to daughter cells during cell division. If the damage is too catastrophic, damaged DNA triggers a response, repairing lesion(s) or inducing apoptosis. Cycling cells control DNA damage *via* a DNA damage response, recognizing DNA lesions and facilitating repair, or halts cell cycle progression in the G1-, G2-, or S-phase, finally resulting in apoptosis. There are several types of DNA damage such as DNA single strand or double strand breaks. Chemotherapy is directly oriented to DNA due to the fact that replicative processes are more subject to genetic anomalies in cancer cells, whose cell division rate is increased in tumors. Curcumin is a DNA binding agent, proven by DNA interaction studies, which shows it binding to minor DNA grooves or at T-bases in the minor groove, G and A bases in the major groove and the phosphate backbone.[173,174] In addition, curcumin can bind RNA, but with a lower affinity than DNA. Curcumin increased ataxia telangiectasia mutated (ATM)-mediated H2AX phosphorylation, proving its DNA double strand break effect.[175,176] The p53 inhibitor MDM2 is inactivated by Chk1, which activates p53.[177,178] This leads to cell cycle arrest by inducing CDKN1A transcription, upregulating p21CIP1, and stimulating apoptosis by Bax, PUMA and NOXA induction.[179,180] These findings are discussed in the context of telomere shortening (single strand DNA breaks) due to ErbB-1 signal inhibiting. Alternative mechanisms for the activation of these proteins are, besides their upregulated expression by ErbB-1 signaling axis, base oxidation, single and double strand breaks. Table 9.5 illustrates the effects of curcumin on DNA and miRNA for various cancer types.[181–199]

9.3.7 Cellular Death

9.3.7.1 Apoptosis

A crucial process in apoptosis is based on changing the mitochondrial membrane potential, increasing the outer membrane and releasing proteins from the space between the inner and outer membranes. Proteins of the anti-apoptotic protein Bcl-2 family and other components coordinate the regulation of this permeabilization. Bcl-2 is integrated into the outer mitochondrial membrane and regulates Bax and Bak activity.

Table 9.5 The effects of curcumin on DNA and miRNA for various cancer types.[a]

Target	Cancer/cell line	Effects	Reference
miRNA	Pancreas	Upregulated miRNA-22 and downregulated miRNA-199a	195
	NSCLC	Upregulation of miR192-5p lead to suppressed P13K/Akt signaling, inhibited proliferation and induced apoptosis, suppressing miR-21 lead to induction of PTEN	196 and 197
	Lung adenocarcinoma	Upregulating miR-186 inhibited progression of tumour	198
	Prostate	Inhibiting miR-208 modulated cell cycle arrest	199
	Nasopharyngeal	Downregulating miR-125a-5p reduced formation of metastases	200
miRNA	Esophageal, bladder, retinoblastoma, colorectal, leukemia, head and neck squamous	Downregulated miR-17-5p, miR-20a, miR-27a, miR-21, miR-34aA (pro-oncogenic) and upregulated miR22, miR-15a/16–1, miR-145, miR-203 (anti-oncogenic) decreased cell proliferation, tumor invasion and increased apoptosis	201
	Pancreas	Downregulated miR-200 and upregulated miR-21 lead to induction of PTEN protein, a marker for loss of tumor aggressiveness, upregulated miR-26a, miR-101, miR-146a,and miR-200b is associated with decreased cell survival and invasive cell migration	202 and 203
DNA	Hepatoma (G2), lung (A549), colon (HCT116)	Induced DNA damage, leading to cell cycle arrest and apoptosis	204
	Colon, pancreas (BxPC-3)	Activated ATM and Chk1, leading to double and single strand DNA breaks	205
	Breast	Preventing nuclear translocation of BRCA1 induced DNA damage and inhibited DNA repair	206
	Lung (A549)	Reduced protein levels of Chk1 and cytosolic cell division cycle 25, increased levels of p53 and p21CIP1, diminished levels of cyclin B1 and cell cycle arrest in G2/M phase	207

[a]↓downregulation/suppression/inhibition; ↑upregulation/expression/induction.

Bax and Bak can move to mitochondria, interrupt the function of Bcl-2, permeabilize the outer mitochondrial membrane and release the yield of the intermembrane space. Apoptosis can be induced by p53 (intrinsic pathway) and through extracellular signals (extrinsic pathway). An interplay between the extrinsic and the intrinsic apoptotic pathways leads to caspase-3, -8 and -9 activation. The signaling pathways of PI3K/Akt, MAPK and NF-κB, regulating cell proliferation and survival, are involved in apoptosis. The strong anticancer effect of curcumin is based on the inhibition of the NF-κB pathway, leading to activation of caspases and repression of cell survival factors. Caspases-3, -7, -8 and -9 can be activated by curcumin in colon cancer, but their reduced activation is related to the mitochondrial pathway.[200–202] Curcumin increases the permeability of the mitochondrial membrane and collapses the membrane potential. The inhibition of Akt phosphorylation, leading to increased apoptosis, is a further curcumin effect.[203] After curcumin treatment, suppressing Bcl-XL, cyclin and CDK1 levels, increased cleavaging PARP (Poly ADP-Ribose Polymerase) and reduced mitochondrial membrane potential were observed.[63,204] Cytotoxicity in prostate cancer cells was induced by an interaction of curcumin and TNFros-related apoptosis-inducing ligand (TRAIL)/Apo2L.[205] In a study with HL-60 leukemia cells curcumin increased levels of p27, p53, caspase-3 and Bax and decreased levels of Bcl-2 and Bcl-XL, followed by apoptosis.[60] In osteosarcoma cells curcumin activated caspase-3 and PARP cleavage.[56] Curcumin increased caspase-3 and caspase-9 activities, resulting in apoptosis of MCF-7 breast cancer cells.[62] Thus, one main factor for curcumin related apoptosis, decreasing mitochondrial membrane potential, followed by an increasing caspase activity, was suggested.[206] For non-small cell lung cancer (NSCLC) cells (NCI-H460) mitochondrial membrane potential decreased, leading to upregulated Bax, Bad, FAS/caspase-8, caspase-3 and reactive oxygen species (ROS) and downregulated Bcl-2, Bcl-XL and XIAP levels after curcumin treatment.[207] For SCLC cells (NCI-H446 and NCI- 688) STAT-3 mediated downregulation of survivin and Bcl-XL was reported after curcumin treatment.

9.3.7.2 Autophagy

The cellular death autophagy characterizes vesicle formation with cellular organelles (autophagosome), promoting an auto phagocytic process. In contrast to apoptosis, autophagy does not promote chromatin condensation and is involved in vacuolization of the cytoplasm. The autophagic death is reversible at the cellular level, but eliminating the stimuli results in interrupted autophagy. The lysosome mediated degradation pathway is crucial for supporting cellular constancy. It is proven that autophagy is crucial as a death inducer in apoptosis resistant cancer cells. Due to this, targeting autophagy is a promising tool against cancer cells. Curcumin can cause autophagy through mechanisms such as beclin-1 dependent

(canonical) and independent (non-canonical) pathways.[208] It was suggested that curcumin mediated autophagic cell death is dependent on the JAK pathway.[209]

9.4 Conclusion

This chapter gives an overview of the biosynthesis of curcumin, molecular targets and the biological mechanism of curcumin. Several biosynthesis pathways are described. The main difference in these pathways is based on the formed intermediates. In this case, the activities of these intermediates are crucial. Unfortunately, there are minimal studies available in the literature and it is important to have more specific research on this topic. Curcumin has a number of molecular targets as described, which makes it an important compound for many biological mechanisms. Curcumin shows antioxidant, anticancer, antiviral, antifungal, antiproliferative, anti-immunomodulatory and anti-inflammatory properties. Since cancer is a disease that is becoming common, we focused mainly on the anticancerous activity of curcumin.

References

1. M. D. C. Ramirez-Ahumada, B. N. Timmermann and D. R. Gang, *Phytochemistry*, 2006, **67**, 2017.
2. Y. Katsuyama, T. Kita, N. Funa and S. Horinouchi, *J. Biol. Chem.*, 2009, **284**, 11160.
3. Y. Katsuyama, M. Matsuzawa, N. Funa and S. Horinouchi, *J. Biol. Chem.*, 2007, **282**, 37702.
4. Y. Katsuyama, M. Matsuzawa, N. Funa and S. Horinouchi, *Microbiology*, 2008, **154**, 2620.
5. Y. Katsuyama, Y. Hirose, N. Funa, Y. Ohnishi and S. Horinouchi, *Biosci. Biotechnol. Biochem.*, 2010, **74**, 641.
6. R. B. Nair, Q. Xia, C. J. Kartha, E. Kurylo, R. N. Hirji, R. Datla and G. Selvaraj, *Society*, 2002, **130**, 210.
7. K. Deepa, T. E. Sheeja, O. B. Rosana, V. Srinivasan, K. S. Krishnamurthy and B. Sasikumar, *Ind. Crops Prod.*, 2017, **97**, 229.
8. P. H. Killian, E. Kronski, K. M. Michalik, O. Barbieri, S. Astigiano and C. P. Sommerhoff, *Carcinogenesis*, 2012, **33**, 2507.
9. E. Kronski, M. E. Fiori, O. Barbieri, S. Astigiano, V. Mirisola and P. H. Killian, *Mol. Oncol.*, 2014, **8**, 581.
10. S. Schwertheim, F. Wein, K. Lennartz, K. Worm, K. W. Schmid and S. Y. Sheu-Grabellus, *Cancer Res. Clin. Oncol.*, 2017, **143**, 1143.
11. A. Mantovani, P. Allavena, A. Sica and F. Balkwill, *Nature*, 208, **454**, 436.
12. A. C. Bharti, N. Donato, S. Singh and B. B. Aggarwal, *Blood*, 2003, **101**, 1053.
13. S. Singh and B. B. Aggarwal, *J. Biol. Chem.*, 1995, **270**, 24995.

14. A. C. Bharti, N. Donato and B. B. Aggarwal, *J. Immunol.*, 2003, **171**, 3863.
15. G. G. Mackenzie, N. Queisser, M. L. Wolfson, C. G. Fraga, A. M. Adamo and P. I. Oteiza, *Int. J. Cancer*, 2008, **123**, 56.
16. M. K. Bae, S. H. Kim, J. W. Jeong, Y. M. Lee, H. S. Kim and S. R. Kim, *Oncol. Rep.*, 2006, **15**, 1557.
17. H. Choi, Y. S. Chun, S. W. Kim, M. S. Kim and J. W. Park, *Mol. Pharmacol.*, 2006, **70**, 1664.
18. B. E. Bachmeier, I. V. Mohrenz, V. Mirisola, E. Schleicher, F. Romeo, C. Hohneke, M. Jochum, A. G. Nerlich and U. Pfeffer, *Carcinogenesis*, 2008, **29**, 779.
19. H. Lu, W. Ouyang and C. Huang, *Mol. Cancer Res.*, 2006, **4**, 221.
20. C. Park, G. Y. Kim, G. D. Kim, B. T. Choi, Y. M. Park and Y. H. Choi, *Oncol. Rep.*, 2006, **15**, 1225.
21. B. B. Aggarwal, S. Shishodia, Y. Takada, S. Banerjee, R. A. Newman and C. E. Bueso-Ramos, *Clin. Cancer Res.*, 2005, **11**, 7490.
22. J. W. Cho, K. S. Lee and C. W. Kim, *Int. J. Mol. Med.*, 2007, **19**, 469.
23. D. Ranjan, C. Chen, T. D. Johnston, H. Jeon and M. Nagabhushan, *Surg. Res.*, 2004, **121**, 171.
24. T. Kobayashi, S. Hashimoto and T. Horie, *Biochem. Pharmacol.*, 1997, **54**, 819.
25. A. J. Fahey, R. R. Adrian and C. S. Constantinescu, *J. Cell. Mol. Med.*, 2007, **11**, 1129.
26. J. A. Burger and A. Peled, *Leukemia*, 2009, **23**, 43.
27. P. J. Roughley and D. A. Whiting, *J. Chem. Soc., Perkin Trans.*, 1973, **1**, 2379.
28. J. Schröder, *Trends Plant Sci.*, 1997, **2**, 373.
29. T. Kita, S. Imai, H. Sawada, H. Kumagai and H. Seto, *Biosci., Biotechnol., Biochem.*, 208, **72**, 1789.
30. R. Renuka and C. Arumughan, *Bioresour. Technol.*, 2007, **98**, 3037.
31. Y. Zhao and A. A. Adjei, *Oncologist*, 2015, **20**, 660.
32. F. Shojaei, *Cancer Lett.*, 2012, **320**, 130.
33. L. M. Ellis and D. J. Hicklin, *Nat. Rev. Cancer*, 208, **8**, 579.
34. N. Ferrara, H. P. Gerber and J. LeCouter, *Nat. Med.*, 2003, **9**, 669.
35. A. K. Singh, G. S. Sidhu, T. Deepa and R. K. Maheshwari, *Cancer Lett.*, 1996, **107**, 109.
36. J. L. Arbiser, N. Klauber, R. Rohan, R. van Leeuwen, M. T. Huang and C. Fisher, *Mol. Med.*, 1998, **4**, 376.
37. R. Mohan, J. Sivak, P. Ashton, L. A. Russo, B. Q. Pham and N. Kasahara, *J. Biol. Chem.*, 2000, **275**, 10405.
38. C. Premanand, M. Rema, M. Z. Sameer, M. Sujatha and M. Balasubramanyam, *Invest. Ophthalmol. Visual Sci.*, 2006, **47**, 2179.
39. G. P. Nagaraju, S. Zhu, J. E. Ko, N. Ashritha, R. Kandimalla and J. P. Snyder, *Cancer Lett.*, 2015, **357**, 557.
40. L. C. Ferreira, A. S. Arbab, B. V. Jardim-Perassi, T. F. Borin, N. N. Gonçalves and R. S. V. Nadimpalli, *Cancer Res.*, 2015, **75**, A02.

41. A. E. Gururaj, M. Belakavadi, D. A. Venkatesh, D. Marmé and B. P. Salimath, *Biochem. Biophys. Res. Commun.*, 2002, **297**, 934.
42. S. Ray, N. Chattopadhyay, A. Mitra, M. Siddiqi and A. Chatterjee, *J. Environ. Pathol., Toxicol. Oncol.*, 2003, **22**, 49.
43. M. Shakibaei, T. John, G. Schulze-Tanzil, I. Lehmann and A. Mobasheri, *Biochem. Pharmacol.*, 2007, **73**, 1434.
44. J. Ravindran, S. Prasad and B. B. Aggarwal, *AAPS J.*, 2009, **11**, 495.
45. K. Krishnaraju, H. Q. Nguyen, D. A. Liebermann and B. Hoffman, *Mol. Cell. Biol.*, 1995, **15**, 5499.
46. A. Krones-Herzig, S. Mittal, K. Yule, H. Liang, C. English and R. Urcis, *Cancer Res.*, 2005, **65**, 5133.
47. S. Mazumder, E. DuPree and A. Almasan, *Curr. Cancer Drug Targets*, 2004, **4**, 65.
48. B. B. Aggarwal, S. Banerjee, U. Bharadwaj, B. Sung, S. Shishodia and G. Sethi, *Biochem. Pharmacol.*, 2007, **73**, 1024.
49. X. Li, K. Kikuchi and Y. Takano, *J. Oncol.*, 2010, **2011**, 963614.
50. S. Shishodia, M. M. Chaturvedi and B. B. Aggarwal, *Curr. Probl. Cancer*, 2007, **31**, 243.
51. P. Anand, C. Sundaram, S. Jhurani, A. B. Kunnumakkara and B. B. Aggarwal, *Cancer Lett.*, 2008, **267**, 133.
52. T. C. Hour, J. Chen, C. Y. Huang, J. Y. Guan, S. H. Lu and Y. S. Pu, *Prostate*, 2002, **51**, 211.
53. C. L. Yang, Y. Y. Liu, Y. G. Ma, Y. X. Xue, D. G. Liu and Y. Ren, *PLoS One*, 2012, **7**, e37960.
54. T. G. Lim, S. Y. Lee, Z. Huang, D. Y. Lim, H. Chen and S. K. Jung, *Cancer Prev. Res.*, 2014, **7**, 466.
55. K. Keyomarsi, S. L. Tucker, T. A. Buchholz, M. Callister, Y. Ding and G. N. Hortobagyi, *Engl. J. Med.*, 2002, **347**, 1566.
56. D. S. Lee, M. K. Lee and J. H. Kim, *Anticancer Res.*, 2009, **29**, 5039.
57. M. J. Park, E. H. Kim, I. C. Park, H. C. Lee, S. H. Woo and J. Y. Lee, *Int. J. Oncol.*, 2002, **21**, 379.
58. Y. Wang, T. Wang, Y. Han, H. Wu, W. Zhao and D. Tong, *Urol. Int.*, 2015, **94**, 464.
59. E. Liu, J. Wu, W. Cao, J. Zhang, W. Liu and X. Jiang, *J. Neuro-Oncol.*, 2007, **85**, 263.
60. T. W. Tan, H. R. Tsai, H. F. Lu, H. L. Lin, M. F. Tsou and Y. T. Lin, *Anticancer Res.*, 2006, **26**, 4361.
61. R. Sahu, S. Batra and S. Srivastava, *Br. J. Cancer*, 2009, **100**, 1425.
62. H. Q. Li, L. J. Jin, F. F. Wu, X. Y. Li, J. S. You and Z. H. Cao, *Afr. J. Pharm. Pharmacol.*, 2012, **6**, 864.
63. S. Shishodia, H. M. Amin, R. Lai and B. B. Aggarwal, *Biochem. Pharmacol.*, 2005, **70**, 700.
64. C. Beevers, F. Li, L. Liu and S. Huang, *Int. J. Cancer*, 2006, **119**, 757.
65. H. S. Liu, C. S. Ke, H. C. Cheng, C. Y. F. Huang and C. L. Su, *Mol. Pharmacol.*, 2011, **80**, 638.

66. L. Moragoda, R. Jaszewski and A. P. Majumdar, *Anticancer Res.*, 2001, **21**, 873.
67. C. Schaaf, B. Shan, M. Buchfelder, M. Losa, J. Kreutzer and W. Rachinger, *Endocr.-Relat. Cancer*, 2009, **16**, 1339.
68. Z. C. Li, L. M. Zhang, H. B. Wang, J. X. Ma and J. Z. Sun, *Tumor Biol.*, 2014, **35**, 111.
69. L. Wan, K. Pantel and Y. Kang, *Nat. Med.*, 2013, **19**, 1450.
70. P. S. Steeg, *Nat. Rev. Cancer*, 2016, **16**, 201.
71. J. Sleeman and P. S. Steeg, *Eur. J. Cancer*, 2010, **46**, 1177.
72. W. Yang, L. Zou, C. Huang and Y. Lei, *Drug Dev. Res.*, 2014, **75**, 331.
73. A. Shehzad, F. Wahid and Y. S. Lee, *Arch. Pharm.*, 2010, **343**, 489.
74. M. K. Shanmugam, G. Rane, M. M. Kanchi, F. Arfuso, A. Chinnathambi and M. E. Zayed, *Molecules*, 2015, **20**, 2728.
75. S. C. Gupta, S. Prasad, J. H. Kim, S. Patchva, L. J. Webb, I. K. Priyadarsini and B. B. Aggarwal, *Nat. Prod. Rep.*, 2011, **28**, 1937.
76. U. Cavallaro and G. Christofori, *Nat. Rev. Cancer*, 2004, **4**, 118.
77. D. Vergara, P. Simeone, D. Latorre, F. Cascione, S. Leporatti and M. Trerotola, *J. Biotechnol.*, 2015, **202**, 3.
78. B. Zhang, H. Zhang and G. Shen, *Jpn. J. Clin. Oncol.*, 2015, **45**, 755.
79. J. A. Galván, I. Zlobec, M. Wartenberg, A. Lugli, B. Gloor, A. Perren and E. Karamitopoulou, *Br. J. Cancer*, 2015, **112**, 1944.
80. S. L. Luo, Y. G. Xie, Z. Li, J. H. Ma and X. Xu, *Tumor Biol.*, 2014, **35**, 5533.
81. M. R. Schneider, F. Hiltwein, J. Grill, H. Blum, S. Krebs and A. Klanner, *Carcinogenesis*, 2014, **35**, 1855.
82. X. Liu and K. M. Chu, *BioMed Res. Int.*, 2014, **2014**, 63738.
83. A. G. Barber, M. Castillo-Martin, D. M. Bonal, A. J. Jia, B. A. Rybicki and A. M. Christiano, *Cancer Med.*, 2015, **4**, 1258.
84. F. Trillsch, S. Kuerti, C. Eulenburg, E. Burandt, L. Woelber and K. Prieske, *Br. J. Cancer*, 2016, **114**, 207.
85. C. C. Chen, M. Sureshbabul, H. W. Chen, Y. S. Lin, J. Y. Lee and Q. S. Hong, *Evidence-Based Complementary Altern. Med.*, 2013, **2013**, 1.
86. R. Kalluri and R. A. Weinberg, *J. Clin. Invest.*, 2009, **119**, 1420.
87. H. Yu, H. Lee, A. Herrmann, R. Buettner and R. Jove, *Nat. Rev. Cancer*, 2014, **17**, 736.
88. D. Eswaran and S. Huang, *Curr. Mol. Med.*, 2009, **9**, 626.
89. K. S. Siveen, S. Sikka, R. Surana, X. Daia, J. Zhang and A. P. Kumar, *Biochim. Biophys. Acta*, 2014, **1845**, 136.
90. S. Shishodia, *Biofactors*, 2013, **39**, 37.
91. L. Hu, L. Xia, H. Zhou, B. Wu, Y. Mu and Y. Wu, *Tumor Biol.*, 2013, **34**, 2573.
92. P. Polakis, *Cold Spring Harbor Perspect. Biol.*, 2012, **4**, 08052.
93. P. J. Morin, *BioEssays*, 1999, **21**, 1021.
94. H. W. Chen, J. Y. Lee, J. Y. Huang, C. C. Wang, W. J. Chen and S. F. Su, *Cancer Res.*, 2008, **68**, 7428.

95. C. Tan, L. Zhang, X. Cheng, X. F. Lin, R. R. Lu, J. D. Bao and H. X. Yu, *Exp. Biol. Med.*, 2015, **240**, 925.
96. C. Y. Zhang, L. Zhang, H. X. Yu, J. D. Bao and R. R. Lu, *Biotechnol. Lett.*, 2013, **35**, 995.
97. L. Zhang, X. Cheng, Y. Gao, C. Zhang, J. Bao and H. Guan, *Exp. Cell Res.*, 2016, **341**, 157.
98. Z. Zhang, H. Chen, C. Xu, L. Song, L. Huang and Y. Lai, *Oncol. Rep.*, 2016, **35**, 2615.
99. R. Blasius, S. Reuter, E. Henry, M. Dicato and M. Diederich, *Biochem. Pharmacol.*, 2006, **72**, 1547.
100. Y. P. Zhang, Y. Q. Li, Y. T. Lv and J. M. Wang, *Genet. Mol. Res.*, 2015, **14**, 1056.
101. C. Zhang, B. Li, X. Zhang, P. Hazarika, B. B. Aggarwal and M. Duvic, *J. Invest. Dermatol.*, 2010, **130**, 2110.
102. S. Uddin, A. R. Hussain, P. S. Manogaran, K. Al-Hussein, L. C. Platanias and M. I. Gutierrez, *Oncogene*, 2005, **24**, 7022.
103. N. Chakravarti, J. N. Myers and B. B. Aggarwal, *Int. J. Cancer*, 2006, **119**, 1268.
104. P. Poma, M. Notarbartolo, M. Labbozzetta, A. Maurici, V. Carina and A. Alaimo, *Int. J. Mol. Med.*, 2007, **20**, 329.
105. A. K. Ghosh, N. E. Kay, C. R. Secreto and T. D. Shanafelt, *Clin. Cancer Res.*, 2009, **15**, 1250.
106. J. Park, V. Ayyappan, E. K. Bae, C. Lee, B. S. Kim and B. K. Kim, *Mol. Oncol.*, 2008, **2**, 317.
107. L. Friedman, L. Lin, S. Ball, T. Bekaii-Saab, J. Fuchs and P. K. Li, *Anticancer Drugs*, 2009, **20**, 444.
108. W. Glienke, L. Maute, J. Wicht and L. Bergmann, *Cancer Invest.*, 2010, **28**, 166.
109. J. H. Seo, K. J. Jeong, W. J. Oh, H. J. Sul, J. S. Sohn and Y. K. Kim, *Cancer Lett.*, 2010, **288**, 50.
110. M. Saydmohammed, D. Joseph and V. Syed, *J. Cell. Biochem.*, 2010, **110**, 447.
111. M. A. Bill, C. Bakan, D. M. Benson Jr, J. Fuchs, G. Young and G. B. Lesinski, *Mol. Cancer Ther.*, 2009, **8**, 2726.
112. L. Wang, Y. Shen, R. Song, Y. Sun, J. Xu and Q. Xu, *Mol. Pharmacol.*, 2009, **76**, 1238.
113. C. Senft, M. Polacin, M. Priester, V. Seifert, D. Kogel and J. Weissenberger, *BMC Cancer*, 2010, **10**, 491.
114. J. Weissenberger, M. Priester, C. Bernreuther, S. Rakel, M. Glatzel and V. Seifert, *Clin. Cancer Res.*, 2010, **16**, 5781.
115. M. G. Alexandrow, L. J. Song, S. Altiok, J. Gray, E. B. Haura and N. B. Kumar, *Eur. J. Cancer Prev.*, 2012, **21**, 407.
116. K. Chiablaem, K. Lirdprapamongkol, S. Keeratichamroen, R. Surarit and J. Svasti, *Anticancer Res.*, 2014, **34**, 1857.
117. D. R. Siwak, S. Shishodia, B. B. Aggarwal and R. Kurzrock, *Cancer*, 2005, **104**, 879.

118. S. Aggarwal, H. Ichikawa, Y. Takada, S. K. Sandur, S. Shishodia and B. B. Aggarwal, *Mol. Pharmacol.*, 2006, **69**, 195.
119. M. S. Woo, S. H. Jung, S. Y. Kim, J. W. Hyun, K. H. Ko and W. K. Kim, *Biochem. Biophys. Res. Commun.*, 2005, **335**, 1017.
120. H. Zong, F. Wang, Q. W. Fan and L. X. Wang, *Mol. Biol. Rep.*, 2012, **39**, 4803.
121. Y. E. Marin, B. A. Wall, S. Wang, J. Namkoong, J. J. Martino and J. Suh, *Melanoma Res.*, 2007, **17**, 274.
122. T. L. Chiu and C. C. Su, *Int. J. Mol. Med.*, 2009, **23**, 469.
123. Q. Liu, W. T. Loo, S. Sze and Y. Tong, *Phytomedicine*, 2009, **16**, 916.
124. M. A. Khan, S. Gahlot and S. Majumdar, *Mol. Cancer Ther.*, 2012, **11**, 1873.
125. S. Liu, Z. Wang, Z. Hu, X. Zeng, Y. Li and Y. Su, *J. Huazhong Univ. Sci. Technol., Med. Sci.*, 2011, **31**, 530.
126. J. L. Dyer, S. Z. Khan, J. G. Bilmen, S. R. Hawtin, M. Wheatley and M. U. Javed, *Cell Calcium*, 2002, **31**, 45.
127. X. Wang, Q. Wang, K. L. Ives and B. M. Evers, *Clin. Cancer Res.*, 2006, **12**, 5346.
128. C. Polytarchou, M. Hatziapostolou and E. Papadimitriou, *J. Biol. Chem.*, 2005, **280**, 40428.
129. M. He, Y. Li, L. Zhang, L. Li, Y. Shen and L. Lin, *Oncol. Rep.*, 2014, **32**, 173.
130. H. J. Kim, S. Y. Park, O. J. Park and Y. M. Kim, *Mol. Med. Rep.*, 2013, **8**, 282.
131. C. P. Prasad, G. Rath, S. Mathur, D. Bhatnagar and R. Ralhan, *Chem.-Biol. Interact.*, 2009, **181**, 263.
132. A. Alonso, J. Sasin, N. Bottini, I. Friedberg, I. Friedberg and A. Osterman, *Cell*, 2004, **117**, 699.
133. R. Roskoski, *Pharmacol. Res.*, 2015, **100**, 1.
134. P. Lahiry, A. Torkamani, N. J. Schork and R. Hegele, *Nat. Rev. Genet.*, 2010, **11**, 60.
135. D. Fabbro, S. W. Cowan-Jacob and H. Moebitz, *Br. J. Pharmacol.*, 2015, **172**, 2675.
136. M. Starok, P. Preira, M. Vayssade, K. Haupt, L. Salomé and C. Rossi, *Biomacromolecules*, 2015, **16**, 1634.
137. G. Chadalapaka, I. Jutooru, R. Burghardt and S. Safe, *Mol. Cancer Res.*, 2010, **8**, 739.
138. J. Downward, *Cell Dev. Biol.*, 2004, **15**, 177.
139. E. Minet, G. Michel, D. Mottet, M. Raes and C. Michiels, *Free Radical Biol. Med.*, 2001, **31**, 847.
140. A. C. Faber, F. J. Dufort, D. Blair, D. Wagner, M. F. Roberts and T. C. Chiles, *Biochem. Pharmacol.*, 2006, **72**, 1246.
141. S. Reuter, S. Eifes, M. Dicato, B. B. Aggarwal and M. Diederich, *Biochem. Pharmacol.*, 208, **76**, 1340.
142. V. Carafa, A. Nebbioso and L. Altucci, *Front. Pharmacol.*, 2012, **3**, 1.

143. A. Hu, J. J. Huang, R. L. Li, Z. Y. Lu, J. L. Duan, W. H. Xu, X. P. Chen and J. P. Fan, *Sci. Rep.*, 2015, **5**, 13429.
144. A. Chen, J. Xu and A. C. Johnson, *Oncogene*, 2006, **25**, 278.
145. S. Wang, S. Yu, W. Shi, L. Ge, X. Yu, J. Fan and J. Zhang, *IUBMB Life*, 2011, **63**, 775.
146. Y. H. Soung and J. Chung, *Mol. Cancer Ther.*, 2011, **10**, 883.
147. C. Ji, C. Cao, S. Lu, R. Kivlin, A. Amaral, N. Kouttab, H. Yang, W. Chu, Z. Bi, W. Di and Y. Wan, *Cancer Chemother. Pharmacol.*, 2008, **62**, 857.
148. J. Kizhakkayil, F. Thayyullathil, S. Chathoth, A. Hago, M. Patel and S. Galadari, *Biochem. Biophys. Res. Commun.*, 2010, **394**, 476.
149. A. R. M. R. Amin, A. Haque, M. A. Rahman, Z. G. Chen, F. R. Khuri and D. M. Shin, *PLoS One*, 2015, **10**, e0124218.
150. A. R. Hussain, M. Al-Rasheed, P. S. Manogaran, K. A. Al-Hussein, L. C. Platanias and K. Al Kuraya, *Apoptosis*, 2006, **11**, 245.
151. Z. Zhao, C. Li, H. Xi, Y. Gao and D. Xu, *Mol. Med. Rep.*, 2015, **12**, 5415.
152. T. F. Wong, T. Takeda, B. Li, K. Tsuiji, M. Kitamura and A. Kondo, *Gynecol. Oncol.*, 2011, **122**, 141.
153. T. F. Wong, T. Takeda, B. Li, K. Tsuiji, A. Kondo and M. Tadakawa, *Int. J. Clin. Oncol.*, 2014, **19**, 354.
154. Y. Akkoç, Ö. Berrak, E. D. Arısan, P. Obakan, A. Çoker-Gürkan and N. Palavan-Ünsal, *Biochem. Pharmacol.*, 2015, **71**, 161.
155. S. H. Jee, S. C. Shen, C. R. Tseng, H. C. Chiu and M. L. Kuo, *J. Invest. Dermatol.*, 1998, **111**, 656.
156. T. Choudhuri, S. Pal, T. Das and G. Sa, *J. Biol. Chem.*, 2005, **280**, 20059.
157. G. Radhakrishna Pillai, A. S. Srivastava, T. I. Hassanein, D. P. Chauhan and E. Carrier, *Cancer Lett.*, 2004, **208**, 163.
158. J. L. Watson, A. Greenshields, R. Hill, A. Hilchie, P. W. Lee and C. A. Giacomantonio, *Mol. Carcinog.*, 2010, **49**, 13.
159. M. Zheng, S. Ekmekcioglu, E. T. Walch, C. H. Tang and E. A. Grimm, *Melanoma Res.*, 2004, **14**, 165.
160. G. Song, Y. B. Mao, Q. F. Cai, L. M. Yao, G. L. Ouyang and S. D. Bao, *Braz. J. Med. Biol. Res.*, 2005, **38**, 1791.
161. Z. Y. He, C. B. Shi, H. Wen, F. L. Li, B. L. Wang and J. Wang, *Cancer Invest.*, 2011, **29**, 208.
162. M. Shi, Q. Cai, L. Yao, Y. Mao, Y. Ming and G. Ouyang, *Cell Biol. Int.*, 2006, **30**, 221.
163. S. Shankar and R. K. Srivastava, *Int. J. Oncol.*, 2007, **30**, 905.
164. A. Liontas and H. Yeger, *Anticancer Res.*, 2004, **24**, 987.
165. J. Wu, Q. Tang, S. Zhao, F. Zheng, Y. Wu and G. Tang, *Int. J. Oncol.*, 2014, **45**, 95.
166. M. Li, Z. Zhang, D. L. Hill, H. Wang and R. Zhang, *Cancer Res.*, 2007, **67**, 1988.
167. Y. W. Kong, D. Ferland-McCollough, T. J. Jackson and M. Bushell, *Lancet Oncol.*, 2012, **13**, e249.
168. A. Lujambio and S. W. Lowe, *Nature*, 2012, **482**, 347.

169. A. M. Croce and G. A. Calin, *Cell*, 2005, **122**, 6.
170. A. Esquela-Kerscher and F. J. Slack, *Nat. Rev. Cancer*, 2006, **6**, 259.
171. P. S. Meltzer, *Nature*, 2005, **435**, 745.
172. J. Krol, I. Loedige and W. Filipowicz, *Nat. Rev. Genet.*, 2010, **11**, 597.
173. A. J. Minn, G. P. Gupta, P. M. Siegel, P. D. Bos, W. Shu and D. D. Giri, *Nature*, 2005, **436**, 518.
174. S. Reuter, S. C. Gupta, B. Park, A. Goel and B. B. Aggarwal, *Genes Nutr.*, 2011, **6**, 93.
175. F. Zsila, Z. Bikadi and M. Simonyi, *Org. Biomol. Chem.*, 2004, **2**, 2902.
176. S. Nafisi, M. Adelzadeh, Z. Norouzi and M. N. Sarbolouki, *DNA Cell Biol.*, 2009, **28**, 201.
177. E. P. Rogakou, D. R. Pilch, A. H. Orr, V. S. Ivanova and W. M. Bonner, *J. Biol. Chem.*, 1998, **273**, 5858.
178. J. W. Harper and S. J. Elledge, *Mol. Cell*, 2007, **28**, 739.
179. J. P. Kruse and W. Gu, *Cell*, 2009, **137**, 609.
180. A. J. Levine, *Cell*, 1997, **88**, 323.
181. A. Villunger, E. M. Michalak, L. Coultas, F. Müllauer, G. Böck and M. J. Ausserlechner, *Science*, 2003, **302**, 1036.
182. M. Sun, Z. Estrov, Y. Ji, K. R. Coombes, D. H. Harris and R. Kurzrock, *Mol. Cancer Ther.*, 2008, **7**, 464.
183. H. Jin, F. Qiao, Y. Wang, Y. Xu and Y. Shang, *Oncol. Rep.*, 2015, **34**, 2782.
184. W. Zhang, W. Bai and W. Zhang, *Clin. Transl. Oncol.*, 2014, **16**, 708.
185. J. Zhang, Y. Du, C. Wu, X. Ren, X. Ti and J. Shi, *Oncol. Rep.*, 2010, **24**, 1217.
186. H. Guo, Y. Xu and Q. Fu, *Tumor Biol.*, 2015, **36**, 8511.
187. W. Gao, J. Y. Chan and T. S. Wong, *Clin. Sci.*, 2014, **127**, 571.
188. G. Mudduluru, J. N. George-William, S. Muppala, I. A. Asangani, R. Kumarswamy and L. D. Nelson, *Biosci. Rep.*, 2011, **31**, 185.
189. S. U. Gandhy, K. Kim, L. Larsen, R. J. Rosengren and S. Safe, *BMC Cancer*, 2012, **12**, 1.
190. S. Saini, S. Arora, S. Majid, V. Shahryari, Y. Chen and G. Deng, *Cancer Prev. Res.*, 2011, **4**, 1698.
191. S. M. Gao, J. J. Yang, C. Q. Chen, J. J. Chen, L. P. Ye and L. Y. Wang, *J. Exp. Clin. Cancer Res.*, 2012, **31**, 27.
192. S. Sreenivasan, K. Thirumalai, R. Danda and S. Krishnakumar, *Curr. Eye Res.*, 2012, **37**, 421.
193. D. Subramaniam, S. Ponnurangam, P. Ramamoorthy, D. Standing, R. J. Battafarano and S. Anant, *PLoS One*, 2012, **7**, 30590.
194. C. C. Yu, L. L. Tsai, M. L. Wang, C. H. Yu, W. L. Lo and Y. C. Chang, *Cancer Res.*, 2013, **73**, 3425.
195. S. Ali, A. Ahmad, S. Banerjee, S. Padhye, K. Dominiak and J. M. Schaffert, *Cancer Res.*, 2010, **70**, 3606.
196. B. Bao, S. Ali, S. Banerjee, Z. Wang, F. Logna and A. S. Azmi, *Cancer Res.*, 2011, **72**, 335.
197. J. Cao, Y. Liu, L. Jia, H. M. Zhou, Y. Kong and G. Yang, *Free Radical Biol. Med.*, 2007, **43**, 968.

198. J. J. Lu, Y. J. Cai and J. Ding, *Mol. Cell. Biochem.*, 2011, **354**, 247.
199. Z. Jiang, S. Jin, J. C. Yalowich, K. D. Brown and B. Rajasekaran, *Mol. Cancer Ther.*, 2010, **9**, 558.
200. D. L. Rowe, T. Ozbay, R. M. O'Regan and R. Nahta, *Breast Cancer*, 2009, **3**, 61.
201. M. H. Pan, W. L. Chang, S. Y. Lin-Shiau, C. T. Ho and J. K. Lin, *J. Agric. Food Chem.*, 2001, **49**, 1464.
202. R. Rashmi, T. R. Santhosh Kumar and D. Karunagaran, *FEBS Lett.*, 2003, **538**, 19.
203. M. S. Squires, E. A. Hudson, L. Howells, S. Sale, C. E. Houghton and J. L. Jones, *Biochem. Pharmacol.*, 2003, **65**, 361.
204. S. Balasubramanian and R. L. Eckert, *Toxicol. Appl. Pharmacol.*, 2007, **224**, 214.
205. D. Deeb, Y. X. Xu, H. Jiang, X. Gao, N. Janakiraman and R. A. Chapman, *Mol. Cancer Ther.*, 2003, **2**, 95.
206. S. S. Lin, H. P. Huang, J. S. Yang, J. Y. Wu, T. C. Hsai and C. C. Lin, *Cancer Lett.*, 208, **272**, 77.
207. S. H. Wu, L. W. Hang, J. S. Yang, H. Y. Chen, H. Y. Lin and J. H. Chiang, *Anticancer Res.*, 2010, **30**, 2125.
208. N. Hasima and B. Ozpolat, *Cell Death Dis.*, 2014, **5**, e1509.
209. M. Katamura, E. Iwai-Kanai, M. Nakaoka, Y. Okawa, M. Ariyoshi and Y. Mita, *J. Clin. Exp. Cardiol.*, 2014, **5**, 337.

CHAPTER 10

The Effect of Turmeric in Gut Diseases

AUGUSTINE AMALRAJ*[a], NIMISHA PULIKKAL SUKUMARAN[a], AKHILA NAIR[a] AND SREERAJ GOPI*[a]

[a]R&D Centre, Aurea Biolabs (P) Ltd, Kolenchery, Cochin–682 311, Kerala, India
*E-mail: augustin14amal@gmail.com, amalraj.a@plantlipids.com, sreerajgopi@yahoo.com

10.1 Introduction

The gastrointestinal tract (GIT) is a tubular tract that runs from the mouth to the anus, including organs such as the pancreas, salivary glands and hepatobiliary system. The gut is under the control of neuro-hormonal system and is divided by thick sphincters as well as being in physical continuity to perform specific functions. The GIT performs various functions, such as nutrient absorption and waste elimination, which is essential for the normal functioning of the human body.[1] There are trillions of microorganisms involved in human gut health, which cover over 1000 bacterial species. This microbial community in the gut is important for the physiology of humans, metabolism and the maintenance of general health.[2–4] There have been many studies which indicate that modifications in the composition of gut microbiota are connected to various diseases such as obesity, liver, diabetes, inflammatory bowel, psoriasis, cancer, neurodegenerative diseases and depression.[4–8]

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

Thus, the gut microbiota has been widely considered as a promising therapeutic target for these gut microbiota-associated diseases.[9–12]

As the GIT is a very sensitive organ, it is effected by numerous factors, which can be either exogenous or endogenous. Disorders can be grouped uinto mild and severe types, where mild are altered bowel habits, celiac disease, lactose intolerance, worm infestation, microbial infection, gastrointestinal reflux disease, dyspepsia, acid peptic ulcer and severe include chronic diarrhea, inflammatory bowel diseases and cancer.[1] Gut health, which is mainly divided into diet, mucosa and commensal flora, is a complex concept. The mucosa is composed of gut-associated lymphoid tissue, digestive epithelium and mucus overlying the epithelium. The gut related commensal bacteria, lymphoid tissue, mucus, and host epithelial cells interact with each other, producing a dynamic and delicate equilibrium with the alimentary tract which indicates well organized functioning of the digestive system.[13,14]

Inflammatory bowel diseases (IBDs) are chronic diseases of the gastrointestinal tract, including ulcerative colitis (UC) and Crohn's disease (CD). These are characterized by recurrent inflammation, influenced by behavior and location and affect millions of people all over the world.[15] Around 12 to 20% people are effected by IBD globally.[16] About 1.6 million people in the United States of America have IBD and the direct and indirect annual costs of the disease is estimated to about $31 billion dollars. The medical costs of CD disease affected patients annually range from $8265 to $18963 and hence there are many patients affected by CD who have to pay large medical bills.[17] It is suspected that the pathophysiology of IBD has immune, genetic, neurological, inflammatory, environmental, and psychological factors but the most prominent of all is the mucosal inflammation (uncontrolled) that produces an abnormal immune response against environmental triggers which are unknown.[18] CD is a systemic, replacing inflammatory disease which affects the gastrointestinal tract comprising stomach, esophagus, colon and small intestine. Most of the CD-suffering patients have perianal lesions. Other features related to CD are inflammatory response associated with lymphoid aggregates, thickness of the bowel wall as well as granulomas.[16] In contrast, changes in acute remission phases are symbolic of UC that affect adolescent and life get deteriorated with the advancement in disease. This advancement can cause impairment of normal physiology and anatomy of the colon due to dysmotility, structuring, anorectal dysfunction and proximal extension consequently leading to urgency, tenesmus and incontinence.[19] These features when amalgamated with the dysplasia commencement, colorectal cancer and extra-intestinal manifestation phenomenon effect the quality of life in patient. UC revolves around colon and symptoms indicate diarrhea and intermittent or continuous blood in stool.[16,19]

Conventionally, IBD symptoms include a periodic abdominal pain, fever, vomiting, bloody stools, weight loss and diarrhea which leads to colorectal cancer and also disturbs the quality of life.[20,21] Even the etiology of IBD is not

fully understood and it is speculated that it is influenced by environmental interaction, immunological factors and genetic factors.[22] Interference in the mode of synthesis, excessive production of reactive oxygen species (ROS), release of anti-inflammatory cytokines such as interleukin (IL)-10, IL-4, IL-11, pro-inflammatory cytokines like tumor necrosis factor α (TNF-α), IL-6, IL-12, IL-1β and interferon γ (IFN-γ), and the transforming growth factor (TGF)-β cause tissue damage and are the prime factors responsible for intestinal inflammation.[21,23,24]

The basic therapeutic strategies involved in the treatment of IBD are the use of anti-inflammatory drugs like immunosuppressants, biological agents, corticosteroids and aminosalicylates. But these medications result in severe adverse effects like diarrhea, nausea, allergic reactions, pancreatitis and lymphopenia which is mainly due to systemic absorption.[25,26] The adverse effects caused by these medications are immense.[21,22,27] Hopefully, natural products may be a promising source of new therapeutic agents in IBD.[15] Statistically, about three-quarters of the globe uses natural remedies on daily basis according to the World Health Organization (WHO). Recently, people with gastrointestinal disorders have been increasingly utilizing herbal supplements for improvement and symptomatic relief in the physiological function of the GIT. Approximately, 8–50% of the population of Western Europe and the USA are using complementary therapy by varying its quality and design.[1,28] There are various studies which report biological properties such as regulation of inflammatory mediators like TNF-α, IL-6, IL-10, IL-1β, cyclooxygenase (COX-2), prostaglandin E2 (PGE-2), nitric oxide (NO) synthase (iNOS) and compounds that possess antioxidant properties.[15,26,29]

10.2 Importance of Turmeric and Its Active Constituent Curcumin

One of the natural herb is Turmeric (*Curcuma longa* Linn) -from the Zingiberaceae family, which is cultivated in subtropical and topical regions around the globe. This herb originates from Sothern Asia, Indonesia, and India. Since time immemorial, Turmeric has been used to maintain oral hygiene. It has been used for medicinal purposes for centuries in countries like China and India, such as for liver and jaundice diseases. Turmeric is scientifically proven to possess a plethora of pharmacological activities such as anti-venom, antioxidant, anti-malarial, anti-proliferative, anti-tumor, anti-inflammatory, anti-aging and anti-angiogenic properties. Additionally, it is also used in the treatment of parasitic infections, skin diseases, ulcers, cold, flu and anti-immune diseases. The bioactive constituents of turmeric consists of curcumin (CUR), bisdemethoxycurcumin (BDMC) and demethoxy curcumin (DMC) which are collectively known as curcuminoids.[30] The recommended daily intake of curcuminoids as food supplements by the World

Health Organization is in the range of 0–3 mg kg^{-1}. The turmeric products and curcuminoids are generally recognized as safe (GRAS) by Food and Drug Administration in the USA. It is suggested that the average intake of turmeric in the Indian diet is about 2–2.5 g per 60 kg individual, which approximately equates to 60–100 mg of CUR.[31] Curcuminoids are extensively used because of numerous biological targets and least side effects that are helpful to improve metabolic diseases, immune disorders as well as cancer. Great attention has been garnered in the Indian Ayurvedic system for inflammatory conditions due to its strong anti-inflammatory properties. The experimental models on CUR have been demonstrated to prevent dextran sodium sulphate (DSS),[33,34] as well as trinitrobenzene sulfonic acid (TNBS).[32] NF-κB inhibition is presumed to be a plausible mechanism behind the anti-inflammatory activities of CUR.[35,36] To elaborate, the IKK (IκB kinase) inhibits both degradation of IκB (inhibitor of NF-κB).[37] In the colonic mucosa the suppression of the activation of NF-κB supports the expression of Th1 and Th2 cytokines for CUR pretreated TNBS induced colitis mice.[36] In Figure 10.1, an overview of the anti-inflammatory effects of CUR are summarized. Another mechanism corresponds to the reduction of COX-2 as well as iNOS production by regulation of neutrophil chemotaxis and inhibition of p38-mitogen activated protein kinase (MAPK) signaling.[38,39] CUR, which contains pleiotropic effects, causes the reduction in myeloperoxidase (MPO) activity (neutrophilic infiltration marker) in the colon and various pro-inflammatory cytokines such as IFN-γ, IL-1β17, IL-1716.[37,40,41]

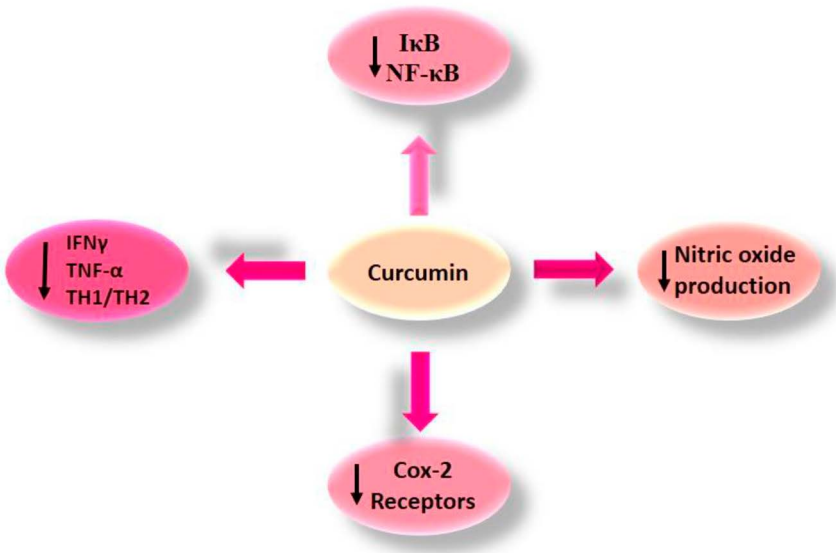


Figure 10.1 An overview of the anti-inflammatory effects of CUR.

10.3 Mechanism of Action of CUR Against Gut Diseases

The GIT is prone to ROS attack as it has access to the outside environment with resident immune cells and intestinal flora as well as dietary factors, which usually forms the potential sources of ROS.[42] Although the epithelial layer forms a protective barrier, GIT is prone to develop inflammatory cytokines and suffer changes in the redox equilibrium *via* production of excessive amounts of ROS and reactive nitrogen species (RNS) through pathogens and ingested materials. Thus, GIT develops inflammation by activating polymorphonuclear neutrophils (PMNs), epithelium and macrophages, which further cause oxidative stress. Oxidative stress is responsible for maintaining active inflammation through induction of transcription factors and redox-sensitive signaling pathways; however, this is not considered the actual cause of inflammation, but a major risk factor in disease progression. Besides, endoplasmic reticulum stress causes intestinal tract inflammation through the accumulation of misfolded proteins causing hyper activation of secretory cells.[43] The main pro-inflammatory pathways that are responsible for the inflammatory responses in the gut include NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), inhibitor of kappa B (I κB), interferon gamma (IFNγ), TNF-α and COX-2.[44] CUR, a pleiotropic polyphenol is considered to modulate various signaling pathways to exert anti-inflammatory properties.[45] The anti-inflammatory properties of CUR are attributed mainly to its interference with the arachidonic acid cascade and blocking of NF-κB activity, which is implicated in the regulation of pro-inflammatory enzymes.[46] Thus, numerous studies of the molecular standpoint support the ability of CUR to lower concentrations of TNF-α, IL-6, COX-1, INF-γ, NF-κB and many other molecules associated with inflammatory processes.[47,48] An overview of the mechanism of action of CUR against gut diseases and the major signaling events involved is summarized and depicted in Table 10.1 and Figure 10.2, respectively.

10.3.1 Inhibition of Transcription Factor NF-κB Activation and IκB Phosphorylation

NF-κB play a pivotal role in inflammation. They comprise p50, p65 and c-Rel subunits in colonic smooth cells. The interaction of the p65 subunit with inhibitory protein IκB, which are secluded in the cytoplasm, helps to camouflage the nuclear localizing sequence of NF-κB.[49] Moreover, there are many stimuli that help to activate these pathways such as viruses, bacterial components such as lipopolysaccride (LPS) and pro-inflammatory cytokines such as IL-1, TNF-α. Indeed, NF-κB pathways control and induce pro-inflammatory cytokines, which form the root cause of inflammation through activation, stimulation and differentiation of lamina propria immune cells. Additionally, TNF-α, which is responsible for activation of the NF-κB pathway, help

Table 10.1 Overview of the mechanism of action of CUR against gut diseases.

Target	Molecular activity	Consequence of action	Reference
Inhibition of nitric oxide Production	Release of chloride ions into the intracellular medium	Ensuing water loss to develop osmotic diarrhea and diffuses into the layers of muscle	16
Regulating shift of TH1/TH2	Increase TH2 and decrease TH1 expression	Reduced lipid peroxidation and iNOS	16
Inhibition of transcription factor NF-κB activation	Inhibiting phosphorylation of IκB on serine 32 and 36	Blocks degradation and activation of NF-κB	51 and 52
Inhibition of COX-2 receptors	Inhibit the COX-2 mRNA	COX-2 expression inhibition by CUR in gastrointestinal cell lines	55 and 56
Inhibition of IFNγ and TNFα	Inhibit apoptosis causing IFNγ signaling	Suppressing the levels of TNF-α	59

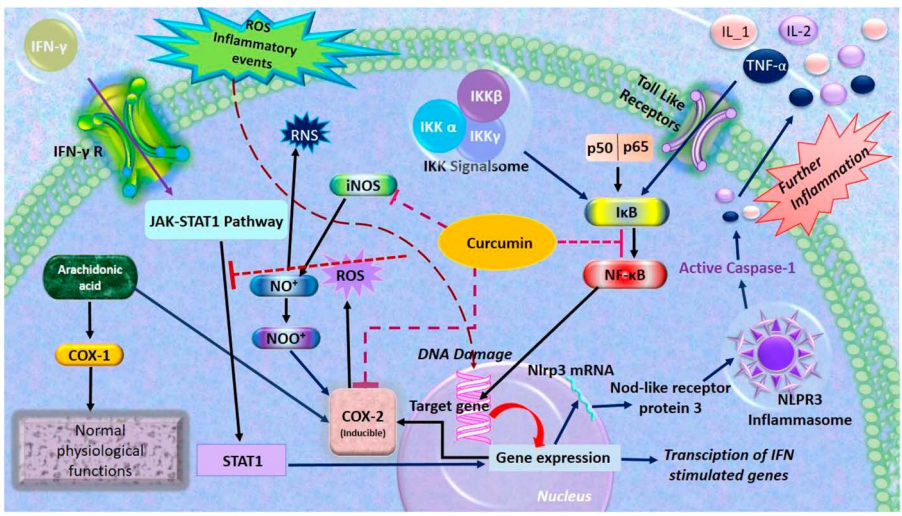


Figure 10.2 The cellular signaling mechanism by which CUR exhibits anti-inflammatory properties.

in the production of ROS. Furthermore, tumor necrosis factor receptor 1 (TNF-R1) along with tumor necrosis factor receptor 2 (TNF-R2) are escalated during inflammation in the intestinal lumen.[50]

In a study it was reported that CUR exhibits NF-κB inhibitory effects by blocking phosphorylation and degrading IκBα with the help of IKK and re-localization of NF-κB into the nucleus. Thus, for the degradation and activation of NF-κB to occur, phosphorylation of IκB is essential. The IKK

signalsome, which is a high molecular mass complex containing several inducible kinases such as the inhibitor of kappa-B kinase a (IKKa) or KKI-1, inhibitor of nuclear kappa-B kinase subunit alpha (IKK-α) or IKK1 and inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β) or IKK-2. These kinases are major factors responsible for phosphorylation to occur on serine 32 and 36.[51] CUR can occlude the degradation and phosphorylation of IκB and downregulate the activation of NF-κB. They also inhibit IκB through TNF and have the capability of downregulating pro-inflammatory cytokines, facilitate transfer of induced NF-αB from cytoplasm to nucleus and manage inflammatory responses by initiating gene transcription and protein synthesis.[52]

10.3.2 Inhibition of Nitric Oxide Production

Nitric oxide (NO) is an inflammatory mediator that is excessively produced in microglial cells *via* certain inflammatory responses. NO facilitates the release of chloride ions into the intracellular medium with ensuing water loss to develop osmotic diarrhea and diffuses into the layers of muscle to cause toxic megacolon.[16] Precisely, these cells are cataloged under brain mononuclear phagocytes and are considered to be the main cause of neurodegenerative diseases, especially the neurotoxicity caused due to the NO released from it. NO production is facilitated by a chain of chemical reactions *via* iNOS. Indeed, it is crucial in stimulating inflammatory responses *via* induction of iNOS[53] and CUR is considered to inhibit the elevation of iNOS expression.[54]

10.3.3 Inhibition of COX-2 Receptors

Cyclooxygenase-2 or prostaglandin endoperoxide synthase (PTGS) are enzymes, denoted as an immediate response to mitogen stimuli and pro-inflammatory mediators. They act in synergy with iNOS to escalate inflammation. The expressions of COX-2 and iNOS are upregulated by activated mitogen activating protein kinase (MAPKs) in intestinal epithelial cells.[38] COX-2 inhibitors are also induced by various agents such as reactive oxygen intermediates and inflammatory cytokines. CUR is reported to inhibit the mRNA and protein expression of COX-2, which supports the previous study of induced (phorbol ester and bile acid) COX-2 expression inhibition by CUR in gastrointestinal cell lines. This benefit has proven CUR to be a potent, alternative non-toxic agent to be used to inhibit COX-2.[55,56]

10.3.4 Inhibition of Cytokines

Cytokines regulate immune responses that are critical factors associated with anti-inflammatory effects. In addition, they are responsible for arbitrating proliferation and activation in the adaptive immune system. Moreover, inflammation is caused due to the imbalance in inflammatory cytokines of intestinal mucosa, which are necessary for normal gut hemostasis.[57]

Commonly, cytokines are classified into two categories: pro-inflammatory and anti-inflammatory cytokines, where pro-inflammatory cytokines are IL-1, IL-12, IL-18, IFN-γ, TNF, granulocyte-macrophage, and anti-inflammatory cytokines are IFN-α, TGF-β, IL-4, IL-10, IL-13.[58] The therapeutically relevant cytokines, which are a prominent link to inflammatory effects, are type II interferon, TNFα, type I and II T-helper cells (TH1/TH2). Nevertheless, the permeability of the intestinal barrier is maintained by epithelial tight junctions (TJ), which are types of multiple protein complexes located at the apical junction of intestinal epithelial cells. Favorably, the cytokines function independently to interrupt the epithelial barrier and collaborate with cellular redistribution and relocalization of transmembrane proteins such as claudins, tricellulin, occludin and junctional adhesion molecules to encourage paracellular permeability. Further, myosin light chain (MLC) kinase-mediated phosphorylation also supports the disruption of epithelial TJ. The depletion of the epithelial barrier is the result of intracellular redistribution that escalates the endocytosis of junctional molecules to enhance the inflammatory invasion of cytokines.[57]

10.3.4.1 Inhibition of IFNγ and TNFα

A prototypical type II interferon, known as IFNγ, is capable of binding to the receptor IFNGR1 (a cell surface receptor) through the JAK-STAT1 pathway. After the activation of the receptor at IFN-γ activation site, STAT1 tends to bind to DNA, resulting in transcription of IFN stimulated genes. These genes are helpful in the defense mechanism against intracellular pathogens. In the same way, during inflammation there is another inflammatory cytokine TNFα that is produced by monocytes/macrophages. It produces a range of signaling functions that is resistant to infection and causes apoptosis or necrosis.[59] CUR exerts its anti-inflammatory effects *via* inhibiting various inflammatory pathways such as IFNγ and TNFα. With the help of a biphasic mechanism of action, CUR is thought to inhibit the IFNγ signaling in epithelial cells. Furthermore, it is capable of suppressing the levels of TNFα, which is considered a major cause of inflammation.[60]

10.3.4.2 Regulating Shift of TH1/TH2

Type 1T helper (TH1) is a main factor for phagocyte dependent protective responses and cell mediated immunity that can develop infection through viruses and intracellular bacteria, whereas type 2T helper cells provide phagocyte independent protective responses and develop infection through gastrointestinal nematodes. They are also responsible for various immunopathological responses. Promisingly, CUR is reported to increase TH2 cytokines and decrease TH1 cytokine expression in colonic mucosa. Moreover, the IFNγ/IL-4 expression responsible for the balance of TH1/TH2 cytokines in circulation and splenocytes are also elevated by CUR.

The decrease in TH1 expression symbolizes the anti-inflammatory effects of CUR, which also reduces lipid peroxidation and iNOS to diminish tissue related injuries.[57]

10.4 Clinical Trials and Studies Conducted on CUR

CUR has long been projected to be a drug candidate for several major human diseases because of its antioxidative, anti-inflammatory and anticancerous effects. Even though preliminary results support the ability of CUR, the data obtained are not conclusive enough to conclude its efficiency as a therapeutic agent. Thus, it is imperative that well-designed clinical trials, supported by better formulations of CUR or novel routes of administration are of subtle importance.[61] Pointedly, a double blind, randomized, randomized, double-blind, single-centre pilot trial was performed on 45 patients with mild to moderate disease activity and distal UC with b25 cm involvement. The use of standardized CUR showed greater improvements in disease activity compared to placebos in patients with mild-to-moderate distal UC.[37] The controlling effects of oral CUR administration were investigated on the gut microbiota of mice (C57BL/6). It was observed that CUR notably affected the families of gut microbial group, which is probably in inclusive of *Bacteroidaceae*, *Prevotellaceae* and *Rikenellaceae*. Taking this into account, in a study, the pathogenic associations between numerous diseases and gut microbiota demonstrated the bioactive properties of CUR.[8] Some of the *in vivo* studies of the anti-inflammatory action of CUR are summarized in Table 10.2. A partially blind, randomized, two-dose, pilot study was conducted to assess the effects of turmeric extract on irritable bowel syndrome (IBS) symptomology in randomized 207 suitable volunteers. A post-study analysis revealed that the abdominal pain/discomfort score reduced significantly and all subjects reported an improvement in symptoms after treatment.[62] Furthermore, turmeric ameliorated the mean macroscopic ulcer score, body weight gain, microscopic ulcer scores, and reduced IL-23 and MPO in the colon of rats treated with acetic acid induced IBD. This study also indicated that the mean serum glutathione level promote reduction in the oxidative stress linked to IBD.[63]

NLRP3 inflammasome mediates IL-1β maturation, and therefore plays a vital role in the development of IBD. Moreover, in DSS-stimulated macrophages, CUR dramatically inhibits NLRP3 inflammasome activation, which is indicated by decreased caspase-1 activation, lowered IL-1β secretion, as well as ASC specks. CUR also prevented major cellular activities leading to NLRP3 inflammasome activation such as intracellular ROS formation, DSS-induced K+ efflux, and cathepsin B release. Administration of CUR reduced the disease assay index, colon length shortening and weight loss, hence improved colitis symptoms. CUR significantly lowered multiple inflammatory cytokine expression such as IL-6, MCP-1, IL-1β, caspase-1 activity, MPO activity and histopathological damage. The inhibitory effect of CUR on

Table 10.2 *In vivo* studies and the efficacy of the anti-inflammatory activities of turmeric.

Active ingredient used	Animal model used	Mechanism of action	Main findings	Reference
CUR	DSS-induced UC mice	Recovery of liver and serum paraoxonase activities.	Higher MPO activity, less body weight loss and longer colon lengths	48
Turmeric	Acetic acid induced IBD rats	Reduced MPO and IL-3	Increase in glutathione level and reduced oxidative stress	62
CUR	DSS-induced colitis in mice	Decreased the expression IL-1β, IL-6, MCP-1, MPO activity, caspase-1 activity	Suppressed DSS-induced NLRP3 inflammasome activation	63
CUR	TNF-α injected mice	Inhibiting neutrophils priming and iNO synthase	Attenuated the hallmarks of oxidative stress, neutrophils influx and ROS-related cellular and histological damages	64
CUR	*Mdr1a*–/– mouse	Downregulation of transcription factors and other regulatory molecules (ERK, FN1, TNFSF12 and PI3K complex)	Reduced immune response and inflammation through decreased neutrophil migration and increased barrier remodeling.	65
CUR	UC-mice	Substantial effects on serum and liver paraoxonase enzyme activity, erythrocyte CA activity, and hepatic cytosolic β-glucosidase activity.	In turn resulted in higher MPO activity, less body weight loss and longer colon lengths compared to therapeutic group indicating preventive role of CUR in IBDs	66
CUR	2,4,6-Trinitrobenzene sulfonic acid -induced colitis rats	Decreased the expression of MMP-1, MMP-3 and TIMP-1	Decrease in colon mucosal injury	67
CUR	TNBS-induced intestinal inflammation rats	Elevating the expression of SOCS-1 and inhibiting JAK/STAT pathways	Reduced apoptosis through the mitochondrial pathway and declined the accumulation of cytochrome C in cytosol	68
CUR	TRPV1 knockout and TNBS-treated Kunming mice	Antagonizing the transient receptor potential vanilloid-1 (TRPV1) receptor	Inhibits visceral nociception	69

DSS-induced colitis blocked the *in vivo* NLRP3 inflammasome activation with particular NLRP3. As CUR could subdue DSS induced activation of NLRP3 inflammasome and diminish DSS-induced colitis in mice forming a potential candidate for IBD therapy.[64] The upregulation of gut mucosal cytokines such as TNF-α and oxidative stress have been related to IBD. The involvement of oxidative stress was evaluated in a colitis model which is immune mediated. TNF-α recruited neutrophils in a dose dependent manner into the abdominal domain utilizing aminoguanidine (AG) when injected intraperitoneally into the mice as a selective inhibitor of iNOS and CUR. TNF-α-toxicity led to a significant increase in MPO, nitrites and malondialdehyde (MDA) levels as well as cell apoptosis in the colon and liver. Treatment with CUR and AG in mice diminished the neutrophils influx, oxidative stress, histological and ROS linked cellular damage in TNF-α, suggesting its potential benefit in oxidative bowel inflammation in IBD.[65]

CUR reduced colon inflammation in a *Mdr1a*−/− mouse model of human inflammatory bowel disease using a combined transcriptomics and proteomics approach. Proteomics and microarray analysis together with regulator effects analysis and ingenuity pathways demonstrated that the anti-inflammatory activity of CUR in a Mdrla−/− mouse colon which may be channeled through the activation of α-catenin. The study also provided evidence to support the action of CUR *via* various molecular pathways that included increased xenobiotic metabolism, reduced immune response and resolution of inflammation by lowered transmission of neutrophil as well as elevated barrier remodeling. The activation of important transcription factors as well as other regulatory molecules like FN1, PI3K, TNFSF12 and ERK during inflammation process were downregulated because of CUR.[66] CUR when therapeutically and prophylactically administered in mice model induced with UC has profound effect on liver and serum paraoxonase enzyme activity, hepatic cytosolic β-glucosidase activity, erythrocyte CA activity. Furthermore, this in turn demonstrated higher MPO activity, longer colon length and lower body weight in contrast to the therapeutic division showcasing the inhibitory role of CUR in IBDs.[67]

CUR decreased the expression of MMP-1, MMP-3 and TIMP-1 in ameliorating inflammatory injury in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats, which is linked to the decrease in its TNF-α inducer. After the treatment of CUR the hydroxyproline content decreased that decreased the mucosal injury in colon which is linked to the decrease in MMPs. So, CUR was effective in the prevention and treatment of IBD.[68] Zhang *et al.* exhibited that CUR possessed antioxidant and anti-inflammatory effects in rat models which are induced with TNBS-induced intestinal inflammation, which suppressed inflammation in the colon as well as weakened the M1/M2 ratio.[69] CUR was involved in intestinal inflammation through elevating the expression of SOCS-1 and inhibiting JAK/STAT pathways. Furthermore, CUR reduced TNBS-induced apoptosis was through the mitochondrial pathway and declined the accumulation of cytochrome C in cytosol.[69] A study explored the possible molecular mechanism of CUR inhibiting visceral

nociception *via* antagonizing the transient receptor potential vanilloid-1 (TRPV1) receptor by using two experimental models: jejunal afferent firing in the *ex vivo* mouse in jejunum preparations (trinitrobenzene sulphonic acid (TNBS) treated Kunming mice, wild type and TRPV1 knockout (KO) mice as well as naïve), and viscero-motor response (VMR) to colorectal distension (CRD) in rats. The *in vivo* and *in vitro* GI nociception is inhibited by CUR, which is indicative of TRPV1 antagonism. These attributes of CUR on TRPV1 can be exploited for the development of new analgesics against FGIDs and other severe diseases.[70]

SIRT1, a NAD^+-dependent protein/histone deacetylase possesses a plethora of pharmacological activities. Although this expression has oncological potential and is overexpressed in colorectal cancer, the molecular mechanism through which it exert colorectal cancer is not known. It is postulated that the SIRT 1 protein expression effects the mRNA in colon cancer cells and CUR has post-translational effects this expression. Therefore, CUR treatment induced proteasomal degradation of SIRT1. Also, cysteine 67 substitution with alanine, which is a protein stable and clonogenic feature of mutant SIRT1, remains undisturbed by CUR. Hence, these studies indicate that CUR provides proteasomal degradation of SIRT1 *via* covalent changes at the cysteine 67 residue of SIRT 1.[71]

CUR altered the aggrandized visceral nociceptive affect to the graded intensity of CRD as well as pellet output and improved the anxiety and depressed type behaviors in acute and chronic stress to imitate the symptoms of IBS, which induced neurochemical and biochemical alterations in rats. The possible mechanism may involve the normalization of neurotransmitters, BDNF (brain-derived neurotrophic factor) and pCREB (phosphorylation of cAMP response element-binding protein) levels; both in colon and hippocampus which indicates that CUR is a potent agent against IBS.[72] The important pathogenesis for gastrointestinal disorders is the disturbance of epithelial TJ with loss of barrier function. CUR induced Heme oxygenase-1 (HO-1) provides protection against different oxidative stresses. In human intestinal epithelial cells the oxidative stress induced intestinal barrier demonstrates the protective effect of CUR, which was elucidated by H_2O_2-induced Caco-2 enterocytic monolayers. CUR is a potential inducer of HO-1 and *via* this mechanism ameliorates the oxidative stress dependent disruption of the intestinal epithelial TJ. This provides a platform for CUR to be used for the treatment of various intestinal diseases.[73] A summary of animal studies conducted to test the efficacy of anti-inflammatory activities of turmeric is presented in Table 10.2.

10.5 Pharmaceutical Formulations of CUR

CUR containing chitosan modified citrus pectinate nanoparticles (MCPCNPs) has demonstrable potential for colon delivery. The MCPCNPs survive acidic pH milieu, with a limited amount of CUR release, but significantly at media showcasing the colon. The synergistic effect of the galectin moiety of pectin

carrier, mucoadhesion and CUR payload portrays the mode of achieving anti-cancer properties to the colon. It is strongly suggested that the formulated MCPCNPs have the potential to be applied as an orally deliverable colon cancer formulation as an alternative treatment of colon cancer.[74] Ung *et al.*[40] investigated whether CUR emulsified in carboxymethyl cellulose and gavaged daily for two weeksat a dose of 200 mg kg^{-1} per day in IL-10 gene deficient mice demonstrated the same effect on spontaneous colitis. Significant reductions in pro-inflammatory cytokine release in intestinal explant cultures were seen in mice treated with the CUR mixture and appeared to have an impact on colitis. But, CUR has added anti-inflammatory effects channeled through a decreased production of potential pro-inflammatory mucosal cytokines.[40] In another clinical pilot scale trial with placebos, bioperine-CUR tablets, and bioperine-turmeric tablets given to healthy subjects subsequent changes in the gut micro biota community architecture were demonstrated at baseline and after four and eight weeks' treatment. Among the responsive subjects, both turmeric as well as CUR altered the gut microbiota in more or less the same manner, suggesting that CUR leads to more changes than turmeric treated subjects.[75]

In vivo, CUR-polymeric nanoparticles (NPs)-DSS treated mice demonstrated significant decrease in both TNF-α secretion and MPO activity portraying a same colonic structural pattern when compared to healthy group. The anchoring of CUR post 12 hours of CUR-NPs resulted a specific colon delivery of CUR as well as ameliorated CUR release at the site of colon.[76] The fabrication of an amphiphilic CUR polymer conjugate containing a disulfide bond acted responsively to the intestinal bacterial reduction environment, which ameliorated the inflammatory progression in the colon and could protect the mice from IBD.[77]

Biodegradable CUR-loaded zein based PVMMA microspheres were produced by spray drying for the intestinal release of orally administrated CUR to improve anti-inflammatory activity. The CUR-loaded zein based PVMMA microspheres caused pro-inflammatory cytokine such as IL-β, COX-2, NOS2 and TNF-α inhibition in LPS stimulated macrophages, which affix the beneficial features of ZN/PVMMA microspheres as CUR delivering system to decrease the inflammatory activity at intestinal section that suffers from IBD.[78] In 2014, Gugulothu *et al.* formulated pH-sensitive nanoparticles of CUR-celecoxib combination as a potential therapy for UC, which was established using a rat model of UC. The synergistic action of the drug combination of celecoxib and CUR reduced the overall toxicity and provided enhanced efficacy for mitigating UC.[79]

CUR containing a polyherbal formulation ingested over a 28 day period resulted in improvements in several gastrointestinal symptoms and overall quality of life, such as GSRS domain ratings for abdominal pain, constipation, diarrhea, indigestion and reflux, WHOQOL-BREF domain ratings for overall quality of life, social relations, environmental health, psychological health, and physical health, and PAC-QOL domain ratings for physical discomfort, psychosocial discomfort, worries and concerns, and life satisfaction.[80]

A novel gut health formulation was prepared by the encapsulation of asafetida-CUR onto the cellulose nanofiber isolated from turmeric spent through spray dry technology with a complete natural matric. The gut health formulation effectively improved characteristic inflammatory bowel disease symptoms and histological scores, attenuated inflammation and maintained intestinal integrity in dextran sulfate sodium-induced ulcerative colitis. The gut health formulation has significant protective effects against dextran sulfate sodium induced colitis that could serve as an effective treatment for amelioration of ulcerative colitis.[14]

10.6 Conclusion

Over the last decade, interest in natural drugs as compounds for drug development has been renewed. Even though the natural products are a rich source of potentially therapeutic drugs, many of them have to be structurally modified so as to optimize their usage as pharmacological agents. Moreover, ailments such as gut diseases affect millions of people worldwide and place a highly significant economic burden on healthcare systems. Supportively, growing scientific evidence for the health benefits of turmeric has made its supplements become more widely accepted and popular around the world. However, anecdotal claims suggest that turmeric relieves acid reflux, but there are few clinical trials to prove these claims. Encouragingly, CUR, the main ingredient of turmeric, has taken precedence over many other compounds and its biomedical research is ever increasing. CUR has a multitude of molecular targets that include transcription factors, receptors, kinases, inflammatory cytokines and other enzymes, which explain its effectiveness against a variety of diseases. The challenges in the pharmacological exploitation of CUR include its absorption, bio-distribution, metabolism and elimination, thereby increasing its bioavailability. In the coming decade, this field of research has to reach milestones with mechanistic underpinnings of its mechanism of action along with insights into its molecular targets, thus promising predictive models of disease course and therapy response, besides development of non-toxic, precision medicine.

Acknowledgements

The contributors gratefully thank the management of Plant Lipids (P) Ltd., Cochin, India, for their support and encouragement. We wish to express our appreciation to our laboratory members for their active help and cooperation.

References

1. R. Arora, P. Malhotra, S. Sundriyal, H. S. Yashavanth, R. J. Pai and M. S. Baliga, in *Bioactive Food as Dietary Interventions for Liver and Gastrointestinal Disease*, ed. R. R. Watson and V. R. Preedy, Academic Press, Elsevier Inc., Cambridge, 2013, p. 301.

2. G. Falony, M. Joossens, S. Vieira-Silva, J. Wang, Y. Darzi, K. Faust, A. Kurilshikov, M. J. Bonder, M. Valles-Colomer, D. Vandeputte, R. Y. Tito, S. Chaffron, L. Rymenans, C. Verspecht, L. De Sutter, G. Lima-Mendez, K. D'hoe, K. Jonckheere, D. Homola, R. Garcia, E. F. Tigchelaar, L. Eeckhaudt, J. Fu, L. Henckaerts, A. Zhernakova, C. Wijmenga and J. Raes, *Science*, 2016, **352**, 560.
3. A. Zhernakova, A. Kurilshikov, M. J. Bonder, E. F. Tigchelaar, M. Schirmer, T. Vatanen, Z. Mujagic, A. V. Vila, G. Falony, S. Vieira-Silva, J. Wang, F. Imhann, E. Brandsma, S. A. Jankipersadsing, M. Joossens, M. C. Cenit, P. Deelen, M. A. Swertz, R. K. Weersma LifeLines cohort study, E. J. Feskens, M. G. Netea, D. Gevers, D. Jonkers, L. Franke, Y. S. Aulchenko, C. Huttenhower, J. Raes, M. H. Hofker, R. J. Xavier, C. Wijmenga and J. Fu, *Science*, 2016, **352**, 565.
4. L. Shen and H. F. Ji, *Crit. Rev. Food Sci. Nutr.*, 2018, **21**, 1.
5. J. W. Arnold, J. Roach and M. A. Azcarate-Peril, *Trends Microbiol.*, 2016, **24**, 887.
6. M. Doulberis, G. Kotronis, D. Gialamprinou, J. Kountouras and P. Katsinelos, *Metab., Clin. Exp.*, 2017, **71**, 182.
7. W. H. Tang, T. Kitai and S. L. Hazen, *Circ. Res.*, 2017, **120**, 1183.
8. L. Shen, L. Liu and H. F. Ji, *Food Nutr. Res.*, 2017, **61**, 1361780.
9. C. A. Woodhouse, V. C. Patel, A. Singanayagam and D. L. Shawcross, *Aliment. Pharmacol. Ther.*, 2017, **47**, 192.
10. L. Brunkwall and M. Orho-Melander, *Diabetologia*, 2017, **60**, 943.
11. P. Maruvada, V. Leone, L. M. Kaplan and E. B. Chang, *Cell Host Microbe*, 2017, **22**, 589.
12. R. Wiest, A. Albillos, M. Trauner, J. S. Bajaj and R. Jalan, *J. Hepatol.*, 2017, **67**, 1084.
13. L. Montagne, J. R. Pluske and D. J. Hampson, *Anim. Feed Sci. Technol.*, 2003, **108**, 95.
14. S. Gopi, A. Amalraj, S. Jude, K. Varma, T. R. Sreeraj, J. T. Haponiuk and S. Thomas, *Mater. Sci. Eng. C*, 2017, **81**, 20.
15. Y. D. Taghipour, R. Bahramsoltani, A. M. Marques, R. Naseri, R. Rahimi, P. Haratipour, A. I. Panah, M. H. Farzaei and M. Abdollahi, *Daru, J. Pharm. Sci.*, 2018, **26**, 229.
16. R. Sreedhar, S. Arumugam, R. A. Thandavarayan, V. Karuppagounder and K. Watanabe, *Drug Discovery Today*, 2016, **21**, 843.
17. A. Schneider, I. Hossain, J. VanderMolen and K. Nicol, *Complement. Ther. Med.*, 2017, **33**, 32.
18. M. Bellini, D. Gambaccini, C. Stasi, M. T. Urbano, S. Marchi and P. Usai-Satta, *World J. Gastroenterol.*, 2014, **20**, 8807.
19. E. G. Vilela, H. O. Torres, F. P. Martins, L. Ferrari Mde, M. M. Andrade and A. S. Cunha, *World J. Gastroenterol.*, 2012, **18**, 872.
20. M. Zhang, X. Wang, M. K. Han, J. F. Collins and D. Merlin, *Nanomedicine*, 2017, **12**, 1927.
21. N. Bribi, F. Algieri, A. Rodriguez-Nogales, T. Vezza, J. Garrido-Mesa, M. P. Utrilla, M. D. M. Contreras, F. Maiza, A. Segura-Carretero, M. E. Rodriguez-Cabezas and J. Gálvez, *Phytomedicine*, 2016, **23**, 901.

22. J. Castro, Y. Ocampo and L. Franco, *J. Crohn's Colitis*, 2015, **9**, 1004.
23. S. J. Hur, S. H. Kang, H. S. Jung, S. C. Kim, H. S. Jeon, I. H. Kim and J. D. Lee, *Nutr. Res.*, 2012, **32**, 801.
24. S. Saeidnia and M. Abdollahi, *Toxicol. Appl. Pharmacol.*, 2013, **273**, 442.
25. A. Beloqui, P. B. Memvanga, R. Coco, S. Reimondez-Troitiño, M. Alhouayek, G. G. Muccioli, M. J. Alonso, N. Csaba, M. de la Fuente and V. Préat, *Colloids Surf., B*, 2016, **143**, 327.
26. M. Zhang, E. Viennois, M. Prasad, Y. Zhang, L. Wang, Z. Zhang, M. K. Han, B. Xiao, C. Xu, S. Srinivasan and D. Merlin, *Biomaterials*, 2016, **101**, 321.
27. S. Mozaffari, S. Nikfar, A. H. Abdolghaffari and M. Abdollahi, *Expert Opin. Biol. Ther.*, 2014, **14**, 583.
28. *Herbal Drugs: A Cancer Chemopreventive and Therapeutic Perspective*, ed. R. Arora, Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, 2010.
29. Y. D. Taghipour, S. Masoomzadeh, M. H. Farzaei, R. Bahramsoltani, Z. Karimi-Soureh, R. Rahimi and M. Abdollahi, *Int. J. Nanomed.*, 2017, **12**, 2689.
30. A. Amalraj, A. Pius, S. Gopi and S. Gopi, *J. Tradit., Complementary Med.*, 2017, **7**, 205.
31. K. Mahmood, K. M. Zia, M. Zuber, M. Salman and M. N. Anjum, *Int. J. Biol. Macromol.*, 2015, **81**, 877.
32. K. Sugimoto, H. Hanai, K. Tozawa, T. Aoshi, M. Uchijima, T. Nagata and Y. Koide, *Gastroenterology*, 2002, **123**, 1912.
33. Y. Deguchi, A. Andoh, O. Inatomi, Y. Yagi, S. Bamba, Y. Araki, K. Hata, T. Tsujikawa and Y. Fujiyama, *Dig. Dis. Sci.*, 2007, **52**, 2993.
34. H. M. Arafa, R. A. Hemeida, A. I. El-Bahrawy and F. M. Hamada, *Food Chem. Toxicol.*, 2009, **47**, 1311.
35. G. C. Jagetia and B. B. Aggarwal, *J. Clin. Immunol.*, 2007, **27**, 19.
36. A. Ukil, S. Maity, S. Karmakar, N. Datta, J. R. Vedasiromoni and P. K. Das, *Br. J. Pharmacol.*, 2003, **139**, 209.
37. V. Singla, V. Pratap Mouli, S. K. Garg, T. Rai, B. N. Choudhury, P. Verma, R. Deb, V. Tiwari, S. Rohatgi, R. Dhingra, S. Kedia, P. K. Sharma, G. Makharia and V. Ahuja, *J. Crohn's Colitis*, 2014, **8**, 208.
38. L. Camacho-Barquero, I. Villegas, J. M. Sánchez-Calvo, E. Talero, S. Sánchez-Fidalgo, V. Motilva and C. Alarcón de la Lastra, *Int. Immunopharmacol.*, 2007, **7**, 333.
39. C. B. Larmonier, M. T. Midura-Kiela, R. Ramalingam, D. Laubitz, N. Janikashvili, N. Larmonier, F. K. Ghishan and P. R. Kiela, *Inflammatory Bowel Dis.*, 2011, **17**, 503.
40. V. Y. Ung, R. R. Foshaug, S. M. MacFarlane, T. A. Churchill, J. S. Doyle, B. C. Sydora and R. N. Fedorak, *Dig. Dis. Sci.*, 2010, **55**, 1272.
41. B. Salh, K. Assi, V. Templeman, K. Parhar, D. Owen, A. Gómez-Muñoz and K. Jacobson, *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2003, **285**, G235.
42. A. Bhattacharyya, R. Chattopadhyay, S. Mitra and S. E. Crowe, *Physiol. Rev.*, 2014, **94**, 329.

43. F. A. Moura, K. Q. Andrade, J. C. F. Santos, O. R. P. Araújo and M. O. F. Goulart, *Redox Biol.*, 2015, **6**, 617.
44. T. Liu, L. Zhang, D. Joo and S. C. Sun, *Signal Transduction Targeted Ther.*, 2017, **2**, 17023.
45. G. R. Irving, A. Karmokar, D. P. Berry, K. Brown and W. P. Steward, *Best Pract. Res., Clin. Gastroenterol.*, 2011, **25**, 519.
46. T. Ali, F. Shakir and J. Morton, *Digestion*, 2012, **85**, 249.
47. R. Mazieiro, R. R. Frizon, S. M. Barbalho and R. A. Goulart, *J. Med. Food*, 2018, **21**, 1077.
48. A. L. Lopresti, *Adv. Nutr.*, 2018, **9**, 41.
49. I. K. Campbell, S. Gerondakis, K. O'Donnell and I. P. Wicks, *J. Clin. Invest.*, 2000, **105**, 1799.
50. X. Liu, S. Yin, Y. Chen, Y. Wu, W. Zheng, H. Dong, Y. Bai, Y. Qin, J. Li, S. Feng and P. Zhao, *Mol. Med. Rep.*, 2018, **17**, 5484.
51. J. A. Taylor, G. D. Bren, K. N. Pennington, S. A. Trushin, S. Asin and C. V. Paya, *J. Mol. Biol.*, 1999, **290**, 839.
52. Y. Zhang and Y. Zeng, *Drug Dev. Res.*, 2019, **80**, 353.
53. K. K. Jung, H. S. Lee, J. Y. Cho, W. C. Shin, M. H. Rhee, T. G. Kim, J. H. Kang, S. H. Kim, S. Hong and S. Y. Kang, *Life Sci.*, 2006, **79**, 2022.
54. T. Awin, N. Buzgaia, S. Z. A. Ghafar, A. Mediani, S. M. M. Faudzi, M. Maulidiani, K. Shaari and F. Abas, *Food Biosci.*, 2019, **29**, 126.
55. F. Zhang, N. K. Altorki, J. R. Mestre, K. Subbaramaiah and A. J. Dannenberg, *Carcinogenesis*, 1999, **20**, 445.
56. A. Goel, C. R. Boland and D. P. Chauhan, *Cancer Lett.*, 2001, **172**, 111.
57. R. Sreedhar, S. Arumugam, R. A. Thandavarayan, V. Karuppagounder and K. Watanabe, *Drug Discovery Today*, 2016, **21**, 843.
58. J. M. Cavaillon, *Cell. Mol. Biol.*, 2001, **47**, 695.
59. H. T. Idriss and J. H. Naismith, *Microsc. Res. Tech.*, 2000, **50**, 184.
60. A. B. Kunnumakkara, B. L. Sailo, K. Banik, C. Harsha, S. Prasad, S. C. Gupta, A. C. Bharti and B. B. Aggarwal, *J. Transl. Med.*, 2018, **16**, 14.
61. C. H. Hsu and A. L. Cheng, *Adv. Exp. Med. Biol.*, 2007, **595**, 471.
62. R. Bundy, A. F. Walker, R. W. Middleton and J. Booth, *J. Altern. Complementary Med.*, 2004, **10**, 1015.
63. S. M. A. Bastaki, M. M. A. Ahmed, A. A. Zaabi, N. Amir and E. Adeghate, *BMC Complementary Altern. Med.*, 2016, **16**, 72.
64. Z. Gong, S. Zhao, J. Zhou, J. Yan, L. Wang, X. Du, H. Li, Y. Chen, W. Cai and J. Wu, *Mol. Immunol.*, 2018, **104**, 11.
65. S. Mouzaoui, I. Rahim and B. Djerdjouri, *Int. Immunopharmacol.*, 2012, **12**, 302.
66. J. M. Cooney, M. P. Barnett, Y. E. Dommels, D. Brewster, C. A. Butts, W. C. McNabb, W. A. Laing and N. C. Roy, *J. Nutr. Biochem.*, 2016, **27**, 181.
67. H. Yildirim, F. B. Sunay, S. Sinan and F. Köçkar, *J. Enzyme Inhib. Med. Chem.*, 2016, **31**, 1583.
68. T. K. Motawi, S. M. Rizk and A. H. Shehata, *J. Physiol. Biochem.*, 2012, **68**, 529.

69. X. Zhang, J. Wu, B. Ye, Q. Wang, X. Xie and H. Shen, *BMC Complementary Altern. Med.*, 2016, **16**, 299.
70. L. Zhi, L. Dong, D. Kong, B. Sun, Q. Sun, D. Grundy, G. Zhang and W. Rong, *Neurogastroenterol. Motil.*, 2013, **25**, e429.
71. Y. H. Lee, N. Y. Song, J. Suh, D. H. Kim, W. Kim, J. Ann, J. Lee, J. H. Baek, H. K. Na and Y. J. Surh, *Cancer Lett.*, 2018, **431**, 219.
72. Y. Yu, S. Wu, J. Li, R. Wang, X. Xie, X. Yu, J. Pan, Y. Xu and L. Zheng, *Metab. Brain Dis.*, 2015, **30**, 47.
73. N. Wang, G. Wang, J. Hao, J. Ma, Y. Wang, X. Jiang and H. Jiang, *Dig. Dis. Sci.*, 2012, **57**, 1792.
74. R. Sabra, N. Billa and C. J. Roberts, *React. Funct. Polym.*, 2018, **123**, 54.
75. C. T. Peterson, A. R. Vaughn, V. Sharma, D. Chopra, P. J. Mills, S. N. Peterson and R. K. Sivamani, *J. Evidence-Based Complementary Altern. Med.*, 2018, **23**, 1.
76. A. Beloqui, R. Coco, P. B. Memvanga, B. Ucakar, A. des Rieux and V. Préat, *Int. J. Pharm.*, 2014, **473**, 203.
77. H. Qiao, D. Fang, J. Chen, Y. Sun, C. Kang, L. Di, J. Li, Z. Chen, J. Chen and Y. Gao, *Drug Delivery*, 2017, **24**, 233.
78. E. Blanco-García, F. J. Otero-Espinar, J. Blanco-Méndez, J. M. Leiro-Vidal and A. Luzardo-Álvarez, *Int. J. Pharm.*, 2017, **518**, 86.
79. D. Gugulothu, A. Kulkarni, V. Patravale and P. Dandekar, *J. Pharm. Sci.*, 2014, **103**, 687.
80. A. L. Lopresti, H. Gupta and S. J. Smith, *BMC Complementary Altern. Med.*, 2018, **18**, 98.

CHAPTER 11

Molecular Docking Studies of Curcumin

Y. BASPINAR*

Ege University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, 35100 Bornova, Izmir, Turkey
*E-mail: yucel.baspinar@ege.edu.tr, yucelbaspinar77@gmail.com

11.1 Introduction

Discovering novel drugs is time-consuming and expensive. Time-consuming, because a typical drug discovery cycle can take up to 12 years or more[1] and expensive with a cost of up to 1 billion US dollars. The drug discovery process is accelerated by rapid developments of combinatorial chemistry and high-throughput screening (HTS) technologies by enabling grand libraries of compounds to be synthesized and screened in a very short time. Many of the identified scores failed due to absorption, distribution, metabolism, excretion and toxicity (ADME/Tox) issues. It was essential to develop alternative strategies for choosing suitable compounds and removing unsuitable elements. Here, the identification of scores by computational methods such as virtual screening (VS) is a crucial step in drug discovery. VS involves database searching for new compounds with a desired biological activity as alternatives to existing ligands. VS is a step by step method with sequential filters for choosing a set of hits with potential biological activity against intended drug targets. The studied compounds do not necessarily exist, thus

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

'testing' them must not consume valuable substance material. Due to these basic requirements, any molecule can be evaluated by VS, in theory. VS databases can contain up to 10 million compounds. These compounds can be obtained from external sources such as libraries from commercial vendors or from public or commercial databases. VS can be used in more and more new and conceptually diverse ways and can be divided into two main categories, ligand-based VS (LBVS) and structure-based VS (SBVS).[2] LBVS uses structure–activity data from known actives to identify candidate compounds for experimental evaluation.[3] LBVS includes searches for similarity and substructure, quantitative structure–activity relationships (QSAR), and pharmacophore and three-dimensional shape matching.[4] In contrast to LBVS, SBVS uses the three-dimensional (3D) structure of the biological target (determined per X-ray crystallography, nuclear magnetic resonance (NMR) or computationally through homology modeling) to dock the candidate molecules and rank them based on their predicted binding affinity or complementarity to the binding site. In this context, the molecular docking studies of curcumin with several receptors such as protein kinase C, tumor necrosis factor-α (TNF-α), cyclooxygenase (COX): COX-1, COX-2, human α1-acid glycoprotein, myeloid differentiation protein-2, histone deacetylases, glyoxalase, proteasom, endoplasmatic reticulum, HIV intergrase/protease, glycogen synthase kinase-3 β, thioredoxin reductase, aldose reductase, casein, albumin, β-lactoglobulin, IG, Bcl- 2, acetylcholine esterase, phospholipase A2, mycobacterial FtsZ protein, DNA, dihydrofolate, ovalbumin and estrogen receptors were reviewed.

11.2 Molecular Properties of Curcumin

Molecular docking studies of curcumin have shown that it can adopt different structural conformations for maximizing hydrophobic contacts with the target molecule. The phenyl moieties of curcumin participate in π–π van der Waals interactions with aromatic amino acids. The phenolic and carbonyl parts of hydrophobic curcumin participate in hydrogen bonding with the targeted molecules. The keto–enol tautomerization of curcumin supports the functional properties such as interacting electrostatically, leading to increased free energies of association.

11.3 Interaction and Binding Mechanism of Curcumin

The properties of curcumin such as adsorption and bioavailability have an impact on its biological and pharmacological activities. Understanding the binding mechanism and interaction between curcumin and the target is essential. The main interaction between the phenolic and carbonyl moieties and the peptide bond is based on hydrogen bonding.[5,6] In the phenolic hydroxyl groups two hydrogen bond donors exist and six acceptors belong to the six oxygen atoms. In addition, hydrophobicity is crucial for

the interaction between protein and curcumin. Van der Waals force and π–π interaction are associated with the two phenyl groups of curcumin and aromatic amino acid residues.[7] The β-sheet breaking of curcumin is affected by hydrogen bonds and hydrophobic interactions, demonstrated with molecular dynamics simulation.[8]

11.4 Molecular Docking Studies of Curcumin

Most of the molecular docking studies of curcumin deal in fact with its analogs. Due to this, the molecular docking studies of curcumin are presented, including information about the interacting amino acid residues and binding energy (Table 11.1). Molecular modeling studies show the binding capacity of curcumin and the active site of various molecules.

The main part of the existing studies with curcumin deal with its anticancer effects. Various receptor interaction studies of curcumin have been reported. Curcumin exerted anti-inflammatory properties by direct binding to the pro-inflammatory molecules' tumor necrosis factor (TNF)-α,[9] cyclooxygenase (COX)-1,[10] COX-2,[11] human α1-acid glycoprotein (AGP)[12] and myeloid differentiation protein 2 (MD-2).[13]

AGP with anti-inflammatory properties is important in immunomodulation,[14,15] bound and transported various endogenous and exogenous compounds like drugs.[16] Circular dichroism (CD), UV-visible absorption and fluorescence spectroscopy measurements showed that curcumin is bound to AGP in a left-handed chiral conformation. CD displacement experiments revealed an interaction between curcumin and F1–S and A, respectively, two genetic variants of AGP. The association constant of this complex estimated that the binding was at the protein surface. Two possible binding sites for curcumin in this complex were suggested, one on the outer region, the open end of the central hydrophobic cavity and another one on the surface cleft through hydrogen bonding and π–π interactions with the phenol and enol groups.[12]

An overexpression of the transmembrane tyrosine kinase ErbB2 (HER2/neu) increased the metastatic potential in cancer cells as well as the resistance against anticancer drugs.[17] Thus, a possible treatment for ErbB2-overexpressing cancers is the use of therapeutics downregulating ErbB2 protein and/or its activity. The effect of curcumin is based on its binding to the ErbB2 kinase domain, indicated by site-directed mutagenesis and molecular docking. The Michael acceptor functionality of curcumin is needed for its covalent association with ErbB2 as well as for the curcumin-mediated ErbB2 depletion.[18] There is a relation between the transport of thyroxin (T4) and retinol in human plasma and the protein transthyretin (TTR). Fluorescence quenching and ANS displacement studies have shown that tertiary and quaternary structural changes of TTR were prevented by forming a TTR-curcumin complex. At low pH, the phenolic and enolic hydroxyl groups of curcumin are protonated and isomerized, hindering curcumin binding to TTR. This indicates that curcumin binds and stabilizes TTR, and the important role of the ligand side chain conformations in binding to TTR emerges.[19]

Table 11.1 Molecular docking studies of curcumin.

Molecular docking molecule	Type of interactions	Interacting residue (s) examples	Binding energy (kcal mol^{-1})	Reference
Protein kinase C	Hydrogen bonds	Leu251, Gln257, Gly254	—	38 and 42
TNF-α	Hydrophobic, hydrogen bonds, covalent	Leu89, Asn90, Asp105, Asn106, Cys129, Tyr201, Lys126	—	9
COX-1, COX-2	Hydrogen bonds	Tyr385, Leu384, Phe518, Met522, Ser530, Tyr355, His90, Leu357, Arg120, Glu524, Val523, Val116, Ala516, Tyr355, Arg120	—	10 and 11
Myeloid differentiation protein-2	Hydrophobic	Cys133, Arg-90, Glu-92 and Tyr-102	—	12 and 43
Histone deacetylases	Hydrophobic, hydrogen bonds	Arg37, Pro35, Ile34, Phe152, Asp29, Tyr100	—	44
Glyoxalase I	Hydrogen bonds	Lys156, Arg122, Arg37, Glu172, Met179	—	36 and 37
Xanthine oxidase		Phe914, Phe1009, Thr1010	—	32
Proteasom	Hydrogen bonds	Thr1, Ser96	—	45
Endoplasmatic reticulum	Hydrogen bonds	Asp254, Arg264, Gln56, Gln267, Leu1040, Ile1041, Ala985	—	46
HIV Integrase/Protease	Hydrogen bonds	Asp64, His67, Thr66, Glu92, Thr93, Asp116, Ser119, Asn120, Lys159, Asp116, Asp64, Asp25, Asp29, Asp30	—	47
Glycogen synthase kinase-3β		Val135, Ile62, Arg141, Lys85	—	34
Thioredoxin reductase	Hydrophobic	His108, Arg351, Lys29, and Leu112	—	48
Aldose reductase		Tyr48, Lys21, Thr19, Gln183, Leu300, and Trp111	—	49
Casein		Trp164, Trp199, Trp143	—	50

Albumin	Hydrophobic, hydrogen bonds	Trp212, Trp134, Trp214	—	51–57
β-Lactoglobulin	Hydrophobic	Trp19, Trp61	—	6 and 58
IG	Hydrogen bond	His35, Arg96, Tyr99, Tyr91, Ala92, Tyr94, Tyr98	—	59
Bcl2		Tyr108, Glu136, Gly141, Asn143, Trp144, Gly145, Arg146, His184, Trp188, Tyr202	—	60 and 61
Acetylcholine esterase	Hydrophobic, electrostatic	Phe330, Trp84 and Asp72	−11.21	62–69
Phospholipase A2	Hydrogen bond	Asp49	−4.32	70 and 71
Mycobacterial FtsZ protein	Hydrogen bond	Arg152, Ile214, Ala218, Gln255, Phe312, Asp349		41 and 72
DNA		G7, T8, C9, T10, C11, C12, G14, A15, G16, A17, C18, C19	−3.82	73 and 74
Dihydrofolate		Phe34, Ile-7, Ala-9, Leu-22 and Val-115, Glu30, Phe 31, Tyr121	−9.02	75
Ovalbumin	Hydrogen bond	Thr92, Pro94, Phe100, Leu102, Ser104, Arg105, Arg127, Gly128 and Trp149	−11.59	76
Estrogen receptor		Glu 1419, Thr 1347	−9.92	77

Studies have shown that curcumin can bind to tubulin,[20] CD13/aminopeptidase N[21] and β-amyloid aggregates.[22,23] In addition, when curcumin is bound to tubulin at a single binding site the binding induces conformational changes in tubulin.[24] Zinc-dependent metalloproteinase APN are crucial in tumor invasion and angiogenesis. Fluorescence and surface plasmon resonance analysis and APN-specific antibody competition assay could prove the binding of curcumin to APN, resulting in inhibition of angiogenesis.[25] UV-visible spectroscopy measurements indicated the binding of curcumin to Aβ-aggregates through the enol form and enolization.[26]

More possible targets resulting from molecular docking studies are summarized in Table 11.1.

11.5 Molecular Docking Studies of Curcumin Analogs

Numerous reports showed that analogs of curcumin can bind to several molecules such as human immunodeficiency virus type-I protease,[27] COX-10,[10,28] DNA polymerase λ,[29] platelet-12-lipoxygenase,[30] COX-2,[11] DNA methyltransferase-1[31] xanthine oxidase,[32] dipeptydyl peptidase-4,[33] glycogen synthase kinase-3β,[34] ribonuclease A,[35] glyoxalase-I,[36,37] protein kinase C,[38] matrix metalloproteinases,[39] CDK2[40] and filamentous temperature-sensitive Z protein.[41]

11.6 Conclusion

Computation, such as high-throughput screening and virtual screening, which are essential tools of modern drug discovery research, has an important role in drug discovery research. Over the last few years several VS methods such as structural queries, pharmacophores, molecular fingerprints, QSAR models, cluster analysis tools, statistical techniques and docking calculations have been developed. VS acts like a filter for large databases in terms of searching for chemical groups, solubility, bioavailability, drug-like and absorption characteristics. There are many track records of novel inhibitors or antagonists of diverse biological targets. VS methods such as partitioning and clustering algorithms that can derive predictive models of biological activity can be adapted for HTS data, which are susceptible to errors. Similarly, attempts were made to interface HTS and VS, such as the application of sequential screening. Minor changes of compounds are computationally selected from major databases and assayed. Due to this, the search for actives is refined in following iterations. The increasing size and number of compound databases and screening targets will result in a combination of computational and biological screening in the field of pharmaceutical research. Molecular docking is an *in silico* method used to develop the homology model for a novel drug candidate. Due to the fact that curcumin

is a well-known drug, using molecular docking in that context can be performed either for investigating the binding capacities of curcumin with novel targeted molecules or for curcumin analogs. Molecular docking is very useful and reasonably reliable for predicting putative binding interactions, activities and affinities of drugs for macromolecules like proteins.

References

1. C. M. Song, S. J. Lim and J. C. Tong, *Briefings Bioinf.*, 2009, **10**, 579.
2. O. Dror, A. Shulman-Peleg, R. Nussinov and H. J. Wolfson, *Curr. Med. Chem.*, 2004, **11**, 71.
3. A. Jahn, G. Hinselmann, N. Fechner and A. Zell, *J. Cheminf.*, 2009, **1**, 14.
4. B. O. Villoutreix, N. Renault, D. Lagorce, O. Sperandio, M. Montes and M. A. Miteva, *Curr. Protein Pept. Sci.*, 2007, **8**, 381.
5. F. Mohammadi, A. Bordbar, A. Divsalar, K. Mohammadi and A. A. Saboury, *Protein J.*, 2009, **28**, 189.
6. F. Mohammadi, A. Bordbar, A. Divsalar, K. Mohammadi and A. A. Saboury, *Protein J.*, 2009, **28**, 117.
7. A. H. Sneharani, S. A. Singh and A. G. Appu Rao, *J. Agric. Food Chem.*, 2009, **57**, 10386.
8. L. N. Zhao, S. Chiu, J. Benoit, L. Y. Chew and Y. Mu, *J. Phys. Chem. B*, 2012, **116**, 7428.
9. S. T. Wua, J. C. Suna, K. J. Leeb and Y. M. Sunc, *Int. J. Eng. Sci. Technol.*, 2010, **2**, 4263.
10. C. Selvam, S. M. Jachak, R. Thilagavathi and A. K. Chakraborti, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1793.
11. S. Padhye, S. Banerjee, D. Chavan, S. Pandye, K. V. Swamy, S. Ali, J. Li, Q. P. Dou and F. H. Sarkar, *Pharm. Res.*, 2009, **26**, 2438.
12. F. Zsila, Z. Bikadi and M. Simonyi, *Bioorg. Med. Chem.*, 2004, **12**, 3239.
13. H. Gradisar, M. M. Keber, P. Pristovsek and R. Jerala, *J. Leukocyte Biol.*, 2007, **82**, 968.
14. T. Fournier, N. N. Medjoubi and D. Porquet, *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.*, 2000, **1482**, 157.
15. T. Hochepied, F. G. Berger, H. Baumann and C. Libert, *Cytokine Growth Factor Rev.*, 2003, **14**, 25.
16. Z. H. Israili and P. G. Dayton, *Drug Metab. Rev.*, 2001, **33**, 161.
17. N. G. Anderson and T. Ahmad, *Front. Biosci.*, 2002, **7**, 1926.
18. Y. Jung, W. Xu, H. Kim, N. Ha and L. Neckers, *Biochim. Biophys. Acta, Mol. Cell Res.*, 2007, **1773**, 383.
19. R. Pullakhandam, P. N. Srinivas, M. K. Nair and G. B. Reddy, *Arch. Biochem. Biophys.*, 2009, **485**, 115.
20. K. K. Gupta, S. S. Bharne, K. Rathinasamy, N. R. Naik and D. Panda, *FEBS J.*, 2006, **273**, 5320.
21. J. S. Shim, J. H. Kim, H. Y. Cho, Y. N. Yum, S. H. Kim, H. J. Park, B. S. Shim, S. H. Choi and H. J. Kwon, *Chem. Biol.*, 2003, **10**, 695.

22. D. Yanagisawa, N. Shirai, T. Amatsubo, H. Taguchi, K. Hirao, M. Urushitani, S. Morikawa, T. Inubushi, M. Kato, F. Kato, K. Morino, H. Kimura, I. Nakano, C. Yoshida, T. Okada, M. Sano, Y. Wada, K. N. Wada, A. Yamamoto and I. Tooyama, *Biomaterials*, 2010, **31**, 4179.
23. F. Yang, G. P. Lim, A. N. Begum, O. J. Ubeda, M. R. Simmons, S. S. Ambegaokar, P. P. Chen, R. Kayed, C. G. Glabe, S. A. Frautschy and G. M. Cole, *J. Biol. Chem.*, 2005, **280**, 5892.
24. P. M. Luthra, R. Kumar and A. Prakash, *Biochem. Biophys. Res. Commun.*, 2009, **384**, 420.
25. K. Dai and J. Lutkenhaus, *J. Bacteriol.*, 1991, **173**, 3500–3506.
26. E. Nogales, K. H. Downing, L. A. Amos and J. Lowe, *Nat. Struct. Biol.*, 1998, **5**, 451.
27. Z. Sui, R. Salto, J. Li, C. Craik and P. R. Ortiz de Montellano, *Bioorg. Med. Chem.*, 1993, **1**, 415.
28. G. Banuppriya, R. Sribalan, V. Padmini and V. Shanmugaiah, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 1655.
29. T. Takeuchi, T. Ishidoh, H. Iijima, I. Kuriyama, N. Shimazaki, O. Koiwai, K. Kuramochi, S. Kobayashi, F. Sugawara, K. Sakaguchi, H. Yoshida and Y. Mizushina, *Genes Cells*, 2006, **11**, 223.
30. J. Jankun, A. M. Aleem, S. Malgorzewicz, M. Szkudlarek, M. I. Zavodszky, D. L. Dewitt, M. Feig, S. H. Selman and E. Skrzypczak-Jankun, *Mol. Cancer Ther.*, 2006, **5**, 1371.
31. Z. Liu, Z. Xie, W. Jones, R. E. Pavlovicz, S. Liu, J. Yu, P. K. Li, J. Lin, J. R. Fuchs, G. Marcucci, C. Li and K. K. Chan, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 706.
32. L. Shen and H. F. Ji, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 5990.
33. E. P. Istyastono, *Indones. J. Chem.*, 2009, **9**, 132.
34. Y. Bustanji, M. O. Taha, I. M. Almasri, M. A. Al-Ghussein, M. K. Mohammad and H. S. Alkhatib, *J. Enzyme Inhib. Med. Chem.*, 2009, **24**, 771.
35. B. K. Sahoo, K. S. Ghosh and S. Dasgupta, *Protein Pept. Lett.*, 2009, **16**, 1485.
36. M. Liu, M. Yuan, M. Lu, X. Bu, H. B. Luo and X. Hu, *Biophys. Chem.*, 2010, **147**, 28.
37. M. Yuan, M. Luo, Y. Song, Q. Xu, X. Wang, Y. Cao, X. Bu, Y. Ren and X. Hu, *Bioorg. Med. Chem.*, 2011, **19**, 1189.
38. A. Majhi, G. M. Rahman, S. Panchal and J. Das, *Bioorg. Med. Chem.*, 2010, **18**, 1591.
39. C. R. Girija, P. Karunakar, C. S. Poojari, N. S. Begum and A. A. Syed, *J. Proteomics Bioinf.*, 2010, **3**, 200.
40. M. R. Borik, N. M. Fawzy, S. M. Abu-Bakr and M. S. Aly, *Molecules*, 2018, **23**, 1398.
41. M. Fujimori, H. Sogawa, S. Ota, P. Karpov, S. Shulga, Y. Blume and N. Kurita, *Chem. Phys. Lett.*, 2018, **692**, 166.
42. J. Das, S. Pany, S. Panchal, A. Majhi and G. M. Rahman, *Bioorg. Med. Chem.*, 2011, **19**, 6196.

43. Z. Wan, G. Chen, L. Chen, X. Liu, W. Fu, Y. Zhang, C. Li, G. Liang and Y. Cai, *Mol. BioSyst.*, 2015, **11**, 1933.
44. G. Bora-Tata, D. Dayangac-Erden, A. S. Demir, S. Dalkara, K. Yelekci and H. Erdem-Yurter, *Bioorg. Med. Chem.*, 2009, **17**, 5219.
45. V. Milacic, S. Banerjee, K. R. Landis-Piwowar, F. H. Sarkar, A. P. Majumdar and Q. P. Dou, *Cancer Res.*, 2008, **68**, 7283.
46. L. Wang, R. Song, Y. Shen, Y. Sun, Y. Gu, Y. Shu and Q. Xu, *Mol. Cancer Ther.*, 2011, **10**, 461.
47. O. Vajragupta, P. Boonchoong, G. M. Morris and A. J. Olson, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 3364.
48. J. Fang, J. Lu and A. Holmgren, *J. Biol. Chem.*, 2005, **280**, 25284.
49. P. Muthenna, P. Suryanarayana, S. K. Gunda, J. M. Petrash and G. B. Reddy, *FEBS Lett.*, 2009, **583**, 3637.
50. A. Sahu, N. Kasoju and U. Bora, *Biomacromolecules*, 2008, **9**, 2905.
51. A. Barik, B. Mishra, A. Kunwar, R. M. Kadam, L. Shen, S. Dutta, S. Padhye, A. K. Satpati, H. Y. Zhang and P. K. Indira, *Eur. J. Med. Chem.*, 2007, **42**, 431.
52. K. I. Priyadarsini, *J. Photochem. Photobiol., C*, 2009, **10**, 81.
53. F. Zsila, Z. Bikadi and M. Simonyi, *Tetrahedron: Asymmetry*, 2003, **14**, 2433.
54. J. S. Mandeville, E. Froehlich and H. A. Tajmir-Riahi, *J. Pharm. Biomed. Anal.*, 2009, **49**, 468.
55. A. C. Pulla Reddy, E. Sudharshan, A. G. Appu Rao and B. R. Lokesh, *Lipids*, 1999, **34**, 1025.
56. A. Kunwar, A. Barik, R. Pandey and K. I. Priyadarsini, *Biochim. Biophys. Acta, Gen. Subj.*, 2006, **1760**, 1513.
57. P. Bourassa, C. D. Kanakis, P. Tarantilis, M. G. Pollissiou and H. A. Tajmir-Riahi, *J. Phys. Chem. B*, 2010, **114**, 3348.
58. G. Kontopidis, C. Holt and L. Sawyer, *J. Dairy Sci.*, 2004, **87**, 785.
59. J. Liu, J. Tian, Z. Hu and X. Chen, *Biopolymers*, 2004, **73**, 443.
60. D. S. Siegel, X. Zhang, R. Feinman, T. Teitz, A. Zelenetz, V. M. Richon, R. A. Rifkind, P. A. Marks and J. Michaeli, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 162.
61. Y. Tu, S. Renner, F. Xu, A. Fleishman, J. Taylor, J. Weisz, R. Vescio, M. Rettig, J. Berenson, S. Krajewski, J. C. Reed and A. Lichtenstei, *Cancer Res.*, 1998, **58**, 256.
62. A. Renuga Parameswari, G. Rajalakshmi and P. Kumaradhas, *Chem.-Biol. Interact.*, 2015, **225**, 21.
63. V. Tello-Franco, M. C. Lozada-Garcia and M. Soriano-Garcia, *Curr. Comput.-Aided Drug Des.*, 2013, **9**, 289.
64. C. Bartolucci, E. Perola, C. Pilger, G. Fels and D. Lamba, *Proteins: Struct., Funct., Genet.*, 2001, **42**, 182.
65. H. M. Greenblatt, G. Kryger, T. Lewis, I. Silman and J. L. Sussman, *FEBS Lett.*, 1999, **463**, 321.
66. M. Levitt and M. F. Perutz, *J. Mol. Biol.*, 1998, **201**, 751.

67. S. A. Botti, C. E. Felder, S. Lifson, J. L. Sussman and A. Silman, *Biophys. J.*, 1999, **77**, 2430.
68. S. Tara, A. H. Elcock, P. D. Kirchhoff, J. M. Briggs, Z. Radic, P. Taylor and J. A. McCammon, *Biopolymers*, 1998, **46**, 465.
69. W. D. Mallender, T. Szegletes and T. L. Rosenberry, *Biochemistry*, 2000, **39**, 7753.
70. K. V. Dileep, I. Tintu and C. Sadasivan, *Interdiscip. Sci.: Comput. Life Sci.*, 2011, **3**, 189.
71. N. Nirmal, G. O. Praba and D. Velmurugan, *Indian J. Biochem. Biophys.*, 2008, **45**, 256.
72. S. Kaur, N. H. Modi, D. Panda and N. Roy, *Eur. J. Med. Chem.*, 2010, **45**, 4209.
73. C. N. N'soukpoé-Kossi, P. Bourassa, J. S. Mandeville, L. Bekale and H. A. Tajmir-Riahi, *J. Photochem. Photobiol., B*, 2015, **151**, 69.
74. S. Nafisi, M. Adelzadeh, Z. Norouzi and M. N. Sarbolouki, *DNA Cell Biol.*, 2009, **28**, 201.
75. Y. Hobani, A. Jerah and A. Bidwai, *Bioinformation*, 2017, **13**, 63.
76. Y. Liu, Y. Cai, D. Ying, Y. Fu, Y. Xiong and X. Le, *Int. J. Biol. Macromol.*, 2018, **116**, 893.
77. R. Pushpalatha, S. Selvamuthukumar and D. Kilimozhi, *J. Young Pharm.*, 2017, **9**, 480.

CHAPTER 12

Biological Activities of Non-curcuminoids

SWEE KEONG YEAP*[a] AND WAN YONG HO[b]

[a]China-ASEAN College of Marine Sciences, Xiamen University Malaysia, 43900 Sepang, Selangor, Malaysia; [b]Faculty of Science and Engineering, University of Nottingham Malaysia, Jalan Broga, 43500 Semenyih, Selangor, Malaysia
*E-mail: skyeap2005@gmail.com

12.1 Introduction

Turmeric (*Curcuma longa* L.) has long been recognised as an important spice for the past 4000 years in India and subsequently in other main regions of the world including China (~700 AD) and Africa (~800–1200 AD).[1] Besides serving as an important ingredient in food preparation, turmeric has been widely used in Ayurvedic[2] and Chinese medicine[3] in treating various ailments including inflammatory related diseases. Curcumin, a curcuminoid that gives the bright yellow colour to *C. longa*, has been reported as the main constituent that contributes to the major bioactivities of this spice.[4] With the advancement in analytical chemistry, more chemical constituents have now been isolated from *C. longa*. To date, more than 200 compounds have been identified[5] where curcuminoids, sesquiterpenes and phenolic compounds are the major groups of active

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

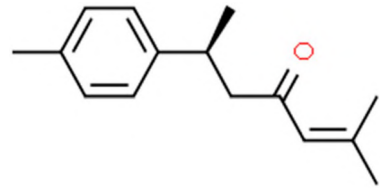
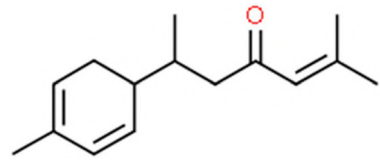
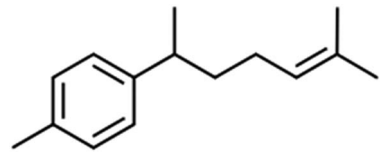
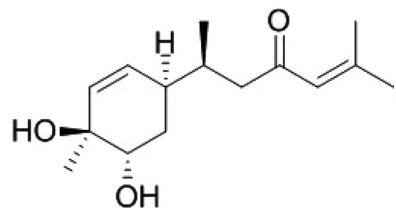
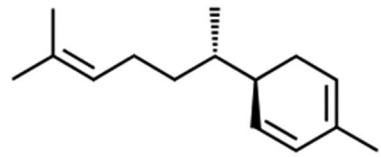
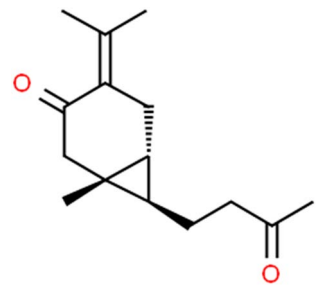
Published by the Royal Society of Chemistry, www.rsc.org

constituents of the spice.[3] Apart from curcumin, demethoxycurcumin and bisdemethoxycurcumin, the three most abundant curcuminoids or compounds[6] in *C. longa*, sesquiterpenes and phenolic compounds have also been implicated in a wide range of biological activities, with some showing a synergistic effect with curcumin when consumed. A number of preclinical and clinical bioactivities of curcumin and some of the major curcuminoids have been widely documented.[4] On the other hand, despite being the second largest group of bioactive compounds in turmeric, many of the non-curcuminoid sesquiterpenes that were reported with promising preclinical bioactivities receive much less attention from the natural product community, and thus less interest for clinical trials. This chapter attempts to provide an overview of the current research progress involving major sesquiterpenes in *C. longa.* Bioactivities reported from preclinical studies of non-curcuminoid sesquiterpenes in *C. longa* are reviewed. Also, the current progress of the clinical trials on β-elemene, the most extensively studied non-curcuminoid sesquiterpenes, will also be discussed in this chapter.

12.2 Non-curcuminoid in Turmeric

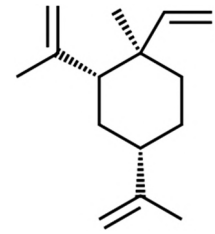
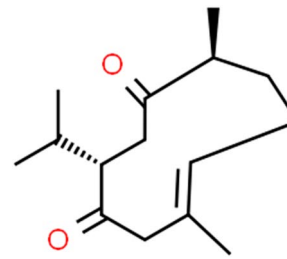
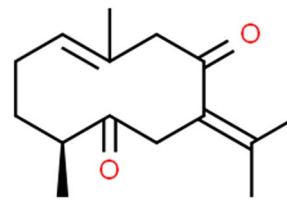
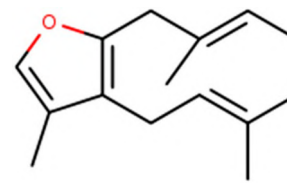
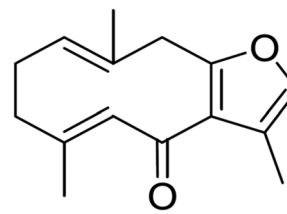
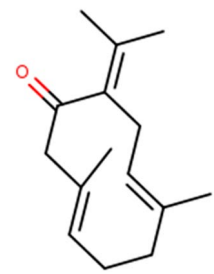
Besides curcuminoid, sesquiterpenes (STs) is another main group of compounds that is responsible for the aroma of *C. longa.*[3] There are different types of sesquiterpenes in *C. longa*, namely bisabolane-type sesquiterpinoids, curcumane-type sesquiterpinoids, elemene-type sesquiterpinoids, germacrane-type sesquiterpinoids and guaiane-type sesquiterpinoids. Among these, bisabolane-type sesquiterpinoids, particularly aromatic-turmerone (ar-turmerone) and curlone, are the most abundant sesquiterpenes in *C. longa.*[6,7] Ar-turmerone receives greater attention than other members in the group due to the discovery of bioactivities especially anti-tumour effects. Bioinformatics prediction based on pharmacokinetics, pharmacodynamics and toxicity profiling (absorption, distribution, metabolism and excretion profiling) suggested that ar-turmerone and β-turmerone are potential druggable molecules with strong binding with inflammatory protein cyclooxygenase-2 (COX-2).[8] On the other hand, curlone is still under-investigated whereby its effect is commonly evaluated in the form of a constituent within standardized extracts which contain other major sesquiterpenes such as ar-turmerone. Thus, bioactivities for curlone are still unclear. Other than turmerone and curlone, β-elemene, which can be isolated from the rhizome of curcuma, is another sesquiterpene that receives great attention due to its anticancer effects, and has attracted study up to clinical levels.[9] Table 12.1 lists the types and chemical structures of the non-curcuminoid sesquiterpenes that are available in *C. longa*. These compounds collectively contribute to the medicinal value of turmeric, which will be discussed in the following sections.

Table 12.1 Types and chemical structures of non-curcuminoid sesquiterpenes (ST) in *C. longa*.

Non-curcuminoid in turmeric	Chemical structure
Bisabolane-type STs	
Ar-turmerone	 10
α/β-Turmerone	 11
α-Curcumene	 12
Bisacurone	 10
Zingiberene	 11
Curcumane type STs	
Curcumenone	 13

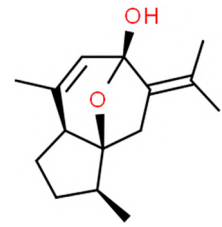
(*continued*)

Table 12.1 (*continued*)

Non-curcuminoid in turmeric	Chemical structure
Elemene type ST	
β-Elemene	 14
Germacrane-type STs	
Curdione	 15
Dehydrocurdione	 13
Furanodiene	 13
Furanodinone	 16
Germacrone	 13

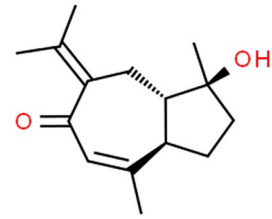
Guaiane-type STs

Curcumenol



13

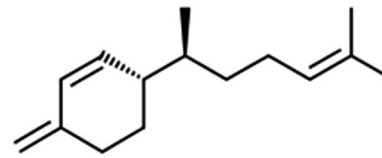
Procurcumenol



17

Other STs

β-sesquiphellandrene



11

12.3 Anticancer Effects of *C. longa* STs

C. longa has been used widely in Ayurvedic[2] and Chinese medicine[3] to treat inflammatory related diseases including cancers. Although the anti-tumour effect was believed to be contributed mainly by curcumin and curcuminoid,[18] recent research suggests that non-curcuminoid STs may exert synergistic anti-tumour effects in combination with curcumin. For instance, an *in vitro* study showed that an equal amount of curcumin showed a higher degree of cytotoxicity to cancer cells when given in the form of *C. longa* extract as compared to its pure form.[19] The cytotoxicity and anti-tumour effect of the STs in cell-based and animal model-based experiments reported to date are listed in Tables 12.2 and 12.3. Table 12.2 shows the *in vitro* cytotoxicity and inhibition concentration that reduced 50% of the cancer cell viability (IC_{50}) value of the respective *C. longa* ST on various types of cancer cell lines, while Table 12.3 shows validation of the *in vitro* anti-tumour effects on animal models. Among the listed compounds, β-elemene was reported to exhibit the broadest cytotoxicity effects against various types of cancer cell lines followed by furanodiene (Table 12.2 and Table 12.3). Based on these current updates, mechanisms of action of different groups of STs and their synergistic effect with other treatments (Table 12.4) are reviewed in the following sections.

Table 12.2 *In vitro* cytotoxicity and IC_{50} value of *C. longa* ST.

Type of ST	Type of cancer	Cell line	Concentration (μM), time (h)
Bisabolane-type STs			
Ar-turmerone	Leukaemia	L1210	231.1, 24;[20] 91.1, 24;[21] 184.9, 48[22]
		HL60	83.2, 24;[20] 46.2, 48;[23] –500, 120[24]
		K562	92.5, 24;[20] 255.2, 24 h[21]
		Molt4B	69.3, 48[23]
		U937	156.7, 24;[21] 138.7, 48[25]
		RBL-2H3	165, 24[21]
	Liver	HepG2	No effect;[26] 299.6, 24[27]
		Hep3B	473.8, 24[27]
		Huh-7	564.9, 24[27]
	Stomach	KATO III	No effect[23]
	Colon	HCT116	741, 48[28]
		HT29	870.9, 48[28]
α-Turmerone	Colon	HCT116	131, 48[28]
		HT29	300.4, 48[28]
α-Curcumene	Breast	MCF-7	206.6, 48[26]
		MDA-MB-231	149.2, 48[26]
	Cervix	SiHa	250, 48[29]
	Leukaemia	HL60	108.6, 48[30]
	Liver	HepG2	162.6, 48[26]
Zingiberene	Breast	MCF-7	842.4, 24[31]
	Brain	N2a	1959, 24[32]
	Cervix	HeLa	298.7, 24[31]
		SiHa	255.3, 24[31]
	Leukaemia	HL60	391.8, 24[31]
Curcumane-type STs			
Curcumenone	Breast	MCF-7	34.1, 72[13,33]
	Cervix	Ca Ski	426.7, 72[13,33]
	Colon	HT-29	183.5, 72[13,33]
	Lung	NCI-H187	65, 24[34]
	Prostate	PC-3	170.7, 72[13,33]
	Umbilical vein endothelial cells	HUVEC	213.4, 72[13,33]
Elemene-type STs			
β-Elemene	Bladder	T24	68.5, 24;[35] 73, 24;[36] 210.4, 24;[37] 371.9, 24[38]
		5637	163.9, 24;[37] 415.9, 24[38]
	Brain	A172	318, 24;[39] 433.6[40]
		C6	342.5, 24[41–44]
		CCF-STTG1	401, 24;[39] 433.6[40]
		U251	288.6, 24[45]
		U-87 MG	237.3, 24;[46] 300.1, 24;[45] 391, 24;[41–44] 431, 24;[39] 611.7, 24;[47] 433.6[40]
	Breast	MCF-7	455, 24;[39] 306.9, 72[48]
		MCF-7/ADR	204.1, 48 h;[49–52] 326.7, 72[48]
		T47D	308, 24[39]
		MDA-MB-231	150, 24 h[53]

	Cervix	HeLa	236, 24;[54] 308, 24;[39] 230.7, 48;[55] 336.2, 48[56]
		ME-180	333, 24[39]
		HTB-33	333, 24[39]
		ShiHa	142.9, 48[57]
	Colon	CCL-222	230, 24[39]
		CCL-225	328, 24[39]
	Gingival squamous	YD-38	342.5, 48[58]
	Leukaemia	HL60	84.9, 120[59]
		K562	256.7, 72[48]
		K562/A02	309.8, 72[48]
	Liver	HepG2	97.9, 72[60]
	Lung	A549	173.2, 24;[61] 234.9, 48;[62] 244.7, 24;[63] 261.8, 24;[64] 298.5, 24;[65] 200, 48[14]
		H460	244.7, 24;[65] 205.5, 48[62]
		NCI-H1299	180, 48[14]
		NCI-H596	465, 24[39]
		NCI-H69	255, 24[39]
		95-D	160, 48[14]
	Lymphoma	Raji	195.7, 48[66]
		Namalwa Burkitt's	146.8, 48[66]
		OCI-LY8	1980.8, 24[67]
	Melanoma	B16F10	447.6, 48[68]
	Nasopharyngeal	C666-1	53.8, 48[69]
		HNE2	63.1, 48[69]
	Osteosarcoma	MG-63	345, 24[70]
		OS-732	93, 48[71]
		Saos-2	305.8, 24[70]
	Ovarian	A2780	318.1, 48[72–76]
		A2780/CP70	342.5, 48[72,73,75,76]
		ES-2	264.2, 48[73]
		OVCAR-3	278.9, 48[73]
		SKOV-3	327.9, 48[73]
		MCAS	381.7, 48[73]
	Prostate	DU145	367, 24[39]
		PC-3	514, 24[39]
	Renal	RCC786-0	712.5, 24[77]
	Sarcoma	A673	185.9, 24[78]
		MHH-ES-1	234.4, 24[78]
	Stomach	SGC-7901	328.8, 24;[79,80] 259.5, 48[55]
		SGC-7901/ADR	261.8, 24[81,82]
		MGC803	391.5, 24[83]
		MKN45	223.1, 24[79]
Germacrane-type STs (84, 85)			
Curdione	Breast	MCF-7	533.1, 72[84]
	Cervix	HeLa	135.4, 12[85]
	Leukaemia	K562	101.5, 12[85]
	Prostate	PC-3	126.9, 12[85]
	Stomach	SGC-7901	84.6, 12[85]

(*continued*)

Table 12.2 (*continued*)

Type of ST	Type of cancer	Cell line	Concentration (μM), time (h)
Dehydrocurdione	Breast	MCF-7	140.8, 72 [13,33]
	Cervix	Ca Ski	93.9, 72 [13,33]
	Colon	HT-29	106.7, 72 [13,33]
	Prostate	PC-3	81.1, 72 [13,33]
	Umbilical vein endothelial cells	HUVEC	102.4, 72 [13,33]
Furanodiene	Breast	MCF-7	171, 72;[13,33] 100, 48;[86] 50, 72 [87]
		MCF-7/ADR	70, 48 h [88,89]
		MDA-MB-231	50, 48 [86]
	Cervix	HeLa	2.8, 12;[85] 2.77, 48 [90]
	Colon	HT-29	212.3, 72 [13,33]
	Connective tissue	HT1080	10.2, 48 [90]
	Leukaemia	HL60	22.2, 48;[90] 30.7, 72 [91]
		K562	69.3, 12 h;[85] 68, 48 [90]
		KG1a	231.1, 48 [92]
		MOLT-4	184.9, 48 [92]
	Liver	HepG2	78.6, 12 h;[85] 7.9, 48;[90] 300, 48 h [93]
		SMMC-7721	69.3, 12 h;[85] 67, 48 [90]
	Lung	A549	32.4, 12 h;[85] 32.4, 48;[90] 170, 48 [14]
		NCI-H1299	140, 48 [14]
		95-D	100, 48 [14]
	Prostate	PC-3	18.5, 12 h;[85] 10.2, 48;[90] 184.9, 72 [13,33]
	Stomach	SGC-7901	18.5, 12 h;[85] 19, 48 [90]
	Umbilical vein endothelial cells	HUVEC	189.5, 72 [13,33]
Furanodienone	Breast	BT-474	30, 48 [94]
		SK-BR-3	20, 48 [94]
		MCF-7	50, 24;[95] 138, 72;[33] 179 [34]
		MCF-7/ADR	52, 48 [88]
		T47D	50, 24 [95]
		MDA-MB-231	>160, 24 [95]
	Colon	HT-29	400, 24 [96]
		RKO	200, 24 [96]
	Lung	NCI-H187	20 [34]
Germacrone	Brain	U87	220, 24 [97]
		U251	250, 24 [97]
	Breast	MCF-7	300, 24;[98] 150, 48;[99] 270.2, 72;[13,33] 202 [34]
		MCF-7/ADR	180, 48 [100]
		MDA-MB-231	300, 24 [98]
	Cervix	Ca Ski	178.6,72 [13,33]
		HeLa	82.4, 12;[85] 168, 48 [101,102]
	Colon	HT-29	196.9, 72 [13,33]

	Leukaemia	K562	64, 12 [85]
		K562/ADR	30, 48 [103]
	Liver	Bel-7402	130;[104,105] 177, 48 [101,102]
		HepG2	160;[104,105] 175, 48 [101,102]
	Lung	A549	68.7, 12;[85] 177, 48 [101,102]
	Prostate	PC-3	27.5, 12;[85] 251.9, 72 [13,33]
	Stomach	SGC-7901	18.3, 12 [85]
	Umbilical vein endothelial cells	HUVEC	338.9, 72 [13,33]
Guaiane-type STs			
Curcumenol	Breast	MCF-7	38.4, 72 [13,33]
		4T1	106.7 [106]
	Cervix	Ca Ski	81.1, 72 [13,33]
	Colon	HT-29	106.7, 72 [13,33]
	Leukaemia	CEMSS	51.2 [107]
	Prostate	PC-3	72.5, 72 [13,33]
	Stomach	AGS	200, 24 [108]
	Umbilical vein endothelial cells	HUVEC	111, 72 [13,33]
Other STs			
β-Sesquiphellandrene	Connective tissue	HT1080	100, 72 [109]
	Lung	A549	5, 72 [110]
		NCI-H1299	7, 72 [110]
	Leukaemia	KBM-5	10, 72 [110]
	Myeloma	U266	6, 72 [110]
		MMI	7, 72 [110]

Table 12.3 *In vivo* anti-tumour effect of *C. longa* ST.

Type of ST	Type of cancer	Cell line/chemical for induction of the model	Active *in vivo* dosage
Bisabolane-type STs			
Ar-turmerone	Sarcoma	S180	50 mg kg^{-1} [111]
		Zebrafish embryos angiogenesis	12.5 μg mL^{-1} [112]
		Matrigel with basic fibroblast growth factor (bFGF)	50 μg mL^{-1} [112]
α-Curcumene	Sarcoma	S180	50 mg kg^{-1} [111]
Elemene-type STs			
β-Elemene	Brain	U87MG and U87MG glioma stem-like cells	β-Elemene 50 mg kg^{-1} + temozolomide 5 mg kg^{-1} [113]
		U87MG glioma stem-like cells	β-Elemene 50 mg kg^{-1} + temozolomide 5 mg kg;[113] 50 mg kg^{-1} [47]

(*continued*)

Table 12.3 (*continued*)

Type of ST	Type of cancer	Cell line/chemical for induction of the model	Active *in vivo* dosage
		Primary glioblastoma stem-like cells	β-Elemene 50 mg kg^{-1} + temozolomide 33 mg kg^{-1} [114]
		C6	100 mg kg^{-1} β-elemene +2 mg kg^{-1} methoxyamine[115]
	Colon	Colo320	40 mg kg^{-1} β-elemene + 4 Gy of radiation[116]
	Gingival squamous	YD-38	45 mg kg^{-1} β-elemene + 5 mg kg^{-1} cisplatin[58]
	Liver	H22	50 mg kg^{-1} [117,118] 100 mg kg^{-1} elemene and 8 mg kg^{-1} Endostar[119]
	Lung	Lewis	100 mg kg^{-1} [120]
		A549	45 mg kg^{-1} elemene + radiation[121–124]
	Lymphoma	Raji and Namalwa Burkitt's	60 mg kg^{-1} [66]
	Nasopharyngeal	C666-1	100 mg kg^{-1} [69]
	Osteosarcoma	OS-732	1.2 mg kg^{-1} [71]
	Pancreatic	7,12 dimerthylbenzanthracene (DMBA)-induced pancreatic carcinoma cells	0.1 mL + IL-23 transfected dendritic cells (2×10^4 cells)[125]
	Sarcoma	A673	100 mg kg^{-1} [78]
		S180	50 mg kg^{-1} [48]
	Stomach	SGC7901/ADR	25 μg kg^{-1} β-elemene and 2 mg kg^{-1} doxorubicin[81,82]
Germacrane-type STs			
Curdione	Breast	MCF-7	50 mg kg^{-1} [84]
Furanodiene	Breast	MCF-7	15 mg kg^{-1} [87]
	Cervix	U14	20 mg kg^{-1} [90] and 40 mg kg^{-1} [85]
	Sarcoma	S180	10 mg kg^{-1} [90]
	Vessel formation	Tg transgenic zebrafish embryos	50 μM[126]
Furanodienone	Brain	G422	80 mg kg^{-1} [127]
	Colon	RKO	2 mg kg^{-1} [96]

12.3.1 Cytotoxic and Anti-tumour Effect of Bisabolane-type of *C. longa* STs

Cancer cell lines from breast, leukaemia, liver, cervix and colon have previously been reported to be sensitive to the treatment by ar-turmerone, α-turmerone, α-curcumene and zingiberene (Table 12.2). Caspase-mediated apoptosis is the most commonly evaluated mechanism among the cell death

Table 12.4 Synergistic effect of *C. longa* ST with drugs or compounds.

Type of ST	Other treatment	Targeting cells	Mode of action
Bisabolane-type STs			
Ar-turmerone α-curcumene	Curcumin	Caco2 (*in vitro*)	Ar-turmerone and α-curcumene are not toxic to $CaCo_2$ cells but enhanced the uptake of curcumin into the cells.[128]
Ar-turmerone	αβ-Turmerone, curcumin	HT29, HCT116, HUVEC (*in vitro*), HT29 (*in vivo*)	Combination of curcumin, αβ-turmerone and ar-turmerone collectively contributed to the strong *in vitro* cytotoxicity on HT29 and HCT116 cells, inhibition of HUVEC tube formation and *in vivo* anti-tumour effect on HT29 cells bearing nude mice of turmeric extract.[28]
Elemene-type STs			
β-Elemene	Cisplatin	YD-38 (*in vitro* and *in vivo*)	β-Elemene reduced IC_{50} value of cisplatin by decreasing expression of phosphor-signal transducer and activator of transcription 3 (p-STAT3), phosphor-Janus kinase 2 (p-JAK2) and B-cell lymphoma 2 (Bcl-2) and increased expression of bcl-2-like protein 4 (Bax) and caspase 3.[58]
		5637, T24, A-172, U87MG, NCI-H69, MCAS, HeLa, ME-180, COLO205, DU145, PC3, H460, MCF-7 and T47D (*in vitro*)	β-Elemene reduced IC_{50} value of cisplatin on the cancer cells through enhancing cisplatin mediated caspase dependent apoptosis.[38,129,130]
		A2780 and resistant A2780/CP70 (*in vitro*)	β-Elemene increased sensitivity of ovarian carcinoma (normal and resistant cells) to the cisplatin treatment through enhancing G2/M arrest (downregulation of cyclin and cyclin-dependent kinase CDC2, cyclin A and cyclin B1 and upregulation of potent cyclin-dependent kinase inhibitor ($p21^{WAF1/CIp1}$) and p53) and inducing apoptosis by blocking cisplatin-induced PI3K/JNK and PI3K/Akt activation. The cell cycle arrest and induction of apoptosis prevented downstream signalling including AP-1, and NF-kB, which promoted DNA repair and cell survival.[72–76]

(*continued*)

Table 12.4 (*continued*)

Type of ST	Other treatment	Targeting cells	Mode of action
		H460, A549 (*in vitro*)	β-Elemene worked synergistically with cisplatin to induce G2/M phase arrest on the non-small cell lung cancer through CHK2-dependent CDC25C/CDC2/cyclin B1 signalling pathway.[62]
	Colchicine, paclitaxel or vinblastin	Colchicine-resistant KB-C2 (*in vitro*)	β-Elemene in combination with either colchicine, paclitaxel or vinblastine (but not alone) selectively targeted the colchicine-resistant cervical cancer cells through inhibiting efflux activity of ABCB1 transporter without affecting its expression.[131]
	Doxorubicin	SGC7901/ADR (*in vitro* and *in vivo*)	β-Elemene increased accumulation of doxorubicin in the cells by suppressing expression of P-gp, phosphorylated Akt, ERK and GST-π and upregulation of E3 ubiquitin ligases, c-Casitas B-lineage lymphoma (Cbl) (c-Cbl and Cbl-b) family of ubiquitin ligase. Moreover, the combination treatment reduced tumour weight and progression.[81,82]
	Endostar	H22 (*in vivo*)	The combination treatment reduced ascites formation through decreasing peritoneal microvascular permeability and reduction of metastatic related genes including VEGF, MMP2 and hypoxia inducible factor 1α (HIF1α).[119]
	Erlotinib (tyrosine kinase inhibitor)	Erlotinib-resistant A549/ER (*in vitro*)	β-Elemene enhanced cytotoxicity of erlotinib to the A549/ER-resistant cells through downregulation of P-gp expression and P-gp-dependent drug efflux.[132]
	Etoposide	A549 (*in vitro*)	The combination treatment promoted apoptosis of the cancer cells by upregulating expression of Bax, p53, p21 and suppressing cyclin D1.[63]
	Gefitinib (EGF receptor tyrosine kinase inhibitor)	A549, H1299 (*in vitro*)	The combination treatment suppressed invasion/migration, epithelial to mesenchymal transition (EMT), stem-like properties and self-renewal capacities of non-small-cell lung cancer by regulating enhancer of zeste homolog 2 (EZH2).[133]

	U251, U87MG (*in vitro*)	β-Elemene enhanced cytotoxicity of 30 μM gefitinib, through decreased phosphorylation of AKT, EGFR and ERK.[45]
Hyperthermia (42 °C)	A549 (*in vitro*)	The hyperthermia treatment sensitized the cytotoxicity of β-elemene by upregulating expression of p21 and Bax; associated with downregulation of Bcl-2 and surviving.[61]
IL-23 transfected dendritic cells	Primary 7, 12 dimerthylbenzanthracene (DMBA)-induced pancreatic carcinoma cells (*in vivo*)	The combination treatment reduced the tumour size and increased survival time.[125]
Methoxyamine	C6 (*in vitro* and *in vivo*)	The combination reduced tumour weight and progression.[115]
Oxaliplatin	Hep3B, Huh7, MHCC97H and MHC-CLM (*in vitro*)	β-Elemene stabilized transporter CTR1 to increase the intake of oxaliplatin into the hepatocellular carcinoma.[134]
Radiation	Colo320 (*in vivo*)	The combination treatment (but not β-elemene alone) reduced tumour weight through induction of Fas mediated apoptosis.[116]
	A549 (*in vitro* and *in vivo*)	β-Elemene enhanced sensitivity of human lung adenocarcinoma A549 cells on radiation *in vitro* and *in vivo* by inhibiting radiation-induced expression of survivin, hypoxia-inducible factor (HIF-1α), mTOR and peroxiredoxin-1 (Prx-1). Prx-1 is a major H_2O_2 scavenger and signalling regulator that confer radioresistance.[121–124]
	U87-MG, T98G, U251, LN229 and C6 (*in vitro*)	β-Elemene increased sensitivity of cancer cells to radiation through decreasing the phosphorylation of ataxia telangiectasia mutated (ATM), AKT and ERK.[135]

(*continued*)

Table 12.4 (*continued*)

Type of ST	Other treatment	Targeting cells	Mode of action
		MKN45 and SGC7901 (*in vitro*)	β-Elemene enhanced sensitivity of human gastric cancer cells to the radiation by upregulating PAK1IP1 and downregulating phosphor-Pak1 and phosphor-ERK1/2 expressions.[136]
	Tamoxifen	MCF-7 and MCF-7/Tam-resistant cells (*in vitro*)	The combination treatment (but not β-elemene alone) induced reversal of tamoxifen resistance in MCF-7 cells through re-expression of oestrogen receptor α (ERα) by downregulating Ras/MAPK/ERK signalling pathway. Upregulation of ER-α expression maintained the epithelial morphology of breast cancer and promoted expression of metastasis-associated protein 3 (MTA3) that downregulation of nuclear transcription factor Snail to reduce the invasion and migration of MCF-7 cells.[137]
	Taxol	A2780 and resistant A2780/CP70 (*in vitro*)	The combination treatment induced G2/M phase arrest and apoptosis on cisplatin-resistant A2780/CP70 cells.[72–76]
	Temozolomide	U87-MG, T98G, U251, LN229 and C6 (*in vitro*)	β-Elemene increased sensitivity of cancer cells to temozolomide through activating activation of glia maturation factor β (GMFβ)[41–44] that inactivated the AKT/ERK surviving pathway.[135]
		U87MG, U87MG stem-like cells, primary glioblastoma stem-like cells (*in vitro* and *in vivo*)	Co-treatment of β-elemene and temozolomide inhibited both glioblastoma parental and stem-like cells.[113,114]
	TRAIL	BGC823 and SGC7901 (*in vitro*)	β-Elemene increased sensitivity of human gastric cancer cells to the TRAIL by promoting DR5 clustering and translocation of caspase 8, DR5 and FADD, which led to cleavage of caspase 8 and formation of death-inducing signalling complex.[138]

Germacrane-type STs			
Furanodiene	Doxorubicin	MCF-7/ADR (*in vitro*)	Furanodiene-sensitized resistant MCF-7 cells to doxorubicin by enhancing the uptake of doxorubicin and inducing both intrinsic apoptosis [by elevating reactive oxygen species (ROS) and calcium level] and extrinsic apoptosis [by elevating tumour necrosis factor alpha (TNFα) level, which promoted caspase 8 cleavage and nuclear factor kappa-light-chain-enhancer of activated B (NF-kB) activation]. However, furanodiene did not affect expression and activity of drug efflux P-glycoprotein (P-gp).[88,89]
		MDA-MB-231 (*in vitro*)	Furanodiene enhanced cytotoxicity of doxorubicin on MDA-MB-231 cells through induction of mitochondria mediated apoptosis.[139] In addition, it attenuated the pro-metastasis effect caused by low concentration of doxorubicin (0.1 μM) in metastatic MDA-MB-231 cells through regulation of integrin αV, focal adhesion kinase/proto-oncogene tyrosine-protein kinase/paxillin (FAK/Src/paxillin), phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt), β-catenin and matrix metallopeptidase 9 (MMP-9) signalling pathways.[140]
	Paclitaxel	95-D (*in vitro*)	Furanodiene showed synergistic anti-proliferative effect with paclitaxel in lung cancer 95-D cells *via* induction of Gap1 phase arrest [downregulation of cyclin D1, cyclin B1, cell division protein kinase 6 (CDK6) and myelocytomatosis (c-myc)]. In addition, the combination treatment also showed potential anti-metastasis effect through downregulation of the metastasis related genes (integrin-β4, focal adhesion kinase and paxillin).[14]

(*continued*)

Table 12.4 (*continued*)

Type of ST	Other treatment	Targeting cells	Mode of action
	Tamoxifen	MCF-7 (*in vitro*)	Furanodiene enhanced the antiproliferation [through downregulating expression of *p*-cyclin D1, cyclin D1, cell division protein kinase 2 (CDK2), cell division protein kinase 6 (CDK6), retinoblastoma protein (p-Rb), phosphorylated-p44 and upregulating cyclin-dependent kinase inhibitor 1B (p27)] and apoptotic [upregulating expression of Bax and BCL2 associated agonist of cell death (Bad)] effects of tamoxifen in MCF-7 cells.[86]
Germacrone	Doxorubicin	MCF-7/ADR, K562/ADR (*in vitro*)	Germacone, which was sensitive to adrianmycin-resistant MCF-7 (MCF-7/ADR) cells but not toxic to adrianmycin-resistant K562 (K562/ADR) cells, reduced expression of multidrug resistance I gene promoter and P-gp protein, which enhanced the apoptosis effect of adriamycin to the resistant cells. Pan *et al.* (2018).[100,103]
	Furanodiene + curdione	MCF-7, MDA-MB-231 (*in vitro*)	Despite poor cytotoxicity of germacrane-type STs on MCF-7 and MDA-MB-231 cells (where no IC_{50} obtained up to 200 μM), combination of germacrone (14 μM)+furanodiene (43 μM) and germacrone (14 μM)+curdione (43 μM)+furanodiene (43 μM) killed more than 50% of MCF-7 and MDA-MB-231 cells *via* upregulation of apoptosis related proteins including Bax/BcL ratio, Poly (ADP-ribose) polymerase (PARP) and cleavage of caspase 9 while downregulating the mitogen-activated protein kinases (p42/p44 MAPK) protein.[15]

determination assays for ar-turmerone (HepG2, HL60 and U937 cells),[23–25] α-curcumene (HL60, MDA-MB-231 and SiHa cells)[26,29,30] and zingiberene (SiHa cells).[31] Among which, ar-turmerone was reported to cause overproduction of intracellular reactive oxygen species (ROS), which activated ERK and

JNK kinase phosphorylation that triggered intrinsic and extrinsic caspase-dependent apoptosis of liver cancer cells.[27]

On the other hand, an anti-metastatic effect of ar-turmerone was also observed whereby the compound obstructed invasion and migration of 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced MDA-MB-231 and MCF-7 cells through the inhibition of MMP-9 and COX-2 expression by blocking NF-kB, PI3K/Akt and ERK1/2 signalling.[141] In another study, the compound inhibited proliferation, tube formation and motility of human microvascular endothelial cells (HUVEC) *via* downregulation of metastasis related genes including matrix metallopeptidase 2 (MMP2), MMP9, vascular endothelial growth factor receptor 3 (VEGFR3), intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM1). This anti-angiogenesis effect was also validated in zebrafish embryos and a basic fibroblast growth factor (bFGF)-challenged mice model.[112] Although ar-turmerone and α-curcumene did not show cytotoxicity towards colon cancer cells, these STs enhanced the uptake of curcumin in colon cancer cells[128] (Table 12.4).

Ar-turmerone is the most extensively evaluated compound among the bisabolane-type of *C. long* STs, both *in vitro* and *in vivo*. Moreover, a comparison to other members of this group shows that ar-turmerone was also the most potent member, with the lowest IC_{50} on various cancer cell lines (Table 12.1). The importance of this compound to *C. longa* should not be underestimated as it has also been shown to enhance the cytotoxicity of curcumin on various cell lines (Table 12.4).

12.3.2 Cytotoxic and Anti-tumour Effect of Elemene-type of *C. longa* ST

Elemene, particularly β-elemene, which can be isolated from *C. longa* and other plants, is one of the most widely studied anticancer STs. In China, β-elemene has been listed in China's Food and Drug Administration for use in clinical trials and treatment of cancer patients.[9,142,143]

12.3.2.1 Bone Cancer

β-Elemene induced caspase-mediated apoptosis and inhibited expression of metastasis related genes [MMP2, MMP9, tissue inhibitors of metalloproteinases metallopeptidase inhibitor 1 (TIMP1), TIMP2, NF-kB, interleukin 8 (IL-8), C-X-C chemokine receptor type 4 (CXCR4), Urokinase (uPA)] in osteosarcoma OS-732 cells.[71] In Ewing sarcoma A673 and MHH-ES-1 cells, β-elemene inhibited insulin-driven growth of the cells by inhibiting phosphorylation of insulin receptor and S6 ribosomal protein while activating the mammalian target of rapamycin (mTOR) and Phosphoinositide 3-kinases (PI3K) signalling pathways.[78] Similarly, the compound was found to induce apoptosis in MG-63 and Saos-2 cells by generating intracellular ROS and activating the PI3K/Akt/mTOR signalling pathway. Although activation of the mTOR signalling pathway also enhanced expression of hypoxia-inducible factor 1-alpha (HIF-1α) that may in

turn rescue cancer cells from β-elemene-induced apoptosis, addition of the HIF-1α inhibitor restored the cytotoxicity of β-elemene.[70]

12.3.2.2 Brain Cancer

Among the *C. longa* STs, β-elemene appears to confer the highest potential to treat brain cancer. A study by Zhang *et al.*[145] showed that β-elemene can traverse the blood–brain barrier to reach a brain tumour site. β-Elemene induced Gap0/Gap1 phase arrest in glioblastoma cells *via* upregulation of glia maturation factor β (GMFβ)-dependent activation of the mitogen-activated protein kinase 3 (MKK3) and 6 (MKK6)-p38 pathways and inactivation of mitogen-activated protein kinase 3 and 1- B-cell lymphoma 2 (ERK1/2-Bcl-2) surviving pathway. On the other hand, the compound disrupted the heat shock protein 90/proto-oncogene serine/threonine-protein kinase (Hsp90/Raf-1) molecular complex and subsequently deactivated the Raf/MEK/ERK pathway leading to suppression of antiapoptotic proteins (Bcl-2, Bcl-xl and X-linked inhibitor of apoptosis) and promotion of both intrinsic/extrinsic apoptosis in the glioblastoma cells.[38,41–44,46,146] β-elemene showed specificity in targeting proliferation, invasion and drug resistance of glioma stem-like cells through downregulation of stemness markers [prominin-1 (CD133) and ATP-binding cassette subfamily G member 2(ABCG2) and Notch1] and mesenchymal markers (*N*-cadherin and β-catenin) alongside upregulation of differentiation related effectors [glial fibrillary acidic protein (GFAP) and sonic hedgehog] and epithelial markers (*E*-cadherin).[47,113,114]

12.3.2.3 Breast Cancer

β-Elemene blocked and reversed transforming growth factor beta 1 (TFG-β1)-mediated epithelial–mesenchymal transition (EMT) by suppressing the expression and phosphorylation of Smad3[147] in breast cancer MCF-7 cells. Furthermore, β-elemene was also cytotoxic to doxorubicin- and doxetaxel-resistant breast cancer (MCF-7/ADR and MCF-7/Doc) cells, particularly to the cancer stem cells' population of these resistant cell lines. The effects were associated with downregulation of ABC transporters such as P-gp, multidrug resistance-associated protein (MRP) and breast cancer resistant protein (BCRP) as well as reduced ABC protein efflux due to weakening of the proteins' functionality. Specificity of β-elemene on the drug-resistant breast cancer cells was claimed to be related to the upregulation of the PTEN signalling pathway *via* modulation of cellular and exosome microRNAs such as miR-34a (upregulated in cells and exosomes) as well as miR-222 and miR-29a (both downregulated in cells).[49–52,148]

12.3.2.4 Lung Cancer

β-Elemene targeted H460 and A549 non-small-cell lung cancer cells through induction of G2/M phase arrest by decreasing expression of cyclin B1 and phosphor-Cdc2(Thr-161) and increasing $p27^{kip1}$ and phosphor-Cdc2 (Tyr-15)

by a Chk2-dependent mechanism.[65] In addition, β-elemene treatment also induced apoptosis in A549 cells by inducing phosphorylation of ERK,[150] inhibiting the PI3K/Akt/mTOR/p70S6K1 signalling pathway[64] and promoting endoplasmic reticulum stress [through up-regulation of endoplasmic reticulum (ER) stress related proteins such as protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), inositol-requiring transmembrane kinase/endoribonuclease 1α (IRE1α), activating transcription factor 6 (ATF6), activating transcription factor 4 (AFT4), CCAAT-enhancer-binding protein homologous protein (CHOP)], which subsequently downregulated the expression of antiapoptotic protein Bcl-2.[120] Further to the effect on a 'normal' A549 lung cancer cell line, β-elemene also induced cell death in cisplatin-resistant A549 (A549/DDP) cells *via* activation of the mitochondrial apoptosis pathways that were associated with reduced cytoplasmic glutathione level and expression of P-gp.[151,152] Other than targeting the lung cancer cells directly, β-elemene was also thought to delay lung cancer progression by preventing M2 macrophages mediated epithelial–mesenchymal transition (EMT) in lung cancer cells.[153]

12.3.2.5 Stomach Cancer

β-Elemene induced apoptosis in gastric cancer SGC7901 cells by dysregulating some of the important pathways including ribosome signalling, peroxisome proliferator-activated receptors (PPARs) signalling pathway, regulation of actin cytoskeleton, phagosome and biosynthesis and metabolism of some amino acids. P21-activated protein kinase-interacting protein 1 (PAK1IP1) and topoisomerase 2-alpha (TOPIIα) are the top upregulated and downregulated proteins in the SGC7901 cells treated with β-elemene.[79,80] On the other hand, β-elemene was also reported to induce apoptosis of MGC803 cells through inhibiting phosphorylation of Akt, mTOR and p70S6K1 and down-regulation of survivin expression.[83]

12.3.2.6 Other Types of Cancer

β-Elemene also showed cytotoxicity to other types of cancer cells as listed in Table 12.5. As β-elemene is the most widely evaluated ST in *C. longa*, it shows the widest cytotoxicity against various cancer cell lines than any other types of *C. longa* ST (Table 12.2). Moreover, Table 12.5 shows that β-elemene induced death of different types of cancer cells through different pathways indicating the potential of β-elemene targeting the heterogeneity of tumour cells.

To date, β-elemene is the second most extensively evaluated compound of *C. longa* after curcumin. It shows *in vitro* cytotoxic and *in vivo* anti-tumour effects on various cancerous cell lines by regulating several pathways such as PI3K/AKT/mTOR, EMT, apoptosis and even cancer stem cells' pathways. This compound showed promising results in small-scale clinical trials[9,142,143] and more importantly, studies show that β-elemene augmented chemotherapy, radiotherapy and immunotherapy of various cancers (Table 12.4).

Table 12.5 Overview of the cytotoxic mechanism of β-elemene on bladder cancer, cervical cancer, oesophageal squamous, liver cancer, lymphoma, melanoma, nasopharyngeal carcinoma, prostate cancer and renal cancer cells.

Types of cancer	Mode of action
Bladder cancer	β-Elemene induced apoptosis in T24 bladder cancer cells through upregulation of phosphatase and tensin homolog (PTEN)[37] and tumour suppressor protein Smad4.[36] On the other hand, it also suppressed expression of B-cell lymphoma-extra-large (Bcl-xl), metastasis-associated protein (MTA-1), survivin[35] and phosphor-AKT[37] of the T24 cells. Cytotoxic effect of β-elemene in another bladder carcinoma BIU-87 cells was claimed to be *via* downregulation of phosphatidylcholine and phosphalidylethanolamine content of membrane and Bcl-2.[144]
Cervical cancer	β-Elemene downregulated the Wingless-related integration site (Wnt)/β-catenin signalling pathway of cervical cancer SiHa cells, which subsequently induced Gap1 phase arrest, promoting p53 mediated apoptosis and suppressed MMP2- and MMP9-mediated invasion.[57]
Oesophageal squamous	β-Elemene targeted oesophageal squamous ECA-109 cells by upregulating expression of long non-coding RNA (lncRNA) CDKN2B-AS1, which subsequently inhibited the expression of human telomerase reverse transcriptase (hTERT).[149]
Liver cancer	β-Elemene induced Gap2/Mitosis (G2/M) phase arrest and Fas/FasL-mediated apoptosis in HepG2 hepatocarcima.[60] In addition, it suppressed c-Met and enhanced histone H1 gene expression in murine hepatocellular carcinoma H22 cells.[117,118]
Lymphoma	β-Elemene induced mitochondria mediated apoptosis in Raji and Namalwa Burkitt's lymphoma cells by upregulating expression of p53 upregulated modulator of apoptosis (PUMA).[66]
Melanoma	β-Elemene inhibited invasion and migration of the melanoma B16F10 cells by downregulating expression of urokinase-type plasminogen activator (uPA), uPA receptor (uPAR), MMP2 and MMP9.[68]
Nasopharyngeal carcinoma	β-Elemene induced G1 phase arrest by inactivation of signal transducer and activator of transcription 3 (Stat3), DNA methyltransferase 1 (DNMT1) and enhancer of zeste homolog 2 (EZH2).[69]
Prostate cancer	β-Elemene induced intrinsic pathway of caspase mediated apoptosis in prostate cancer DU145 cells.[39]
Renal cancer	β-Elemene induced apoptosis of renal cancer RCC786-0 cells through inhibition of MAPK/ERK and PI3K/Akt/mTOR signalling pathway.[77]

12.3.3 Cytotoxic and Anti-tumour Effect of Germacrane-type of *C. longa* STs

12.3.3.1 Breast Cancer

Curdione, furanodiene, furanodienone and germacrone, which belong to germacrane-STs in *C. longa*, is another important group of compounds that possess anticancer potential. Curdione, furanodiene and germacrone

showed cytotoxicity on oestrogen receptor positive breast cancer MCF-7 cells by inducing intrinsic caspase apoptosis through downregulation of oestrogen receptor alpha (ERα) and upregulation of tumour protein p53 (TP53) and AT-mutated protein (pATM) expression.[84,87,154] Lim *et al.*[99] showed that germacrone exerted an apoptotic effect on MCF-7 by decreasing the ERα occupancy at oestrogen response elements and preventing downstream coregulators and RNA Pol II from binding to the target genes. Furanodiene[86,87] and germacrone[98] showed a similar cytotoxic effect on oestrogen positive MCF-7 and triple negative MDA-MB-231 breast cancer cells. Zhong *et al.*[98] showed that germacrone differentially induced G1 arrest in MDA-MB-231 cells and G2/M arrest in MCF-7. Apoptotic effect of germacrone on MDA-MB-231 was mediated by leakage of cytochrome c, overexpression of Bcl-2 related ovarian killer (Bok) and cleavage of caspase 3. In targeting triple negative MDA-MB-231 cells, furanodiene appeared to be more outstanding than germacrone as indicated by its lower IC_{50} values in both cell lines than that of germacrone (Table 12.1). In addition, furanodiene exhibited an anti-invasive effect in metastatic MDA-MB-231 cells through blocking NF-kB-dependent Wnt/β-catenin, MMP9 and VEGF activation.[155,156] It was suggested that the anti-metastatic effect to inhibit migration, invasion and tube formation of endothelial cells may also be contributed by the ability of furanodiene to suppress pAkt (Ser473), p-Erk ½ (Thr202/Tyr204), ICAM-1 and p-p85 (Ser428) expression of the cells.[125] On the other hand, furanodienone was more sensitive to oestrogen (of MCF-7 and T47D cells) and human epidermal growth factor receptor 2 (HER2) (of BT474 and SKBR3 cells)-positive cells than the triple negative MDA-MB-231 breast cancer cells.[94,95] Furanodienone induced apoptosis in oestrogen receptor positive breast cancer cells by targeting ERα and oestrogen-responsive elements, which suppressed the transcription of the E2-responsive genes including myelocytomatosis (c-myc), cyclin D1 and Bcl-2 genes.[95] On the other hand, furanodienone killed Her2 overexpressing cells by inhibiting the phosphorylation of Her2, epidermal growth factor receptor (EGFR), Akt and Glycogen synthase kinase 3 beta (Gsk3β) and promoting expression of cyclin-dependent kinase inhibitor $p27^{kip1}$.[94] Besides showing cytotoxicity against drug-sensitive breast cancer cell lines, furanodiene, furanodienone and germacrone showed cytotoxicity on doxorubicin-resistant MCF-7/ADR cells.[88,100,157] Mechanistic studies showed that furanodiene induced AMP-activated protein kinase (AMPK) mediated apoptosis and downregulation of Her2 expression.[157] On the other hand, germacrone induced p53 mediated apoptosis by sensitizing the doxorubicin treatment through suppression of the P-glycoprotein and multidrug resistance 1 gene promoter in MCF-7/ADR cells and K562/ADR cells.[100,103]

12.3.3.2 Other Types of Cancer

Germacrane-STs also showed cytotoxicity on other types of cancer including brain cancer, colon cancer, leukaemia, liver cancer and lung cancer as listed in Table 12.6.

Table 12.6 Overview of the cytotoxic mechanism of germacrane-STs on brain cancer, colon cancer, leukaemia, liver cancer and lung cancer cells.

Types of cancer	Mode of action
Brain cancer	Germacrone induced G1 phase arrest (*via* upregulation of p21 and downregulation of cyclin D1 and CDK2) and apoptosis (increase of p53 and decrease of Bcl-2) in U87MG and U251 glioblastoma cells.[97]
Colon cancer	Furanodienone induced G1 phase arrest (through over-expression of $p21^{Cip1}$, which suppresses the cyclin D1, CDK4/6 and cyclin E, CDK2) and ROS-induced JNK/P38 phosphorylation, which mediated both intrinsic and extrinsic apoptosis in colorectal cancer cells *in vitro* and *in vivo*.[96]
Leukaemia	Furanodiene killed HL-60 leukaemia through induction of tumour necrosis factor receptor 1 (TNFR1)-mediated extrinsic apoptotic pathway.[91] Moreover, it targeted KG1a and MOLT-4 leukaemia cells by suppressing the expression of Wilms' tumour protein.[92]
Liver cancer	Furanodiene and germacrone were sensitive to liver cancer cells. Furanodiene inducted G2/M phase arrest and p38 MAPK activated apoptosis in HepG2 cells (Xiao *et al.*, 2007) while germacrone arrested HepG2 and Bel7402 cells at Gap2 phase by decreased cyclin B1 expression and increased p21 expression. Germacrone also induced apoptosis on both hepatoma cells through upregulation of Bax/Bcl-2 or Bcl-xL ratio and p53 expression. Higher concentration of germacrone (240 μM, at 24 h) induced apoptosis of HepG2 cells by inhibiting pSTAT3, pJAK2.[104,105]
Lung cancer	Furanodiene was also sensitive to lung cancer cells through inducing G1 phase arrest (by downregulating the expression of cyclin D1 and CDK6), endoplasmic reticulum stress-induced apoptosis (by upregulating binding immunoglobulin protein and C/EBP homologous protein) and suppressing migration/invasion of the 95-D lung cancer cells.[14,158] Co-treatment of furanodiene with paclitaxel showed a synergistic effect on lung cancer cells[14] (Table 12.4) indicating that furanodiene is a potential adjuvant for lung cancer treatment.

Germacrane-type STs of *C. longa* showed cytotoxicity against various types of cancer cell lines. More specifically, most of the studies involving germacrene-type of *C. longa* STs focused on breast cancer cell lines ranging from the evaluation of drug-resistant breast cancer cells through promotion of apoptosis; downregulation of inflammation-mediated EMT; to suppression of the P-glycoprotein and multidrug resistance 1 gene promoter, both individually or in combination with chemotherapeutic drugs such as doxorubicin and cisplatin.

12.3.4 Cytotoxic and Anti-tumour Effect of Guaiane and Other Types of *C. longa* STs

Many of the studies evaluating the anti-tumour effect of curcumenol (Guaiane-type STs) and β-sesquiphellandrene remain on the *in vitro* cell-based level.[13,33,106–110] Nevertheless, both compounds showed the potential as anticancer agents in the *in vitro* studies. Based on *in silico* prediction, low IC_{50} value of curcumenol on MCF-7 cells[13,33] (Table 12.1) may be contributed by its ability to bind to the oestrogen receptor alpha.[159] In addition, curcumenol also exhibited cytotoxicity and anti-invasion effects on oestrogen-negative breast cancer cells.[106] β-sesquiphellandrene, on the other hand, was reported to confer high selectivity in the induction of caspase-mediated apoptosis on p53 expressing cancer cells.[110] Thus, both compounds are worth exploring further for their anticancer mechanisms and *in vivo* efficacy.

12.3.5 Synergistic Effect of *C. longa* STs with Other Cancer Treatments

Ar-turmerone, α-curcumene, β-elemene, furanodiene and germacrone were shown to work synergistically with various other drugs including cisplatin, cholchicine, paclitaxel, vinblastine, doxorubicin, endostar, erlotinib, etoposide, gefitinib, interleukin-23 expressing dendritic cells, methoxyamine, oxaliplatin, radiation, tamoxifen, taxol, temozolomide and TNF-related apoptosis-inducing ligand (TRAIL) to kill various types of cancer cells including the drug-resistant cancer cell lines (Table 12.4). Furthermore, combinations of different types of *C. longa* STs showed enhanced anti-inflammatory and anticancer effects. For instance, Murakami *et al.*[160] showed that a combination of β-atlantone, germacrone, ar-turmerone, α-turmerone, β-turmerone and curcumin inhibited COX-2 and inducible nitric oxide synthase (iNOS) expressions in lipopolysaccharides (LPS)-treated RAW264.7 cells and abolished dimethylhydradine-initiated and dextran sulfate sodium-promoted colon carcinogenesis in mice.[160] This finding also justified why turmeric extract showed a greater anticancer effect than curcumin alone.

12.3.6 Current Progress of the Clinical Trial of *C. longa* STs

To date, four clinical studies involving *C. longa* STs have been registered in Clinicaltrials.gov under the U.S. National Library of Medicine and all of the interventions include β-elemene. These on-going clinical trials include one on newly diagnosed malignant gliomas (Phase 3), one on non-small-cell lung cancer (Phase 2, co-treated with EGFR-Erlotinib, Gefitinib and Icotinib) and another two on advanced liver cancer. A high number of clinical trials for evaluation of β-elemene are expected as the compound has been listed by China's Food and Drug Administration for their locally registered clinical trials.[9,142,143] However, some of these clinical trials that used *C. longa* STs,

particularly β-elemene as an adjuvant for conventional cancer therapy, which were performed in China, are not registered with Clinicaltrials.gov. These studies generally involved a lower number of participants but were successfully completed with positive outcomes.[9,142,143] For instance, patients with acute myeloid leukaemia received two courses of 400 mg β-elemene plus HAA chemotherapy (harringtonine, aclacinomycin and cytarabine) were reported to show 80.8% effectiveness while HAA chemotherapy alone showed 52.9% effectiveness.[142] Another study by Ma *et al.*[9] showed that treatment with β-elemene plus chemoradiotherapy and temozolomide after surgery prolonged median progression-free survival, overall survival and haematologic toxicities caused by chemoradiotherapy of newly diagnosed glioblastoma.

On the other hand, no other *C. longa* STs reviewed in this chapter have progressed to clinical trial based on the record in Clinicaltrials.gov. However, a report showed that curdione has been used clinically to treat cervical cancer in China. It is expected that more clinical trials using *C. longa* STs as treatment for cancer and even other diseases will be carried out in the near future if the outcomes of the aforementioned trials are positive.[10]

12.3.7 Limitations of the Current Anticancer Studies of *C. longa* STs

Despite the numerous reports acclaiming the potential anticancer effects of *C. longa* STs against a broad spectrum of cancers, translation of these compounds into clinical usage are still scarce due to some technical and biological limitations.

Technically, better standardization of *C. longa* STs is required for reliable preclinical and clinical evaluations. Biologically, many of the experiments halted at screening level whereby additional evaluation such as mechanism of action, *in vivo* efficacy and pharmacokinetic validation studies are still lacking. In addition, high variation of IC_{50} values across different studies that tested the same compound on the same cell lines is also noticeable (Table 12.2). For example, the IC_{50} value of ar-turmerone on leukaemia L1210 cells reported by Baik *et al.*[20] was 2.5-fold higher than that of Ji *et al.*[21] This variation may be contributed by different culture conditions such as cell seeding number and the type of experiment used to obtain the IC_{50} values.

On the other hand, although ar-turmerone, α-turmerone and β-turmerone showed cytotoxicity on several cancer cells lines and an anti-inflammatory effect on TNF-α-induced NF-kB activation, their efficacies were still much lower than that of curcumin.[161] To overcome this, chemical modification such as molecular hybridization of the active groups may help to enhance the bioactivity and efficacy of these *C. longa* STs.[162] Another biological limitation is that the cancer cells may establish an escape mechanism after being treated with the *C. longa* STs. For instance, cancer cells treated with β-elemene,[53,64,77,83] furanodiene[158] and β-elemene + gefitinib[45] have been reported to activate autophagy (evidenced by morphological changes and

upregulation of light chain 3-II and Atg5-Atg12 expression) that functions as a cytoprotective mechanism, which may subsequently mediate multidrug resistance in cancer. Activation of autophagy in *C. longa* STs may be due to the deactivation of the mTOR pathway by the compounds.[83] To overcome this, the addition of an autophagy inhibitor such as chloroquine was suggested to be used as co-treatment with *C. longa* STs.[163]

Clinically, the main setback is that the number of registered and non-registered clinical trials using these compounds as treatment is too low compared to curcumin. Although the outcomes of the clinical trials as listed in Section 12.3.6 were encouraging, a systemic review suggested that more randomized control trials for β-elemene that adopt good clinical practice standards are needed as most of the previous trials lack methodological quality.[143]

12.4 Immunomodulation Effect of *C. longa* STs

Traditional usage of *C. longa* in treating immune related diseases such as inflammation and to strengthen the body's immune system provides us with a clue that the compounds present in plants may carry strong immunomodulatory effects including immune-stimulation and anti-inflammation activities. Curcumin has been well recognized for its immunomodulatory effects particularly as an anti-inflammatory agent.[164] In this section, the immunostimulatory/immunosuppressive and anti-inflammatory effect of different *C. longa* STs are discussed.

Ar-turmerone and α-turmerone have been shown to promote proliferation and cytokines (interleukin-2, interferon-γ and TNF-α) production of peripheral blood mononuclear cells.[26] In addition, ar-turmerone also stimulated IL-12 and TNFα production of dendritic cells.[165] This immunostimulatory effect of ar-turmerone helped to delay the disease progression of leukaemia P388D1 cells-challenged mice by activating T cells proliferation and IL-2 production.[166] However, Ferreira *et al.*[167] and Oh *et al.*[168] reported that ar-turmerone caused cytotoxicity in the peripheral blood mononuclear cell (PBMC), inhibited interferon-γ and interleukin-2 production of $CD4^+$ T lymphocytes, and suppressed natural killer cell cytolytic activity. Thus, additional preclinical experiments that set out to identify the effective dosage of ar-turmerone for future clinical usage is necessary.

Most of the health benefits of *C. longa* have been attributed to its anti-inflammatory effects, particularly contributed by curcumin, the major active curcuminoid in turmeric.[18] However, other active compounds such as STs were also reported with anti-inflammatory effects, which may collectively contribute to this effect of the *C. longa* plant.

Nitric oxide (NO) is the mediator and regulator of inflammatory response. High concentrations of NO rapidly oxidize to reactive nitrogen oxide species (RNS), which may cause activation of COX-2 to produce inflammatory mediator prostaglandin E2 (PGE2).[169,170] In addition, the RNS generated may also promote lipid peroxidation and apoptosis of macrophages and

thus mediate local inflammation.[169] Among the *C. longa* STs, tumeronol A, tumerone Q, ar-turmerone, β-turmerone, bisacumol, bisacurone, β-elemene, curcumenone, curdione, dehydrocurdione, furanodiene, germacrone and curcumenol have been reported to inhibit NO production in LPS-stimulated macrophage cells *in vitro*.[171–178] In addition, suppression of PEG2 and COX2 were also observed in LPS-induced macrophages treated by tumeronol A, ar-turmerone, β-turmerone, curcumenol and furanodienone.[171,176,179] Ohnishi *et al.*[177] proposed that inhibition of NO by dehydrocurdione treatment was through induction of heme oxygenase (HO)-1 antioxidant enzyme production. Another study by Fang *et al.*[173] suggested that β-elemene suppressed pro-inflammatory mediators (interleukin-1β, interleukin-6, interleukin-10, TNF-α and iNOS) expression by downregulating the Wnt/β-catenin signalling pathway. Furthermore, β-elemene was capable of inhibiting TNF-α and IL-6 production in low density lipoprotein (LDL)-stimulated macrophages.[180] Other than macrophages, ar-curcumene and β-elemene were also reported to suppress LPS-mediated inflammation in bronchial epithelial cells and PBMC-derived neutrophils through inhibition of interleukin-8 production.[181,182]

Animal studies further validated the anti-inflammatory effects of ar-turmerone, furanodiene and furanodienone. Ar-turmerone treatment helped to ameliorate the disease progression of imiquimod-induced psoriasis-like skin disease in mice by suppressing transfer of CD8 T cells in the epidermis and expression of NF-kB, COX-2, p38 MAPK, TNFα, interleukin-6, interleukin-17, interleukin-22 and interleukin-23.[183] Furthermore, furanodiene and furanodienone were able to suppress 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mice ear inflammation.[184]

A more encouraging observation was made by Kawasaki *et al.*,[185] which showed concurrent intake of bisacurone and curcumin benefited the emotional states and reduced the level of serum liver enzyme [aspartate transaminase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (γGTP)] and serum inflammatory markers [c-reactive protein (CRP), serum amyloid A (SAA), interleukin-6, TNF-α and PGE2] in healthy human participants with a moderately high body mass index.

Collectively, preclinical and clinical findings supported the involvement of STs in the anti-inflammatory effects of *C. longa.* However, a study by Matsuda *et al.*[174] pointed out that curcumin remains the strongest inflammation inhibitor in *C. longa.* Although the immunomodulatory and anti-inflammatory effect of *C. longa* shows lower efficacy than curcumin, these compounds may collectively and synergistically contribute to the strengthening of immunity and suppression of chronic disease inflammation when patients consume turmeric crude extract, which was reported to have an even greater anti-inflammatory effect than curcumin only.[18] Despite non-curcuminoid compounds being reported to inhibit a certain level of anti-inflammatory effect, the use of *C. longa* extract containing both curcuminoid and STs can achieve a better anti-inflammatory result.

12.5 Hepatoprotective Activity of *C. longa* STs

Evidenced by the traditional practice of *C. longa* to treat liver disease, several STs from the plant have been shown to confer a hepatoprotective effect. A preliminary *in vitro* study showed that bisacurone and ar-turmerone protected hepatoma HepG2 cells.[186] The use of an ethanol challenged mice model in *in vivo* study suggested that bisacurone prevented liver inflammation by suppressing TNF-α and interleukin-6 production. In addition, it also suppressed the hepatic lipid peroxidation by activating hepatic antioxidant superoxide dismutase and glutathione.[187] Other than bisabolane-type STs, Kimura *et al.*[188] also reported that curcumenone prevented ethanol drunkenness by enhancing liver alcohol dehydrogenase activity in human subjects.

Apart from protection against ethanol-induced liver damage, *C. longa* STs also exhibited hepatoprotection against chemicals. Germacrone was shown to protect primary rat hepatocytes from galactosamine (D-GalN)-induced cytotoxicity[189] while curcumenone, curdione, dehydrocurdione, furanodiene, germacone and curcumenol were able to protect mice from D-GalN/LPS-induced liver damage at 50 mg kg^{-1} body weight of treatment.[190,191]

β-Elemene was reported to ameliorate liver fibrosis *via* inhibition of plasma angiotensin II (ANG II) secretion. The compound also suppressed expression of ATIR, CD44 and alpha-smooth muscle actin (αSMA) in hepatic stellate cells, which are involved in the production of collagen that cause liver fibrosis.[192,193] This effect was further validated using the carbon tetrachloride (CCL_4)-induced liver fibrosis rat model. The β-elemene-treated rat showed a lower level of collagen disposition in the liver and plasma level of ANG II.[194]

12.6 Neuroprotective Effect of *C. longa* STs

Generally, the neuroprotective effect of *C. longa* extract was claimed to be contributed by curcumin and curcuminoids.[195] However, there is also much evidence that suggested the role of STs having a neuroprotective effect. β-Secretase, for instance, was proposed as a potential pharmacological target for the treatment of Alzheimer's disease due to the ability to initiate amyloid beta (Aβ) generation by cleaving a β-site amyloid precursor protein.[196] On the other hand, ar-turmerone (IC_{50} 92 μM), α-turmerone (IC_{50} 39 μM) and β-turmerone (IC_{50} 62 μM) were able to inhibit β-secretase *in vitro.*[197] In addition, both ar-turmerone and germacrone were able to inhibit AChE activity,[199,200] which suggests the possibility of using these compounds as an acetylcholinesterase inhibitor that is widely used to treat Alzheimer's disease to achieve symptomatic improvement.[198]

The cyto/neuroprotective effect of *C. longa* STs is mainly contributed by the antioxidant and antiapoptotic effect on the neuron cells. Among the *C. longa* STs, curcumenone, curcumenol, procurcumenol, dehydrocurdione and germacrone were able to protect neuroblastoma NG108-15 cells from H_2O_2-induced apoptosis *in vitro*.[17] In addition, zingiberene also shows protection

to primary rat cerebral cortex neuron cells from H_2O_2-induced apoptosis by increasing the total antioxidant capacity in the cells.[32,201] β-elemene protected cobalt chloride ($CoCl_2$)-induced apoptosis of motor neurons VSC4.1 cells through activating phosphorylation of the PI3K-AKT-mTOR pathway and inhibition of Rho kinase activation (inhibition on phosphorylation of LIMK, confilin) that promoted expression of the growth associated protein 43 (GAP-43) and neurite outgrowth. Moreover, 320 μg kg^{-1} body weight of β-elemene promoted motor neuron survival, reduced the area of lesion cavity, promoted locomotor behavioural recovery and reduced neuronal apoptosis (CHOP, cleaved caspase 12, Bax, cleave caspase 3) in the spinal cord ventral horn of a spinal cord injury rat model through suppression of ER stress and inflammation (interleukin-6 and interleukin-1β) while promoting expression of GAP-43.[202,203] Curdione treatment was found to promote the cognitive function and neuronal morphologic recovery of rats subjected to two hours of middle cerebral artery occlusion. This effect may be contributed by the activation of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX) that prevented lipid peroxidation and apoptosis (Bax, cytochrome-c, cleave caspase 3 and cleave caspase 9) in the ischaemic cortex of mice.[204] Ar-turmerone promoted proliferation and differentiation of primary foetal rat neural stem cells. In addition, greater mobilization of proliferating endogenous neural stem cells from the subventricular zone and hippocampus was observed in adult rats intracerebroventricularly injected by ar-turmerone.[205]

Immune activation, especially those *via* microglia cells in the central nervous system, plays an important role in the regulation of homeostasis of the brain and defence against pathogens' invasion. However, persistent inflammation caused by failure in the normal resolution mechanisms of neurodegenerative diseases including Alzheimer's disease, multiple sclerosis, Parkinson's disease and amyotrophic lateral sclerosis may lead to progression of the diseases. Thus, the ability to ameliorate neuroinflammation may be beneficial to the management of neurodegenerative diseases.[206] Furanodienone was found to suppress NO generation in LPS-stimulated microglial BV2 cells.[16] Furthermore, ar-turmerone and curcumenol were also reported to inhibit LPS-induced neuroinflammation in BV2 cells by blocking activation of toll-like receptor 4 (TLR4)-mediated NF-kB, JNK and p38 MAPK, which are associated with downregulation of pro-inflammatory mediator/cytokines [TNF-α, interleukin-1β, interleukin-6, monocyte chemoattractant protein-1 (MCP-1), MMP-9, iNOS and COX2] expression.[141,207,208] Furthermore, ar-turmerone also showed similar *anti*-neuroinflammatory effect in an Aβ-induced BV2 cells model.[209] This effect was validated in the LPS challenged mice model where ar-turmerone attenuated the LPS mediated neuroinflammation and memory impairment by targeting TNF-α and interleukin-1β production in the cortex and hippocampus.[208] Furthermore, Zhang *et al.*[145,210] and Meng *et al.*[211] reported that β-elemene suppressed neuroinflammation in the myelin oligodendrocyte glycoprotein (MOG_{33-35}) peptide-challenged mice models with multiple sclerosis and traumatic brain injury. In the multiple

sclerosis model, β-elemene suppressed neuroinflammation by downregulating interleukin-6, interleukin-17, interleukin-23, retinoic acid-related orphan receptor gamma t (RORγt) and interferon-γ production by T cells in the optic nerve and spinal cord through inhibiting T-helper 17 and promoting expansion in T regulatory cells.[145,210] Whereas in the traumatic brain injury model, β-elemene prevented inflammatory-mediated apoptosis in the brain.[211] Curdione, another ST, also showed an ability to improve the cognitive function of aged mice after partial hepatectomy through enrichment of the endogenous antioxidant enzymes (SOD, CAT and GSH-Px) and suppression of inflammation (NF-kB, interleukin-1β, interleukin-6 and TNFα) in the hippocampus.[212] Among *C. longa* STs, ar-turmerone and β-elemene may be the most potent compounds for treatment of neuro-related diseases owing to their ability to pass through the blood–brain barrier.[145,213]

12.7 Analgesic/Depressive Activity of *C. longa* STs

Turmeric has been traditionally used for the control of pain and treatment for epilepsy. Although curcumin was reported to have anticonvulsant activity, its application is still limited by poor absorption and rapid metabolism in humans. Thus, other compounds such as STs from turmeric have been proposed as a potential lead compound contributing to this effect of turmeric.[214] Ar-turmerone and α/β-turmerone were able to reduce locomotor activity of pentylenetetrazole (PTZ)-induced convulsant in a zebrafish and mice model through downregulation of seizure marker c-fos expression.[213,214] Moreover, Gong *et al.*[215] also reported that β-elemene decreased pain-related behaviours (including paw withdrawal mechanical threshold and paw withdrawal thermal latency) in a bone cancer-related pain rat model by downregulating expression of *N*-methyl-D-aspartate receptor 2B subunit (NR2B). Another ST curcumenol showed central nervous system depressing action against hexobarbital-induced necrosis in a rat model[216] and reduced abdominal constriction induced by acetic acid (0.6%) in a mouse model.[217,218] Among the germacrane-type STs, furanodiene was shown to help alleviate different types of pain more effectively in female than males clinically.[219] On the other hand, dehydrocurdione and germacrone reduced the number of writhes[220–222] and abdominal constriction[218] in mice challenged with acetic acid. Furthermore, germacrone-STs treatment also helped to increase sleeping time and reduced stress-induced ulcers in mice.[220]

12.8 Cardioprotective Activity of *C. longa* STs

Overexpression of endothelial adhesion molecule VCAM-1 of the endothelial cells by phosphorylation of PI3K/Akt and activation of NF-kB signalling is associated with the early initiation of atherosclerosis by promoting monocyte accumulation. Thus, VCAM-1 has been proposed as a molecular target in the treatment of atherosclerosis. Bisacurone was found to inhibit

TNF-α-mediated production of VCAM-1 in HUVEC cells by blocking phosphorylation of PI3K/Akt and deactivation of the NF-kB signalling pathway.[230]

Also, β-elemene was reported to ameliorate progression of atherosclerosis, neointimal hyperplasia, serum lipid profile and infiltration of macrophage in both balloon angioplasty-induced endothelial injury and diet-induced atherogenic animal models[180,231,232] by alleviating the level of vascular oxidative stress and preventing pro-inflammatory cytokines production. This cardioprotective effect was achieved *via* inhibited migration of vascular smooth muscle, prevention of oxidative stress-induced apoptosis in endothelial cells[231] and maintenance of the endothelial function through enhancement of plasma nitrite/nitrate levels through promotion of phosphorylation of $eNOS^{ser1177}$ and AKT signalling.[232]

Moreover, ar-turmerone, bisacumol (bisabolane-type ST), curcumenone (curcumane-type ST), curdione, dehydrocurdione, furanodiene, germacrone (germacrane-type STs) and curcumenol (guaiane-type ST) showed *in vitro* inhibition of K^+-induced contraction of a rat aortic ring, indicating that many of the *C. longa* STs show vasorelaxant activity and are thus potential antihypertension treatment agents.[233,234]

In addition, ar-turmerone and curdione were also reported to show anticoagulant activities. Ar-turmerone was able to inhibit collagen- and arachidonic acid-induced (but not the thrombin-induced aggregation) rabbit platelet aggregation.[235] Unlike ar-turmerone, curdione was able to inhibit thrombin-induced platelet activation by downregulating Talin 1 and β1-tubulin expression in the integrin signalling pathway.[236] Collectively, amelioration of atherosclerosis, cyto-protection of endothelial cells, vasorelaxing and anticoagulant activities of these STs contribute to the cardioprotective potential of *C. longa*.

12.9 Other Bioactivities of *C. longa* STs

Other bioactivities of *C. longa* STs are summarized in Table 12.7. Among the listed *C. longa* STs, ar-turmerone showed the widest bioactivities including anti-aging, anti-venom, choleretic and hypo-glycaemic effects than other listed STs.

12.10 Other Non-curcuminoids and their Bioactivities

Although this chapter focuses on the bioactivities of non-curcuminoids STs in *C. longa*, evidence shows that many other classes of non-curcuminoids such as calebin A, tetrahydrocurcumin, *etc.* also show prominent bioactivities, especially anticancer effects.

Calebin A is a monomeric phenylpropene derivative in *C. longa.*[5] *In vitro* studies showed that calebin A possessed neuroprotective activity by exerting protection against β-amyloid insult[250] and anti-obesity effect *via* inhibition of adipogenesis and hepatic steatosis by activating AMPK signalling.[251] Besides,

Table 12.7 Other bioactivities of C. longa STs.

Bioactivities	Remarks
Anti-aging	*C. longa* extract has been widely used as an active ingredient in cosmetic products. This activity may be contributed by the whitening effect of ar-turmerone and anti-aging effect of germacrone. Park *et al.*[223] reported that ar-turmerone inhibited alpha-melanocyte stimulating hormone (α-MSH) and 3-isobuty-1-methxlzanthine (IBMX)-induced melanogenesis and tyrosinase activity of B16F10 melanoma cells by inhibiting expression of tyrosinase, tyrosinase-related protein 1 (TRP-1), tyrosinase related protein 2 (TRP-2) and cyclic adenosine monophosphate (cAMP)-responsive element binding protein. The whitening effect of ar-turmerone is even better than curcumin. On the other hand, germacrone was reported to suppress expression of MMP1, MMP2 and MMP3 in keratinocytes, which contributed to skin aging.[224] In addition, germacrone also enhanced the expression of the longevity gene SIRT1 in keratinocytes.[200]
Anti-androgenic	Germacrone inhibited 5α-reductase that converted testosterone to dihydrotestosterone (IC_{50}: 420 μg mL^{-1}), which may contribute to the antiproliferation effect on testosterone-induced LNCaP cells. This effect may be contributed by the anti-prostate cancer effect[13,33,85] or treatment of male pattern baldness.[239]
Anti-arthritis	The anti-arthritis effect of *C. longa* was correlated with its anti-inflammatory effect contributed by curcuminoid and STs present in the plant. Dehydrocurdione was reported to ameliorate adjuvant-induced chronic arthritis in rats by suppressing COX-2 mediated inflammation.[222] On the other hand, β-elemene also showed *in vitro* anti-arthritis potential by inducing apoptosis in primary human rheumatoid arthritis fibroblast-like synoviocytes *via* p38 MAPK phosphorylation and ROS-mediated mitochondria apoptosis.[241]
Anti-fibrosis	β-Elemene inhibited proliferation of human airway granulation fibroblasts through suppression of the Wnt/β-catenin pathway [downregulation of Wnt3a, β-catenin, alpha smooth muscle actin (α-SMA), transforming growth factor beta (TGF-β), Col-I gene expression].[242] Clinical trial of direct injection of β-elemene showed reduction of airway granulation through induction of antiproliferation and apoptosis of airway primary fibroblast.[243]
Anti-obesity	Germacrone exhibited potential preclinical anti-obesity effect where it suppressed adipogenesis and increased lipolysis in 3T3-L1 adipocytes through downregulation of adipogenesis-related genes [Kruppel-like factor 4 (KLF4), Kruppel-like factor 5 (KLF5), CCAAT-enhancer-binding proteins alpha (C/EBP-α), CCAAT-enhancer-binding proteins beta (C/EBP-β), CCAAT-enhancer-binding proteins delta (C/EBP-δ), PPAR-γ] and upregulation of AMPKα, Acetyl-CoA carboxylase (ACC) and carnitine palmitoyltransferase I (CPT1).[228] In addition, 30 days' treatment with germacrone attenuated body weight gain, visceral fat pad weight, fasting plasma glucose, serum insulin and leptin, serum lipid profile and liver fat accumulation by improving fatty acid β-oxidation through activation of the AMPKα signalling pathway of the high-fat-diet-induced obese mice model.[229]

(*continued*)

Table 12.7 (*continued*)

Bioactivities	Remarks
Anti-pyretic	Dehydrocurdione showed antipyretic activity with fever reduction caused by injection of baker yeast in mice.[222]
Anti-venom	Ar-turmerone (600 μg/mouse) reduced 50% lethality in mice challenged with 12.5 μg of venom.[167] Topical application of ar-turmerone (1 g/rabbit) also attenuated oedema, necrosis and local haemorrhage after Bothrops alternatus envenomation (125 μg/rabbit) challenge in rabbits.[240]
Anti-viral	*C. longa* extract exhibited an antiviral effect, which was mainly contributed by curcumin and curcuminoid.[244] Other than curcuminoid, α-curcumene, furadienone and germacrone may also contribute to the antiviral property of *C. longa* extract. α-Curcumene was reported with *in vitro* antiviral effect against influenza A (H1NI and H3N2) viruses[12] while furadienone was sensitive to influenza A (H1N1) and Coxsackie viruses.[245] Germacrone showed an antiviral effect to a broader range of viruses including influenza A (H1N1 and H3N2), influenza B,[246] feline calicivirus strain F9,[247] porcine parvovirus[248] and porcine reproductive and respiratory syndrome virus,[249] through inhibition of virus replication.
Choleretic	Ar-turmerone was reported to enhance bile flow and total bile acids excretion in a bile fistula rat model.[238]
Hypoglycaemic	*C. longa* extract was reported to have a hypoglycaemic effect, which is mainly contributed by curcuminoids in the plant.[225] Potential role of STs in antidiabetic activity of *C. longa* is still under investigation whereby PPARγ ligand binding activity[225] and inhibition of α-glucosidase and α-amylase[226] of ar-turmerone were associated with the sensitization of glucose metabolism. In addition, curcumenol and curdione showed a potential hypoglycaemic effect by enhancing glucose consumption of liver cancer HepG2 cells.[227] More preclinical studies are needed to validate the antidiabetic potential of the *C. longa* STs.
Wound healing	Pre-treatment of Zingiberene reduced the lesion index of hydrochloric acid/ethanol-induced gastric lesions in rats.[237]

calebin A also showed an *in vitro* cytotoxic effect on human colorectal cancer, malignant peripheral nerve sheath tumour, leukaemia, lymphoma, lung adenocarcinoma, breast adenocarcinoma, squamous cell carcinoma, myeloma and drug-resistant human gastric cancer cell lines. Calebin-induced death of the above cancerous cell lines *via* suppression of inflammation, proliferation, invasion, metastasis while activating histone acetyltransferase, cell cycle arrest, chemosensitivity subsequently led to apoptosis.[252–257] Among the tested cancerous cells, the effect on colon cancer was further validated *in vivo*.[255]

Tetrahydrocurcumin, metabolites of curcumin, also possessed various bioactivities including antioxidant, anti-inflammation, antiviral, antibacterial, antidiabetic and anti-tumour effects.[258] Although tetrahydrocurcumin has a similar chemical structure to curcumin, its efficacy and regulation of

the mechanism of its bioactivities are different compared to curcumin. For example, tetrahydrocurcumin showed a better antioxidant effect than curcumin.[258] This activity has contributed to its neuroprotective effect against glutamate-induced oxidative stress in hippocampal cells.[259] On the other hand, tetrahydrocurcumin was reported to have poor binding to various cancer molecular targets such as β-catenine, cyclooxygenase-2, NF-kB, cyclin D1, VEGF, PGE2 *etc.*, which contributed to lower tumour cell inhibition than curcumin.[258]

Although curcuminoids and STs are the two major classes of compounds in turmeric, many minor and less popular metabolites such as calebin A and tetrahydrocurcumin among the 200 other compounds that have been identified from *C. longa* have also been proven for their bioactivities and are worth further evaluation.

12.11 Conclusion

The current updates support the theory that different types of STs from *C. longa* possess numerous bioactivities such as anti-inflammation, antitumour and neuroprotective effects. Further to that, enhanced effects were also observed when these STs were applied together with other compounds including curcumin. In addition, the ability of some of these STs, including ar-turmerone and β-elemene, to pass through the blood–brain barrier is remarkable for the treatment of brain diseases.[145] With supporting evidence from preclinical and small-scale human trials, more studies to evaluate the clinical efficacy and the detailed mode-of-action of these non-curcuminoid STs would be desirable.

References

1. S. Prasad and B. B. Aggarwal, *Herbal Medicine: Biomolecular and Clinical Aspects*, ed. I. F. Benzie and S. Wachtel-Galor, CRC Press/Taylor & Francis, Boca Raton (FL), 2nd edn, 2011, ch. 13, Available from: https://www.ncbi.nlm.nih.gov/books/NBK92752/.
2. A. Singh, C. Shekhar, V. K. Singh and K. R. C. Reddy, *Indian J. Agric. Allied Sci.*, 2017, **3**, 78.
3. R. Li, C. Xiang, X. Zhang, D. A. Guo and M. Ye, *Curr. Pharm. Biotechnol.*, 2010, **6**, 256.
4. A. Amalraj, A. Pius, S. Gopi and S. Gopi, *J. Tradit., Complementary Med.*, 2017, **7**, 205.
5. S. Balaji and B. Chempakam, *Food Chem. Toxicol.*, 2010, **48**, 2951.
6. X. G. He, L. Z. Lin, L. Z. Lian and M. Lindenmaier, *J. Chromatogr. A*, 1998, **818**, 127.
7. L. Y. Wang, M. Zhang, C. F. Zhang and Z. T. Wang, *Acta Pharmacol. Sin.*, 2008, **43**, 724.
8. T. Zakerali and S. Shahbazi, *Assay Drug Dev. Technol.*, 2018, **16**, 397.

9. C. Ma, W. Zhou, Z. Yan, M. Qu and X. Bu, *OncoTargets Ther.*, 2016, **9**, 7521.
10. A. Nair, A. Amalraj, J. Jacob, A. B. Kunnumakkara and S. Gopi, *Biomolecules*, 2019, **9**, 13.
11. N. S. Dosoky and W. N. Setzer, *Nutrients*, 2018, **10**, 1196.
12. Y. Cheng, J. Y. Mai, T. L. Hou, J. Ping and J. J. Chen, *Mol. Med. Rep.*, 2016, **14**, 3704.
13. O. A. Ahmed Hamdi, S. N. Syed Abdul Rahman, K. Awang, N. Abdul Wahab, C. Y. Looi, N. F. Thomas and S. N. Abd Malek, *Sci. World J.*, 2014, **2014**, 321943.
14. W. S. Xu, Y. Y. Dang, J. J. Guo, G. S. Wu, J. J. Lu, X. P. Chen and Y. T Wang, *Evidence-Based Complementary Altern. Med.*, 2012, **2012**, 426521.
15. Q. Kong, F. Sun and X. Chen, *Cell J.*, 2013, **15**, 160.
16. M. C. Marcotullio, F. messina, M. Curini, A. Macchiarulo, M. Cellanetti, D. Ricci, L. Giamperi, A. Bucchini, A. Minellil, A. L. Mierla and I. Bellezza, *Molecules*, 2011, **16**, 10357.
17. O. A. A. Hamdi, L. J. Ye, M. N. A. Kamarudin, H. Hazni, M. Paydar, C. Y. Looi, J. A. Shilpi, H. A. Kadir and K. Awang, *Rec. Nat. Prod.*, 2015, **9**, 349.
18. S. J. Hewlings and D. S. Kalman, *Foods*, 2017, **6**, 92.
19. J. H. Kim, S. C. Gupta, B. Park, V. R. Yadav and B. B. Aggarwal, *Mol. Nutr. Food Res.*, 2012, **56**, 454.
20. K. U. Baik, S. H. Jung and B. Z. Ahn, *Arch. Pharmacal Res.*, 1993, **16**, 254.
21. M. Ji, J. Choi, J. Lee and Y. Lee, *Int. J. Mol. Med.*, 2004, **14**, 253.
22. W. G. Oh, K. U. Baik, S. H. Jung and B. Z. Ahn, *Arch. Pharmacal Res.*, 1992, **15**, 256.
23. Y. Aratanechemuge, T. Komiya, H. Moteki, H. Katsuzaki, K. Imai and H. Hibasami, *Int. J. Mol. Med.*, 2002, **9**, 481.
24. S. H. Paek, G. J. Kim, H. S. Jeong and S. K. Yum, *Arch. Pharmacal Res.*, 1996, **19**, 91.
25. H. S. Lee, *J. Appl. Biol. Chem.*, 2009, **52**, 212.
26. G. G. L. Yue, B. C. L. Chan, P. M. Hon, M. Y. H. Lee, K. P. Fung, P. C. Leung and C. B. S. Lau, *Food Chem. Toxicol.*, 2010, **48**, 2011.
27. S. B. Cheng, L. C. Wu, Y. C. Hsieh, C. H. Wu, Y. J. Chan, L. H. Chang, C. M. J. Chang, S. L. Hsu, C. L. Teng and C. C. Wu, *J. Agric. Food Chem.*, 2012, **60**, 9620.
28. G. G. L. Yue, L. Jiang, H. F. Kwok, J. K. M. Lee, K. M. Chan, K. P. Fung, P. C. Leung and C. B. S. Lau, *J. Funct. Foods*, 2016, **20**, 565.
29. Y. Shin and Y. Lee, *Toxicol. Res.*, 2013, **29**, 257.
30. K. Kimura, Y. Sakamoto, N. Fujisawa, S. Uesugi, N. Aburai, M. Kawada, S. Ohba, T. Yamori, E. Tsuchiya and H. Koshino, *Bioorg. Med. Chem.*, 2012, **20**, 3887.
31. Y. Lee, *Toxicol. Res.*, 2016, **32**, 225.
32. B. Togar, H. Turkez, A. Tatar, A. Hacimuftuoglu and F. Geyikoglu, *Cytotechnology*, 2014, **67**, 939.
33. O. A. A. Hamdi, E. H. Anouar, J. A. Shilpi, Z. B. K. Al Trabolsy, S. B. M. Zain, N. S. S. Zakaria, M. Zulkefeli, J. F. F. Weber, S. N. A. Malek, S. N. S. A. Rahman and K. Awang, *Int. J. Mol. Sci.*, 2015, **16**, 9450.

34. R. Chokchaisiri, P. Pimkaew, P. Piyachaturawat, R. Chalermglin and A. Suksamrarn, *Rec. Nat. Prod.*, 2014, **8**, 46.
35. X. Chen, Y. Wang, H. Luo, Z. Luo, T. Zhang, N. Yang, X. Long, H. Xie, W. Qiu, B. Zhang, J. Ding and L. Yang, *Mol. Med. Rep.*, 2012, **6**, 989.
36. X. Lu, Y. Wang, H. Luo, W. Qiu, H. Han, X. Chen and L. Yang, *Mol. Med. Rep.*, 2013, **7**, 513.
37. B. Cai, L. Ma, S. Nong, Y. Wu, X. Guo and J. Pu, *Oncol. Lett.*, 2018, **16**, 6019.
38. Q. Q. Li, G. Wang, H. Liang, J. M. Li, F. Huang, P. K. Agarwal, Y. Zhong and E. Reed, *Anticancer Res.*, 2013, **33**, 1421.
39. Q. Q. Li, G. Wang, F. Huang, M. Banda and E. Reed, *J. Pharm. Pharmacol.*, 2010, **62**, 1018.
40. Q. Q. Li, R. X. Lee, H. Liang and Y. Zhong, *Anticancer Res.*, 2013, **33**, 65.
41. Y. Q. Yao, X. Ding, Y. C. Jia, C. X. Huang, Y. Z. Wang and Y. H. Xu, *Cancer Lett.*, 2008, **264**, 127.
42. T. Zhu, Y. Zhao, J. Zhang, L. Li, L. Zou, Y. Yao and Y. Xu, *Int. J. Oncol.*, 2011, **38**, 419.
43. T. Zhu, Y. Xu, B. Dong, J. Zhang, Z. Wei, Y. Xu and Y. Yao, *Oncol. Rep.*, 2011, **26**, 405.
44. Y. S. Zhao, T. Z. Zhu, Y. W. Chen, Y. Q. Yao, C. M. Wu, Z. Q. Wei, W. Wang and Y. H. Xu, *J. Neuro-Oncol.*, 2012, **107**, 307.
45. L. Mu, T. Wang, Y. Chen, X. Tang, Y. Yuan and Y. Zhao, *Int. J. Oncol.*, 2016, **49**, 1427.
46. C. L. Li, L. Chang, L. Guo, D. Zhao, H. B. Liu, Q. S. Wang, P. Zhang, W. Z. Du, X. Liu, H. T. Zhang, Y. Liu, Y. Zhang, J. H. Xie, J. G. Ming, Y. Q. Cui, Y. Sun, Z. R. Zhang and C. L. Jiang, *Asian Pac. J. Cancer Prev.*, 2014, **15**, 10407.
47. T. Zhu, X. Li, L. Luo, X. Wang, Z. Li, P. Xie, X. Gao, Z. Song, J. Su and G. Liang, *J. Transl. Med.*, 2015, **13**, 356.
48. X. F. Ding, M. Shen, L. Y. Xu, J. H. Dong and G. Chen, *Oncol. Lett.*, 2013, **5**, 1554.
49. H. B. Xu, L. Li, J. Fu, X. P. Mao and L. Z. Xu, *Pharmacology*, 2012, **89**, 303.
50. C. Y. Tang, L. X. Zhu, J. D. Yu, Z. Chen, M. C. Gu, C. F. Mu, Q. Liu and Y. Xiong, *Eur. J. Pharm. Sci.*, 2018, **120**, 20.
51. J. Zhang, H. Zhang, L. Chen, D. W. Sun, C. F. Mao, W. Chen, J. Z. Wu, S. L. Zhong, J. H. Zhao and J. H. Tang, *Cell. Physiol. Biochem.*, 2014, **34**, 2027.
52. J. Zhang, H. Zhang, Y. F. Yao, S. L. Zhong, J. H. Zhao and J. H. Tang, *Cell. Physiol. Biochem.*, 2015, **36**, 2274.
53. C. Guan, W. Liu, Y. Yue, H. Jin, X. Wang and X. J. Wang, *Int. J. Clin. Exp. Pathol.*, 2014, **7**, 3948.
54. Y. Sun, G. Liu, Y. Zhang, H. Zhu, Y. Ren and Y. M. Shen, *Bioorg. Med. Chem.*, 2009, **17**, 1118.
55. L. Xu, S. Tao, X. Wang, Z. Yu, M. Wang, D. Chen, Y. Jing and J. Dong, *Bioorg. Med. Chem.*, 2006, **14**, 5351.
56. G. Liu, Z. Kong and Y. Shen, *Med. Chem. Res.*, 2013, **22**, 3536.

57. L. Wang, Y. Zhao, Q. Wu, Y. Guan and X. Wu, *Mol. Med. Rep.*, 2018, **17**, 4299.
58. C. Huang and Y. Yu, *Med. Sci. Monit.*, 2017, **23**, 1507.
59. Z. Yu, R. Wang, L. Xu, S. Xie, J. Dong and Y. Jing, *PLoS One*, 2011, **6**, e15843.
60. Z. J. Dai, W. Tang, W. F. Lu, J. Gao, H. F. Kang, X. B. Ma, W. L. Min, X. J. Wang and W. Y. Wu, *Cancer Cell Int.*, 2013, **13**, 27.
61. Z. Wu, T. Wang, Y. Zhang, Z. Zheng, S. Yu, S. Jing, S. Chen, H. Jiang and S. Ma, *Exp. Ther. Med.*, 2017, **13**, 3153.
62. Q. Q. Li, G. Wang, F. Huang, J. M. Li, C. F. Cuff and E. Reed, *Med. Oncol.*, 2013, **30**, 488.
63. F. Zhang, L. Xu, X. Qu, M. Zhao, B. Jin, J. Kang, Y. Liu and X. Hu, *Mol. Med. Rep.*, 2011, **4**, 1189.
64. J. Liu, X. J. Hu, B. Jin, X. J. Qu, K. Z. Hou and Y. P. Liu, *J. Pharm. Pharmacol.*, 2012, **64**, 146.
65. G. Wang, X. Li, F. Huang, J. Zhao, H. Ding, C. Cunningham, J. E. Coad, D. C. Flynn, E. Reed and Q. Q. Li, *Cell. Mol. Life Sci.*, 2005, **62**, 881.
66. T. Hu and Y. Gao, *Biomed. Pharmacother.*, 2018, **106**, 1557.
67. D. Li, X. Wu and X. Liang, *Biomed. Res.*, 2017, **28**, 5418.
68. H. Shi, L. Liu, L. Liu, J. Geng, Y. Zhou and L. Chen, *Melanoma Res.*, 2014, **24**, 99.
69. J. J. Wu, Q. Tang, L. J. Yang, Y. Q. Chen, F. Zheng and S. S. Hann, *Sci. Rep.*, 2017, **7**, 509.
70. D. Liang, M. Yang, B. Guo, L. Yang, J. Cao and X. Zhang, *J. Cancer Res. Clin. Oncol.*, 2012, **138**, 1865.
71. M. Fang, X. Mei, H. Yao, T. Zhang, T. Zhang, N. Lu, Y. Liu, W. Xu and C. Wan, *Oncol. Lett.*, 2018, **15**, 3957.
72. X. Li, G. Wang, J. Zhao, H. Ding, C. Cunningham, F. Chen, D. C. Flynn, E. Reed and Q. Q. Li, *Cell. Mol. Life Sci.*, 2005, **62**, 894.
73. R. X. Lee, Q. Q. Li and E. Reed, *Anticancer Res.*, 2012, **32**, 3103.
74. Q. Q. Li, R. X. Lee, H. Liang, Y. Zhong and E. Reed, *Med. Oncol.*, 2013, **30**, 424.
75. Q. Q. Li, R. X. Lee, H. Liang, G. Wang, J. M. Li, Y. Zhong and E. Reed, *Int. J. Oncol.*, 2013, **43**, 721.
76. B. Zou, Q. Q. Li, J. Zhao, J. M. Li, C. F. Cuff and E. Reed, *Anticancer Res.*, 2013, **33**, 929.
77. Y. H. Zhan, J. Liu, X. J. Qu, K. Z. Hou, K. F. Wang, Y. P. Liu and B. Wu, *Asian Pac. J. Cancer Prev.*, 2012, **13**, 2739.
78. D. Wu, D. Lv, T. Zhang, L. Guo, F. Ma, C. Zhang, G. Lv and L. Huang, *Endocr.-Relat. Cancer*, 2019, **26**, 187.
79. J. S. Liu, S. C. He, Z. L. Zhang, R. Chen, L. Fan, G. L. Qiu, S. Chang, L. Li and X. M. Che, *Oncol. Rep.*, 2014, **32**, 2635–2647.
80. J. S. Liu, X. M. Che, S. Chang, G. L. Qiu, S. C. He, L. Fan, W. Zhao, Z. L. Zhang and S. F. Wang, *World J. Gastroenterol.*, 2015, **21**, 9945.
81. Y. Zhang, X. J. Qu, Y. P. Liu, K. Z. Hou and J. Liu, *World Chin. J. Dig.*, 2011, **19**, 1394.

82. Y. Zhang, X. D. Mu, E. Z. Li, Y. Luo, N. Song, X. J. Qu, X. J. Hu and Y. P. Liu, *Int. J. Mol. Sci.*, 2013, **14**, 10075.
83. J. Liu, Y. Zhang, J. Qu, L. Xu, K. Hou, J. Zhang, X. Qu and Y. Liu, *BMC Cancer*, 2011, **11**, 183.
84. J. Li, W. H. Bian, J. Wan, J. Zhou, Y. Lin, J. R. Wang, Z. X. Wang, Q. Shen and K. M. Wang, *Asian Pac. J. Cancer Prev.*, 2014, **15**, 9997.
85. Z. Ba, Y. Zheng, H. Zhang, X. Sun and D. Lin, *Chin. J. Cancer Res.*, 2009, **21**, 154.
86. Z. F. Zhong, Y. B. Li, S. P. Wang, W. Tan, X. P. Chen, M. W. Chen and Y. T. Wang, *J. Cell. Biochem.*, 2012, **113**, 2643.
87. Z. Zhong, Y. Dang, X. Yuan, W. Guo, Y. Li, W. Tan, J. Cui, J. Lu, Q. Zhang, X. Chen and Y. Wang, *Cell. Physiol. Biochem.*, 2012, **30**, 778.
88. Z. Zhong, H. Yu, S. Wang, Y. Wang and L. Cui, *Chin. Med.*, 2018, **13**, 44.
89. Z. F. Zhong, H. B. Yu, C. M. Wang, W. A. Qiang, S. P. Wang, J. M. Zhang, H. Yu, L. Cui, T. Wu, D. Q. Li and Y. T. Wang, *Front. Pharmacol.*, 2017, **8**, 648.
90. X. Y. Sun, Y. P. Zheng, D. H. Lin, H. Zhang, F. Zhao and C. S. Yuan, *Am. J. Chin. Med.*, 2009, **37**, 589.
91. E. Ma, X. Wang, Y. Li, X. Sun, W. Tai, T. Li and T. Guo, *Cancer Lett.*, 2008, **271**, 158.
92. S. Anuchapreeda, N. Khumpirapang, K. Rupitiwiriya, L. Tho-iam, A. Saiai, S. Okonogi and T. Usuki, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 410.
93. Y. Xiao, F. Q. Yang, S. P. Li, J. L. Gao, G. Hu, S. C. Lao, E. L. Conceicao, K. P. Fung, Y. T. Wang and S. M. Y. Lee, *Cancer Biol. Ther.*, 2007, **6**, 1044.
94. Y. W. Li, G. Y. Zhu, X. L. Shen, J. H. Chu, Z. L. Yu and W. F. Fong, *Cancer Chemother. Pharmacol.*, 2011, **68**, 1315.
95. Y. W. Li, G. Y. Zhu, X. L. Shen, J. H. Chu, Z. L. Yu and W. F. Fong, *J. Cell. Biochem.*, 2011, **112**, 217.
96. Y. Jiang, X. Wang and D. Hu, *Cell Death Dis.*, 2017, **8**, e2815.
97. B. Liu, Y. Q. Gao, X. M. Wang, Y. C. Wang and L. Q. Fu, *Mol. Med. Rep.*, 2014, **10**, 1046.
98. Z. Zhong, X. Chen, W. Tan, Z. Xu, K. Zhou, T. Wu, L. Cui and Y. Wang, *Eur. J. Pharmacol.*, 2011, **667**, 50.
99. M. S. Lim, S. Y. Choung and K. W. Jeong, *Phytother. Res.*, 2016, **30**, 2036.
100. X. H. Xie, H. Zhao, Y. Y. Hu and X. D. Gu, *Exp. Ther. Med.*, 2014, **8**, 1611.
101. J. Wu, Y. Feng, C. Han, W. Huang, Z. Shen, M. Yang, W. Chen and L. Ye, *Oncotarget*, 2017, **8**, 15149.
102. L. Ye, J. Wu, W. Chen, Y. Feng and Z. Shen, *RSC Adv.*, 2017, **7**, 3760.
103. J. Pan, D. Miao and L. Chen, *Chem.-Biol. Interact.*, 2018, **288**, 32.
104. Y. Liu, Q. Zheng, B. Fang, W. Wang, F. Ma, S. Roshan, A. Banafa, M. Chen, J. Chang, X. Deng, K. Li, G. Yang and G. He, *J. Huazhong Univ. Sci. Technol., Med. Sci.*, 2013, **33**, 339.
105. Y. Liu, W. Wang, B. Fang, F. Ma, Q. Zheng, P. Deng, S. Zhao, M. Chen, G. Yang and G. He, *Eur. J. Pharmacol.*, 2013, **698**, 95.
106. X. H. Han, Y. Y. Ye, B. F. Guo and S. Liu, *Chin. J. Integr. Med.*, 2012, **10**, 67.

107. M. Aspollah Sukari, T. S. Wah, S. M. Saad, N. H. Lajis and T. Y. Y. Hin, *Nat. Prod. Res.*, 2010, **24**, 838.
108. E. B. Jung, T. A. Trinh, T. K. Lee, N. Yamabe, K. S. Kang, J. H. Song, S. Choi, S. Lee, T. S. Jang, K. H. Kim and G. S. Hwang, *J. Ethnopharmacol.*, 2018, **213**, 48.
109. H. Matsuda, S. Nakamura, J. Iwami, X. Li, Y. Pongpiriyadacha, M. Nakai, M. Kubo, Y. Fukuyama and M. Yoshikawa, *Chem. Pharm. Bull.*, 2011, **59**, 365.
110. A. K. Tyagi, S. Prasad, W. Yuan, S. Li and B. B. Aggarwal, *Invest. New Drugs*, 2015, **33**, 1175.
111. H. Itokawa, F. Hirayama, K. Funakoshi and K. Takeya, *Chem. Pharm. Bull.*, 1985, **33**, 3488.
112. G. G. L. Yue, H. F. Kwok, J. K. M. Lee, L. Jiang, K. M. Chan, L. Cheng, E. C. W. Wong, P. C. Leung, K. P. Fung and C. B. S. Lau, *J. Funct. Foods*, 2015, **15**, 243.
113. H. Feng, J. Wang, H. Jiang, X. Mei, Y. Zhao, F. Chen, Y. Qu, K. Sai, C. Guo, Q. Yang, Z. Zhang and Z. Chen, *Stem Cells Transl. Med.*, 2017, **6**, 830.
114. T. Z. Zhu, X. M. Li, L. H. Luo, Z. Q. Song, X. Gao, Z. Q. Li, J. Y. Su and G. B. Liang, *Int. J. Oncol.*, 2014, **45**, 699.
115. Y. Zhu, J. Hu, F. Shen, H. Shen, W. Liu and J. Zhang, *J. Neuro-Oncol.*, 2013, **113**, 375.
116. J. Chen, J. She, G. Wang and S. Han, *J. Xi'an Jiaotong Univ., Med. Sci.*, 2014, **35**, 394.
117. F. Bao, J. Qiu and H. Zhang, *Mol. Med. Rep.*, 2012, **6**, 185.
118. Y. Qin, Y. Guo, W. Wei, B. Wang, H. Jin, J. Sun, X. Qi, S. Ren and Y. Zuo, *Prev. Nutr. Food Sci.*, 2012, **2**, 91.
119. Z. Y. Jiang, S. K. Qin, X. J. Yin, Y. L. Chen and L. Zhu, *Exp. Ther. Med.*, 2012, **4**, 277.
120. Y. Liu, Z. Y. Jiang, Y. L. Zhou, H. H. Qiu, G. Wang, Y. Luo, J. B. Liu, X. W. Liu, W. Q. Bu, J. Song, L. Cui, X. B. Jia and L. Feng, *Biomed. Pharmacother.*, 2017, **93**, 490.
121. L. J. Li, L. F. Zhong, L. P. Jiang, C. Y. Geng and L. J. Zou, *Phytother. Res.*, 2011, **25**, 1095.
122. G. Li, B. Xie, X. Li, Y. Chen, Q. Wang, Y. Xu, M. Xu-Welliver and L. Zou, *Cancer Biother. Radiopharm.*, 2012, **27**, 56.
123. G. Li, B. Xie, X. Li, Y. Chen, Y. Xu, M. Xu-Welliver and L. Zou, *Oncol. Rep.*, 2015, **33**, 1427.
124. E. Tong, Y. Xu, G. Li, K. Zou and L. Zou, *Afr. J. Tradit., Complementary Altern. Med.*, 2013, **10**, 18.
125. G. Tan, Z. Wang, L. Che and S. Yin, *Front. Med. China*, 2007, **1**, 41.
126. Z. F. Zhong, P. M. Hoi, G. S. Wu, Z. T. Xu, W. Tan, X. P. Chen, L. Cui, T. Wu and Y. T. Wang, *J. Ethnopharmacol.*, 2012, **141**, 721.
127. H. Ge and H. Ji, *Chin. J. New Drugs*, 2015, **24**, 1053.
128. G. G. L. Yue, S. W. Cheng, H. Yu, Z. S. Xu, J. K. M. Lee, P. M. Hon, M. Y. H. Lee, E. J. Kennelly, G. Deng, S. K. Yeung, B. R. Cassileth, K. P. Fung, P. C. Leung and C. B. S. Lau, *J. Med. Food*, 2012, **15**, 242.

129. Q. Q. Li, G. Wang, E. Reed, L. Huang and C. F. Cuff, *Basic Clin. Pharmacol. Toxicol.*, 2010, **107**, 868.
130. Q. Q. Li, G. Wang, M. Zhang, C. F. Cuff, L. Huang and E. Reed, *Oncol. Rep.*, 2009, **22**, 161.
131. H. Q. Guo, G. N. Zhang, Y. J. Wang, Y. K. Zhang, K. Sodani, T. T. Talele, C. R. Ashby Jr and Z. S. Chen, *Oncol. Rep.*, 2014, **31**, 858.
132. L. Lin, L. Li, X. Chen, B. Zeng and T. Lin, *Oncol. Lett.*, 2018, **16**, 3380.
133. H. Cheng, X. Ge, S. Zhuo, Y. Gao, B. Zhu, J. Zhang, W. Shang, D. Xu, W. Ge and L. Shi, *Front. Pharmacol.*, 2018, **9**, 1413.
134. X. Li, Z. Lin, B. Zhang, L. Guo, S. Liu, H. Li, J. Zhang and Q. Ye, *Sci. Rep.*, 2016, **6**, 21010.
135. S. Liu, L. Zhou, Y. Zhao and Y. Yuan, *Oncol. Rep.*, 2015, **34**, 943.
136. J. S. Liu, X. M. Che, G. Qiu, L. Fan, W. Zhao, S. He, S. Chang and S. Wang, *J. Xi'an Jiaotong Univ., Med. Sci.*, 2015, **36**, 840.
137. B. Zhang, X. Zhang, B. Tang, P. Zheng and Y. Zhang, *Breast Cancer Res. Treat.*, 2012, **136**, 399.
138. L. Xu, T. Guo, X. Qu, X. Hu, Y. Zhang, X. Che, H. Song, J. Gong, R. Ma, C. Li, Y. Fan, Y. Ma, K. Hou, P. Wu, H. Dong and Y. Liu, *Cell Biol. Int.*, 2018, **42**, 1377.
139. Z. F. Zhong, W. A. Qiang, C. M. Wang, W. Tan and Y. T. Wang, *Eur. J. Pharmacol.*, 2016, **774**, 10.
140. Z. F. Zhong, W. Tan, K. Tian, H. Yu, W. A. Qiang and Y. T. Wang, *Oncol. Rep.*, 2017, **37**, 2016.
141. S. Y. Park, Y. H. Kim, Y. Kim and S. J. Lee, *J. Cell. Biochem.*, 2012, **113**, 3653.
142. C. Zheng, X. Cai, S. Wu, Z. Liu, Y. Shi and W. Zhou, *Pak. J. Med. Sci.*, 2014, **30**, 1270.
143. X. Peng, Y. Zhao, X. Liang, L. Wu, S. Cui, A. Guo and W. Wang, *Contemp. Clin. Trials*, 2006, **27**, 70.
144. C. Li, M. Li, Q. Zhou, X. Shu, Y. Liu and G. Han, *Chin. Tradit. Herb. Drugs*, 2007, **38**, 886.
145. R. Zhang, A. Tian, H. Zhang, Z. Zhou, H. Yu and L. Chen, *J. Mol. Neurosci.*, 2011, **44**, 31.
146. T. Z. Zhu, X. M. Li, L. H. Luo, Y. H. Xu, P. Cao, Y. Liu and G. B. Liang, *Mol. Med. Rep.*, 2014, **10**, 1122.
147. X. Zhang, Y. Li, Y. Zhang, J. Song, Q. Wang, L. Zheng and D. Liu, *PLoS One*, 2013, **8**, e58719.
148. Y. Dong, L. Li, L. Wang, T. Zhou, J. W. Liu and Y. J. Gao, *Genet. Mol. Res.*, 2015, **14**, 2347.
149. Z. Hu, H. Wu, Y. Li, Q. Hou, Y. Wang, S. Li, B. Xia and S. Wu, *Anticancer Drugs*, 2015, **26**, 531.
150. L. Li, L. Xu, X. Qu, M. Zhao, P. Yu, J. Kang, Y. Liu and X. Hu, *Mol. Med. Rep.*, 2011, **4**, 1243.
151. C. Yao, J. Jiang, Y. Tu, S. Ye, H. Du and Y. Zhang, *Thorac. Cancer*, 2014, **5**, 304.
152. C. C. Yao, Y. R. Tu, J. Jiang, S. F. Ye, H. X. Du and Y. Zhang, *Oncol. Rep.*, 2014, **31**, 2131.

153. X. Yu, M. Xu, N. Li, Z. Li, H. Li, S. Shao, K. Zou and L. Zou, *Biochem. Biophys. Res. Commun.*, 2017, **490**, 514.
154. Q. Kong, Y. Ma, J. Yu and X. Chen, *Sci. Rep.*, 2017, **7**, 15543.
155. Z. Zhong, W. Tan, X. Chen and Y. Wang, *Eur. J. Pharmacol.*, 2014, **737**, 1.
156. Y. Wang, X. Liu, X. Wang, F. Li, J. Li and W. Cao, *Biomed. Res.*, 2012, **23**, 231.
157. Z. F. Zhong, W. Tan, W. W. Qiang, V. L. Scofield, K. Tian, C. M. Wang, W. A. Qiang and Y. T Wang, *Mol. BioSyst.*, 2016, **12**, 1626.
158. W. S. Xu, T. Li, G. S. Wu, Y. Y. Dang, W. H. Hao, X. P. Chen, J. J. Lu and Y. T Wang, *Am. J. Chin. Med.*, 2014, **42**, 243.
159. R. Mustarichie, J. Levitas and J. Arpina, *Med. J. Indones.*, 2014, **23**, 15.
160. A. Murakami, I. Furukawa, S. Miyamoto, T. Tanaka and H. Ohigashi, *BioFactors*, 2012, **39**, 221.
161. S. K. Sandur, M. K. Pandey, B. Sung, K. S. Ahn, A. Murakami, G. Sethi, P. Limtrakul, V. Badmaev and B. B. Aggarwal, *Carcinogenesis*, 2007, **28**, 1765.
162. X. L. Liu, C. Yang, W. H. Zhang, G. Zhou, X. T. Ma, B. Lin, M. Zhang, Y. Zhou and T. T. Feng, *Tetrahedron Lett.*, 2016, **57**, 1385.
163. Y. J. Li, Y. H. Lei, N. Yao, C. R. Wang, N. Hu, W. C. Ye, D. M. Zhang and Z. S. Chen, *Chin. J. Cancer*, 2017, **36**, 52.
164. S. C. Gautam, X. Gao and S. Dulchavsky, *Adv. Exp. Med. Biol.*, 2007, **595**, 321.
165. Y. Tan, Y. Meng, H. Zhang, C. Sun, Q. Wang, X. Yang, W. Zheng, H. Zhou and F. Shan, *Hum. Vaccines Immunother.*, 2012, **8**, 1416.
166. D. Kim, Y. Suh, H. Lee and Y. Lee, *Int. J. Mol. Med.*, 2013, **31**, 386.
167. L. A. F. Ferreira, O. B. Henriques, A. A. S. Andreoni, G. R. F. Vital, M. M. C. Campos, G. G. Habermehl and V. L. G. de Moraes, *Toxicon*, 1992, **30**, 1211.
168. S. Oh, A. R. Han, H. R. Park, E. J. Jang, H. K. Kim, M. G. Jeong, H. Song, G. H. Park, E. K. Seo and E. S. Hwang, *Chem. Biodiversity*, 2014, **11**, 1034.
169. J. W. Coleman, *Int. Immunopharmacol.*, 2001, **1**, 1397.
170. C. Chen, *Nat. Chem. Biol.*, 2010, **6**, 401.
171. K. Kawasaki, C. Okuda-Hanafusa, M. Aoyagi, K. Taoka, N. Yamamoto, K. Muroyama, S. Murosaki and Y. Yamamoto, *Biosci., Biotechnol., Biochem.*, 2018, **82**, 2109.
172. T. Yuan, C. Zhang, C. Qiu, G. Xia, F. Wang, B. Lin, H. Li and L. Chen, *Nat. Prod. Res.*, 2018, **32**, 1887.
173. Y. Fang, Y. Kang, H. Zou, X. Cheng, T. Xie, L. Shi and H. Zhang, *Fitoterapia*, 2018, **124**, 92.
174. H. Matsuda, T. Morikawa, I. Toguchida, K. Ninomiya and M. Yoshikawa, *Chem. Pharm. Bull.*, 2001, **49**, 1558.
175. Y. Lee, *BMB Rep.*, 2009, **42**, 96.
176. H. H. Chae, S. N. Min, Y. L. Woo and K. L. Sang, *Planta Med.*, 2002, **68**, 545.
177. M. Ohnishi, T. Urasaki, K. Egusa, C. Kunobu, T. Harada, R. Shinkado, H. Nishi, S. Maehara, C. Kitamura, T. Hata, K. Ohashi, H. Shibuya and A. Inoue, *Phytother. Res.*, 2018, **32**, 892.

178. Y. Lou, F. Zhao, H. He, K. F. Peng, X. H. Zhou, L. X. Chen and F. Qiu, *J. Asian Nat. Prod. Res.*, 2009, **11**, 737.
179. K. Tanaka, Y. Kuba, A. Ina, H. Watanabe and K. Komatsu, *Chem. Pharm. Bull.*, 2008, **56**, 936.
180. Y. Zhong, J. Liu, W. M. Huo, W. L. Duan, X. Wang and L. Shang, *Chin. J. Nat. Med.*, 2015, **13**, 415.
181. J. A. Podlogar and E. J. Verspohl, *Phytother. Res.*, 2012, **26**, 333.
182. E. Sieniawska, P. Michel, T. Mroczek, S. Granica and K. Skalicka-Wozniak, *Food Chem. Toxicol.*, 2019, **125**, 161.
183. Y. L. Li, Z. Y. Du, P. H. Li, L. Yan, W. Zhou, Y. D. Tang, G. R. Liu, Y. X. Fang, K. Zhang, C. Z. Dong and H. X. Chen, *Int. Immunopharmacol.*, 2018, **64**, 319.
184. H. Makabe, N. Maru, A. Kuwabara, T. Kamo and M. Hirota, *Nat. Prod. Res.*, 2006, **20**, 680.
185. K. Kawasaki, I. Fukuhara, K. Muroyama and S. Murosaki, *Jpn. Pharmacol. Ther.*, 2017, **45**, 243.
186. C. Megumi, K. Muroyama, H. Sasako and N. Tsuge, *Food Sci. Technol. Res.*, 2017, **23**, 275.
187. R. Uchio, Y. Higashi, Y. Kohama, K. Kawasaki and T. Hirao, *J. Nutr. Sci.*, 2017, **6**, e3.
188. Y. Kimura, M. Sumiyoshi and T. Tamaki, *Fitoterapia*, 2013, **84**, 163.
189. H. Matsuda, T. Morikawa, K. Ninomiya and M. Yoshikawa, *Bioorg. Med. Chem.*, 2001, **9**, 909.
190. H. Matsuda, K. Ninomiya, T. Morikawa and M. Yoshikawa, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 339.
191. T. Morikawa, H. Matsuda, K. Ninomiya and M. Yoshikawa, *Biol. Pharm. Bull.*, 2002, **25**, 627.
192. L. Yang, R. Zhu, Q. Zhu, D. Dan, Y. E. Jin, K. Xu and X. Hao, *Front. Med. China*, 2009, **3**, 36.
193. J. Zheng, L. Ma, Q. Ren, L. Li, Y. Zhang, H. Shi, Y. Liu, C. Li, Y. Dou, S. Li, H. Zhang and M. Yang, *BMC Gastroenterol.*, 2014, **14**, 224.
194. R. Zhu, L. Yang, L. Shen, J. Ye, J. Liu and S. Hu, *J. Huazhong Univ. Sci. Technol., Med. Sci.*, 2009, **29**, 177.
195. S. Yuliani, M. Mustofa and G. Partadiredja, *Nutr. Neurosci.*, 2018, **7**, 1.
196. H. Wang, R. Li and Y. Shen, *Trends Pharmacol. Sci.*, 2013, **34**, 215.
197. S. Matsumura, K. Murata, N. Zaima, Y. Yoshioka, M. Morimoto, H. Kugo, A. Yamamoto, T. Moriyama and H. Matsuda, *Nat. Prod. Commun.*, 2016, **11**, 1785.
198. M. B. Colovic, D. Z. Krstic, T. D. Lazarevic-Pasti, A. M. Bondzic and V. M. Vasic, *Curr. Neuropharmacol.*, 2013, **11**, 315.
199. M. Fujiwara, N. Yagi and M. Miyazawa, *J. Agric. Food Chem.*, 2010, **58**, 2824.
200. C. Zhang, J. Ji, M. Ji and P. Fan, *Phytochem. Lett.*, 2015, **12**, 215.
201. B. Togar, H. Turkez, A. Stefano, A. Tatar and D. Cetin, *Hum. Exp. Toxicol.*, 2014, **34**, 135.
202. J. Wang, H. Li, Y. Ren, Y. Yao, J. Hu, M. Zheng, Y. Ding, Y. Y. Chen, Y. Shen, L. L. Wang and Y. Zhu, *Cell. Physiol. Biochem.*, 2018, **49**, 595.

203. J. Wang, H. Li, Y. Yao, Y. Ren, J. Lin, J. Hu, M. Zheng, X. Song, T. Zhao, Y. Y. Chen, Y. Shen, Y. J. Zhu and L. L. Wang, *Neuroscience*, 2018, **383**, 12.
204. X. J. Li, L. Liang, H. X. Shi, X. P. S. Sun, J. Wang and L. S. Zhang, *Neuropsychiatr. Dis. Treat.*, 2017, **13**, 1733.
205. J. Hucklenbroich, R. Klein, B. Neumaier, R. Graf, G. R. Fink, M. Schroeter and M. A. Rueger, *Stem Cell Res. Ther.*, 2014, **5**, 100.
206. W. W. Chen, X. Zhang and W. J. Huang, *Mol. Med. Rep.*, 2016, **13**, 3391.
207. J. Y. Lo, M. N. A. Kamarudin, O. A. A. Hamdi, K. Awang and H. A. Kadir, *Food Funct.*, 2015, **6**, 3550.
208. M. Chen, Y. Y. Chang, S. Huang, L. H. Xiao, W. Zhou, L. Y. Zhang, C. Li, R. P. Zhou, J. Tang, L. Lin, Z. Y. Du and K. Zhang, *Mol. Nutr. Food Res.*, 2018, **62**, 1700281.
209. S. Y. Park, M. L. Jin, Y. H. Kim, Y. Kim and S. J. Lee, *Int. Immunopharmacol.*, 2012, **14**, 13.
210. R. Zhang, A. Tian, X. Shi, H. Yu and L. Chen, *Int. Immunopharmacol.*, 2010, **10**, 738.
211. X. Meng, N. Li, Y. Zhang, D. Fan, C. Yang, H. Li, D. Guo and S. Pan, *Transl. Neurosci.*, 2018, **9**, 33.
212. H. Shi, L. Shi and J. X. Li, *J. Xi'an Jiaotong Univ., Med. Sci.*, 2017, **38**, 749.
213. A. M. Orellana-Paucar, T. Afrikanova, J. Thomas, Y. K. Aibuldinov, W. Dehaen, P. A. M. De Witte and C. V. Esguerra, *PLoS One*, 2013, **8**, e81634.
214. A. M. Orellana-Paucar, A. S. K. Serruys, T. Afrikanova, J. Maes, W. De Borggraeve, J. Alen, F. Leon-Tamariz, I. M. Wilches-Arizabala, A. D. Crawford, P. A. M. de Witte and C. V. Esguerra, *Epilepsy Behav.*, 2012, **24**, 14.
215. L. Gong, H. Cai, Q. Zhou and X. Kong, *Trop. J. Pharm. Res.*, 2018, **17**, 597.
216. K. H. Shin, K. Y. Yoon and T. S. Cho, *Korean J. Pharmacogn.*, 1994, **25**, 221.
217. D. de Fatima Navarro, M. M. de Souza, R. A. Neto, V. Golin, R. Niero, R. A. Yunes, F. Delle Monache and V. Cechinel Filho, *Phytomedicine*, 2002, **9**, 427.
218. C. R. Pamplona, M. M. de Souza, S. Machado Mda, V. Cechinel Filho, D. Navarro, R. A. Yunes, F. Delle Monache and R. Niero, *Z. Naturforsch., C*, 2006, **61**, 6.
219. A. Germano, A. Occhipinti, F. Barbero and M. E. Maffei, *BioMed Res. Int.*, 2017, **2017**, 3804356.
220. M. Yamazaki, Y. Maebayashi, N. Iwase and T. Kaneko, *Chem. Pharm. Bull.*, 1988, **36**, 2075.
221. C. F. Hossain, M. Al-Amin, A. S. M. Sayem, I. H. Siragee, A. M. Tunan, F. Hassan, M. M. Kabir and G. N. N. Sultana, *BMC Complementary Altern. Med.*, 2015, **15**, 191.
222. T. Yoshioka, E. Fujii, M. Endo, K. Wada, Y. Tokunaga, N. Shiba, H. Hohsho, H. Shibuya and T. Muraki, *Inflammation Res.*, 1998, **47**, 476.
223. S. Y. Park, M. L. Jin, Y. H. Kim, Y. H. Kim and S. J. Lee, *Arch. Dermatol. Res.*, 2011, **303**, 737.
224. J. H. Park, M. A. A. Mohamed, Y. J. Jung, S. Shrestha, T. H. Lee, C. H. Lee, D. Han, J. Kim and N. I. Baek, *Arch. Pharmacal Res.*, 2015, **38**, 1752.

225. M. Kuroda, Y. Mimakl, T. Nishiyama, T. Mae, H. Kishida, M. Tsukagawa, K. Takahashi, T. Kawada, K. Nakagawa and M. Kitahara, *Biol. Pharm. Bull.*, 2005, **28**, 937.
226. P. C. Lekshmi, R. Arimboor, P. S. Indulekha and A. Nirmala Menon, *Int. J. Food Sci. Nutr.*, 2012, **63**, 832.
227. C. X. Zhou, L. S. Zhang, F. F. Chen, H. S. Wu, J. X. Mo and L. S. Gan, *Fitoterapia*, 2017, **121**, 141.
228. Y. R. Guo and S. Y. Choung, *J. Pharm. Pharmacol.*, 2017, **69**, 202.
229. Y. R. Guo and S. Y. Choung, *J. Med. Food*, 2017, **20**, 46.
230. D. I. Sun, I. T. Nizamutdinova, Y. M. Kim, X. F. Cai, J. J. Lee, S. S. Kang, Y. S. Kim, K. M. Kang, G. Y. Chai, K. C. Chang and H. J. Kim, *Int. Immunopharmacol.*, 2008, **8**, 1272.
231. L. Wu, G. Wang, S. Tang, G. Long and T. Yin, *Cardiovasc. Drugs Ther.*, 2011, **25**, 233.
232. M. Liu, X. Chen, J. Ma, W. Hassan, H. Wu, J. Ling and J. Shang, *Biomed. Pharmacother.*, 2017, **95**, 1789.
233. M. Yoshikawa, T. Murakami, T. Morikawa and H. Matsuda, *Chem. Pharm. Bull.*, 1998, **46**, 1186.
234. H. Matsuda, T. Morikawa, K. Ninomiya and M. Yoshikawa, *Tetrahedron*, 2001, **57**, 8443.
235. H. S. Lee, *Bioresour. Technol.*, 2006, **97**, 1372.
236. D. Zhang, W. Qiao, Y. Zhao, H. Fang, D. Xu and Q. Xia, *Fitoterapia*, 2017, **116**, 106.
237. Y. Johji, M. Michihiko, Q. R. Huang, M. Hisashi and F. Hajime, *J. Ethnopharmacol.*, 1988, **23**, 299.
238. Y. Wang, L. Wang, X. Zhu, D. Wang and X. Li, *J. Food Sci.*, 2016, **81**, H1800.
239. N. Suphrom, G. Pumthong, N. Khorana, N. Waranuch, N. Limpeanchob and K. Ingkaninan, *Fitoterapia*, 2012, **83**, 864.
240. M. M. Melo, G. G. Habermehl, N. J. F. Oliveira, E. F. Nascimento, M. M. B. Santos and M. Lucia, *Arq. Bras. Med. Vet. Zootec.*, 2005, **57**, 7.
241. S. Zou, C. Wang, Z. Cui, P. Guo, Q. Meng, X. Shi, Y. Gao, G. Yang and Z. Han, *Pharmacol. Rep.*, 2016, **68**, 7.
242. C. Xue, L. L. Hong, J. S. Lin, X. Y. Yao, D. H. Wu, X. P. Lin, J. M. Zhang, X. B. Zhang and Y. M. Zeng, *Biosci. Rep.*, 2018, **38**, BSR20171386.
243. L. Hong, Y. Zeng and D. Yang, *Respiration*, 2016, **92**, 329.
244. S. Z. Moghadamtousi, H. A. Kadir, P. Hassandarvish, H. Tajik, S. Abubakar and K. Zandi, *BioMed Res. Int.*, 2014, **2014**, 186864.
245. N. S. Nor Azman, M. S. Hossan, V. Nissapatorn, C. Uthaipibull, P. Prommana, K. T. Jin, M. Rahmatullah, T. Mahboob, C. S. Raju, H. M. Jindal, B. Hazra, M. R. Mohd Abd Razak, V. K. Prajapati, R. K. Pandey, N. Aminudin, K. Shaari, N. H. Ismail, M. S. Butler, V. V. Zarubaev and C. Wiart, *Exp. Parasitol.*, 2018, **194**, 67.
246. Q. Liao, Z. Qian, R. Liu, L. An and X. Chen, *Arch. Virol.*, 2013, **100**, 578.
247. H. Wu, Y. Liu, S. Zu, X. Sun, C. Liu, D. Liu, X. Zhang, J. Tian and L. Qu, *Arch. Virol.*, 2016, **161**, 1559.

248. Y. Chen, Y. Dong, Y. Jiao, L. Hou, Y. Shi, T. Gu, P. Zhou, Z. Shi, L. Xu and C. Wang, *Arch. Virol.*, 2015, **160**, 1415.
249. J. Feng, X. Bai, T. Cui, H. Zhou, Y. Chen, J. Xie, Q. Shi, H. Wang and G. Zhang, *Curr. Microbiol.*, 2016, **73**, 317.
250. D. S. H. L. Kim and J. Y. Kim, *Bioorg. Med. Chem.*, 2001, **11**, 2541.
251. C. S. Lai, S. N. Liao, M. L. Tsai, N. Kalyanam, M. Majeed, A. Majeed, C. T. Ho and M. H. Pan, *Mol. Nutr. Food Res.*, 2015, **59**, 1883.
252. C. Buhrmann, B. Popper, A. B. Kunnumakkara, B. B. Aggarwal and M. Shakibaei, *Nutrients*, 2019, **11**, 2904.
253. M. J. Lee, Y. J. Tsai, M. Y. Lin, H. L. You, N. Kalyanam, C. T. Ho and M. H. Pan, *Phytomedicine*, 2019, **57**, 377.
254. A. K. Tyagi, S. Prasad, M. Majeed and B. B. Aggarwal, *Phytomecidine*, 2017, **34**, 171.
255. W. S. Liou, C. Lin, P. S. Lee, N. Kalyanam, C. T. Ho and M. H. Pan, *J. Funct. Foods*, 2016, **26**, 781.
256. B. B. Aggarwal, W. Yuan, S. Li and S. C. Gupta, *Mol. Nutr. Food Res.*, 2013, **57**, 1529.
257. Y. Li, S. Li, Y. Han, J. Liu, J. Zhang, F. Li, Y. Wang, X. Liu and L. Yao, *Eur. J. Pharmacol.*, 2008, **591**, 252.
258. B. B. Aggarwal, L. Deb and S. Prasad, *Molecules*, 2015, **20**, 185.
259. C. H. Park, J. H. Song, S. N. Kim, J. H. Lee, H. J. Lee, K. S. Kang and H. H. Lim, *Molecules*, 2020, **25**, 144.

CHAPTER 13

Toxicology Aspects of Turmeric

SWAPNIL P. BORSE[a,b], ABHISHEK S. KULKARNI[c], HEMANT KOSHIA[d], KAMALA K. VASU[e] AND MANISH NIVSARKAR*[b]

[a]AYUSH-Center of Excellence, Center for Complementary and Integrative Health [CCIH], Interdisciplinary School of Health Sciences, Savitribai Phule Pune University [SPPU], Pune, Maharashtra, 411007, India; [b]Department of Pharmacology and Toxicology, B. V. Patel Pharmaceutical Education and Research Development (PERD) Centre, Thaltej, Ahmedabad, Gujarat, 380054, India; [c]Department of Neutraceuticals, Krishna's Navjeevan Group, Aurangabad, Maharashtra, 431003, India; [d]Food, Drugs and Cosmetics Administration, Department of Health and Family Welfare, Government of Gujarat, Gandhinagar, Gujarat, India; [e]Department of Medicinal Chemistry, B. V. Patel Pharmaceutical Education and Research Development (PERD) Centre, Thaltej, Ahmedabad, Gujarat, 380054, India
*E-mail: manishnivsarkar@gmail.com

13.1 Turmeric and Toxicology: An Overview

Turmeric (*Curcuma longa*), from the Zingiberaceae family, is an important herb. Its rhizomes are widely used throughout the world as a principal spice and drug.[1] Particularly in the Indian subcontinent it is widely used in diet and as a complementary alternative system of medicine (CAM). In Ayurveda, it has been considered as *Dashemani Lekhaniya* (emaciating), *Kusthagna* (anti-dermatosis), *Visaghna* (anti-poisonous).[2,3] Turmeric is also used in many Hindu ritual processes such as topical application on the bodies of the bride

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

and groom before a marriage ceremony. Hindu females apply it on their forehead during their day-to-day rituals.[1] Therefore, it is a common mindset that turmeric is a holy and totally safe substance. The same assumptions are made for its major and important phytoconstituent, curcumin. However, as rightly said by the physician Paracelsus (1493–1541), who is regarded as the father of toxicology, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy".[4] Therefore, this chapter focuses on understanding the toxicological aspect of turmeric for safe and efficacious use as a food component and drug to manage disease conditions.

13.2 Factors Affecting the Toxicological Aspects of Turmeric

The phytoconstituents present in turmeric are many and their proportion varies with respect to the many confounding factors that include but are not limited to the geographical variation, method of cultivation and collection, isolation, preparation and storage, environmental contamination, infection, soil condition, plant–soil–environment–microbiota interplay, preparation and/or formulation, *etc.*[5–9]

Adulteration is a major issue associated with spices, especially with turmeric. The adulteration may be intentional or unintentional. The intentional adulterations are mostly riskier and toxic compared to unintentional. Table 13.1 describes possible adulterants of turmeric and their toxic effects. Traditionally, there are simple tests that are used to identify these adulterations in turmeric. Nowadays, many sophisticated microscopic, DNA barcoding, and/or analytical methods are available for the detection of adulteration

Table 13.1 Toxic adulterants used in turmeric.

Adulterant	Toxicity	Simple detection test	Reference
Metanil yellow, chalk powder	Metanil yellow, Carcinogenic and Neurotoxic	Addition of few drops of water and concentrated hydrochloric acid to the turmeric powder in glass beaker gives a pink color. The stable pink color after water addition confirms the presence of metanil yellow, whereas release of small bubbles during this reaction indicates the presence of chalk powder.	69–72
Lead chromate	Carcinogenic, Genotoxic	Addition of teaspoon turmeric powder in a glass of water leaks streaks of water-soluble color	69–73

or counterfeit products of turmeric.[10–16] These analytical methods include but are not limited to IR (infrared spectroscopy), NMR (nuclear magnetic resonance spectroscopy), MS (mass spectroscopy), HPLC (high performance liquid chromatography), LC-MS (liquid chromatography–mass spectroscopy) and LC-MS/MS (liquid chromatography–mass spectroscopy–mass spectroscopy).[10–16]

13.2.1 Interactions of Turmeric as a Therapeutic Adjuvant

The interactions can be beneficial, harmful or even toxic and it depends on the dose, time, duration, frequency, disease, individualization, concomitantly administered drug/food/herb, *etc.* Turmeric is taken as a therapeutic adjuvant with the assumption that it is fully safe to consume with different drugs and/or herbal formulations. However, this may or may not always be the case. This depends on the intensity and type of drug(s)–herb(s)–disease(s) interactions.[17] Turmeric contains hundreds of phytoconstituents.[18] Balaji *et al.*, reported almost 200 plus chemical constituents in turmeric.[18] Their proportion may vary with respect to geographical location, cultivation and collection, preparation, storage, *etc.*[19] Therefore, it is very important to note that turmeric does not act alone; it is a combination of all these multiple compounds. However, studying the probable interactions for individual phytoconstituents, enriched fractionated extracts, typical mixtures of isolated phytoconstituents, complete turmeric (rhizome/oil), and different formulations of turmeric should be screened separately and on a case by case basis for clinically translational understanding about beneficial, harmful or fatal interactions. Based on the literature it has been found that turmeric and/or its major phytoconstituent curcumin can interact with many drugs. Among these phytoconstituents, curcumin has been extensively studied. Many researchers seem to be confused and/or have assumed that turmeric and curcumin are replaceable with each other, but this is not the case.[6,19–21]

Therefore, the drugs or phytoconstituents that have the highest probability of concomitant administration should be screened first. For instance, as curcumin or turmeric is consumed for many chronic diseases such as cancer, diabetes, arthritis, stress, pain, common cold, *etc.* then preferably drugs from these classes should be studied.[22,23] The interactions are of mainly two types, pharmacokinetic (absorption, distribution, metabolism and excretion) and pharmacodynamic (synergistic, antagonistic, additive, no effect) interactions.[17] The pharmacokinetic interactions take place mainly because of either induction or inhibition of drug metabolizing enzymes (DMEs) and drug transporters (DTs).[17] Thereby, the substrate drug, interacting drug and/or their metabolites can affect each other's pharmacokinetics–pharmacodynamics (PK-PD) and toxicokinetics–toxicodynamics (TK-TD).[17]

This section focuses on harmful or toxic interactions where turmeric or its major phytoconstituents are being consumed. This concomitant

administration assumed turmeric/curcumin as potential therapeutic adjuvants to maximize the benefit from primary ongoing treatment. But instead of benefits, harmful or toxic effects may occur.

13.2.1.1 Turmeric–Drug–Disease–Herb–Food Interactions

Turmeric and especially curcumin hold the potential to inhibit or downregulate the important DMEs and DTs. Curcumin and turmeric have been reported to be inhibitors or downregulators of ABCC5 (MRP5), glutathione S-transferase, CYP2C9, CYP1A2, CYP2A6, CYP3A4, CYP2B6, CYP1A2, CYP2D6, P-glycoprotein, *etc.*[24–31] Curcumin along with its derivatives are shown to inhibit the activity of several drug metabolizing enzymes such as CYP450, glutathione-S-transferase and UDP-glicuronosyltransferase, which could be responsible for an undesirable increase in plasma concentration of drugs metabolized through these enzymes such as morphine, digoxin, acetoaminophen *etc.*[27,32–37] The effect of indomethacin and reserpine may be reduced due to concomitant administration with turmeric. To enhance the bioavailability of curcumin, it is generally combined with piperine, but at the same time it inhibits CYP3A4 non-competitively. CYP3A4 inhibition adversely affects the metabolism of drugs used more commonly by elderly patients such as amiodarone, quinidine *etc.* thereby increasing the risk of death by threatening ventricular arrhythmias.[38] Geraniol, another constituent of turmeric, has been reported to act as an inducer and substrate of CYP2B6, CYP1A1 and CYP3A5.[24,39,40] Thereby, turmeric holds strong potential to alter the PK-PD and/or TK-TD of the drugs that are the substrates of these DMEs and/or DTs such as warfarin, paclitaxel, docetaxel, etoposide, everolimus, midazolam, sufasalazine, norfloxacin, sulindac, losartan, tacrolimus, clopidrogel, saquinavir, resveratrol, rusuvastatin, talinolol, dextromethorphan, flurbiprofen, paracetamol, *etc.*[24–31,41] When pancreatic cancer patients were administered crude curcumin powder 3.6 g day^{-1} given with gemcitabine (1 g m^{-2}, i.v., weekly for three to four weeks) some of them were observed to have severe stomach pain. Thereby, for these patients the treatment of curcumin was discontinued due to a consequence of harmful pharmacodynamic herb–drug–disease interaction.[31] Curcumin increases the systemic exposure of tacrolimus and thereby associated side effects may also increase.[31,42] Dietary curcumin may inhibit chemotherapy induced programmed cell death, *i.e.* apoptosis in breast cancer patients through blockade of the c-Jun NH_2-terminal kinase pathway and inhibition of reactive oxygen species generation.[43] Warfarin has a very narrow therapeutic window, co-administration of curcumin elevates C_{max} and AUC. A small increase in the serum levels can cause bleeding.[44] Turmeric, due to its intense absorptive and fluorescent properties, may interfere with laboratory tests that use dye and thereby may cause misinterpretation of the laboratory findings. Table 13.2 depicts pharmacokinetic alteration that occurred due to the interaction of turmeric with commonly used drugs covering cardiovascular, anticoagulant, NSAID (non-steroidal anti-inflammatory drugs) and anticancer drugs.

Table 13.2 Turmeric–drug interactions.

Drugs	Compound/preparation	Pharmacokinetic alteration	Pharmacokinetic model	Mechanism	Reference
Losartan (40 mg kg^{-1}, p.o. and 1 mg kg^{-1}, i.v.)	Curcumin (100 mg kg^{-1}, p.o.)	C_{max} increased by 3.5-fold AUC increased by 1.7-fold	Sprague Dawley rats	Inhibition P-gp and inhibits CYP3A4	74 and 75
Talinolol (50 mg, p.o.)	Curcumin (300 mg kg^{-1}, p.o.)	Reduction in C_{max} and AUC Increased in clearance	Open labeled model in healthy volunteers	Inhibition P-gp	76 and 77
Celiprolol (30 mg kg^{-1}, p.o.)	Curcumin (60 mg kg^{-1}, p.o.)	C_{max} increased by 1.9 AUC increased by 1.3 Clearance reduced by 22%	Sprague Dawley rats	Inhibition P-gp	78
Rosuvastatin (5 mg kg^{-1}, p.o. in rats and dogs)	Curcumin (500 mg kg^{-1}, p.o. in rats and 100 mg kg^{-1}, p.o. in dogs)	C_{max} increased 1.3-fold AUC increased by 2.2	Sprague Dawley rats and Beagle dogs	Inhibition of OATP transporter	79
Paclitaxel (20 mg kg^{-1}, p.o.)	Curcumin (50 mg kg^{-1}, p.o.)	AUC increased by 4.1-fold	SKOV3 human ovarian adenocarcinoma bearing female nu/nu mice	Downregulation of P-gp and CYP3A2	80
Docetaxel (0.2–10 μM)	Curcumin (10 0 mg kg^{-1}, p.o.)	AUC increased by 1.86-fold $t_{½}$ increased by 1.55-fold Clearance reduced by 52.1%	Sprague Dawley rats	Inhibition of OATP1B1 and OATP1B3	81
Warfarin (0.2 mg kg^{-1}, p.o.)	Curcumin (25, 50 and100 mg kg^{-1}, p.o.)	C_{max} increased by 1.5-fold AUC increased by 1.6-fold Clearance reduced by 57.14%	Wistar rats	—	82
Norfloxacin (100 g kg^{-1}, p.o.)	Curcumin (60 mg kg^{-1}, p.o.)	AUC increased by 1.5-fold	New Zealand white rabbits	—	82

13.3 Marketed Turmeric Formulations and Toxicological Aspects

Turmeric formulations have not only occupied an appreciable place in the FMCG market but also in the health and nutrition market. Various preparations of turmeric are available that include rhizomes, standardized extracts, blended powders, tablets, capsules, syrups, liquid drops, oils, juices, creams, gels, soaps, shampoos, balms, antiseptic tapes and many more. These formulations have discreet doses but at the same time they have very bold claims. Heavy doses must undergo toxicological as well as interaction studies. Adequate data is required when it comes to justification of these claims. Claims should be backed up with well-planned trials and significant results to use the ultimate conclusion as a claim.

Turmeric at the dose of 10 μg mL^{-1} leads to dose and time dependent induction of chromosome aberrations in several mammalian cells. Reports also suggest that curcumin at therapeutic doses can induce DNA damage and chromosomal alterations both *in vitro* and *in vivo*.[45] For example, curcumin at doses of 2.5 μg mL^{-1} and 5 μg mL^{-1} leads to DNA damage in mitochondrial and nuclear genome in cells.[46] Oleoresin present in turmeric that contains 79–85% curcumin was administered to rats and mice for two years to study the toxicological and carcinogenic effects. The study ended with the following conclusion based on the relevant observations: the study underlines carcinogenic activity in female rats, female mice and male rats, based on incidences of clitoral gland adenomas and hepatocellular adenomas at 10 000 ppm in female rats, increased incidence of hepatocellular adenomas at 10 000 ppm in female mice and carcinomas of the small intestine and hepatocellular adenomas at 2000 ppm and 10 000 ppm in male mice. Turmeric oleoresin ingestion also increased incidence of ulcers, hyperplasia and inflammation of stomach, cecum and colon in male rates and cecum and thyroid gland follicular cell hyperplasia in female rats.[47] The study also underlines carcinogenic activity in female rats, female mice and male rats, based on incidences of clitoral gland adenomas and hepatocellular adenomas in female rats and carcinomas of the small intestine and hepatocellular adenomas in male mice.[47] A report published in 2010 shows the possible promotion of lung cancer by curcumin.[48] Curcumin is also found to be an active iron chelator *in vivo*; this suggests the potential to affect iron metabolism and caution is required for anemic conditions or related pharmacotherapeutic management.[49]

13.4 Methods for Early Prediction of Toxicity

13.4.1 Chemoinformatics

Herbal drugs like turmeric contain hundreds of compounds and thereby predicting combined actions of such drugs in biological systems is a challenge. Therefore, the techniques and approaches such as chemoinformatics can be of great help to understand and predict the TK-TD.[50] Chemoinformatics uses information techniques and computer science as an *in silico* technique.[18,50]

Chemoinformatics were used to study the hundreds of phytoconstituents of turmeric. Balaji S., *et al.* reported almost more than 200 chemical constituents in turmeric. Their proportion may vary with respect to geographical location, cultivation and collection, preparation, storage, *etc.* Therefore, it is very important to note that turmeric does not act alone; it is a combination of all these multiple compounds.[18] Balaji. *et al.*, analyzed 200 plus molecules from turmeric for bacterial mutagenicity, rodent carcinogenicity and human hepatotoxicity. Among these 200, 16 molecules, namely 1,3,5,11-bisabolatetraene, 3-hydroxy-1, 10-bisaboladien-9-one, *ar*-turmerone, bisacurone, bisacurone (A, B and C), bisacurone epoxide and turmeronol B, 4S-dihydrocurcumenone, (E)-1,7-diphenyl-1-hepten-5-one, (E)-1,7-diphenyl-3-hydroxy-1-hepten-5-one,1b,4b,5b,10b-zedoaronediol, isospathulenol, isozedoarondiol and zedoarondiol were found to be non-mutagenic, non-carcinogenic, non-hepatotoxic. Among these 184 remaining compounds, 136 were mutagenic, 64 hepatotoxic and 153 were carcinogenic.[18]

13.4.2 Ayur-informatics

The 'lock and key' theory in which the drug key acts on the single target lock is the basis of modern pharmacology. This has led to the synthesis of one drug for one target for one disease. The major disadvantage of this approach is the side effects of drugs synthesized in that way. This guided scientists to explore innovative approaches in drug design with multi targeted as well as multi compound drugs. This has led to a new branch in pharmaceutical science called 'network pharmacology'. Network pharmacology utilizes the advancements in computational biology and systems biology to generate networks of interactions of drugs and their molecular targets. The Indian traditional system of medicine, Ayurveda, has practiced herbal formulations for thousands of years. But the exact mechanisms of action (PK-PD or TK-TD) of these drugs have not been explored very much. In this scenario, the integration of drug discovery and bioinformatics with the network pharmacology approach will give a new perspective and larger acceptance to Ayurveda.[51,52] So far, network pharmacology has been compared to that of network toxicology. Ayurvedic approaches and Ayurvedic understanding of toxicity can be coupled with network pharmacology or network toxicology to predict the toxicity of herbal drugs/formulations.[51–53]

13.5 Management of Turmeric Toxicity

13.5.1 Traditional Methods to Ensure the Safety of Turmeric and Its Formulations

Although turmeric is always regarded as safe, traditional systems of medicine like Ayurveda consider its pharmaco-toxicological potential and ability to affect the homeodynamics. Depending on the phenotypic individualization, an Ayurvedic physician decides the vehicle for turmeric administration such as lukewarm water *versus* warm milk.[54] Thereby they ensure the safety

and effectiveness by minimizing even hidden detrimental effects, if any.[54,55] Briefly, in Ayurveda phenotypic grouping, individualization is of three main types, *viz. Vatta*, *Pitta* and *Kapha*. Ayurvedic understanding of health and disease prediction/predisposition has much evidence-based support.[56–58] For instance, according to Ayurveda, *Kapha* phenotype resembles a poor metabolizer, *Vatta* intermediate metabolizers, whereas *Pitta* resembles fast metabolizers.[58,59] Therefore, if an individual with *Pitta* Prakriti has an acidity problem then turmeric is prescribed with one glass of milk plus one teaspoon of cow ghee. Thereby, turmeric will give a *Rasayana* effect (immunizer/revitalize) along with management of the hyperacidity problem. Whereas for the *Vatta* phenotype, an individual with anorexia and difficulty in bowel habits may be prescribed turmeric with lukewarm water. If the latter type of turmeric formulation is given to earlier issue, then the issue of hyperacidity may increase. Another way to manage toxicity and/or long-term side effects is dose optimization with respect to the circadian clock and alteration in the climate/climate zone. For instance, the dose of turmeric can be increased or decreased depending on the winter or summer season. It has also been noted that the dose given by Ayurvedic/herbal practitioners is much less than the toxic dose (8 mg kg^{-1} day^{-1}).[19] But, at the same time, due to its easy availability and misconceptions about safety, there are always chances of daily/chronic consumption of turmeric by patients with or without informing physicians, thereby exposing them to turmeric associated toxicities. The common toxicities associated with long-term high dose of turmeric can cause nausea, diarrhea, increased serum alkaline phosphatase and lactate dehydrogenase, *etc.*[19]

13.6 Turmeric – Hype or Hope

Turmeric, an ordinary spice from the kitchen, holds the crown of the golden curry spice, and as one of the most powerful search engines on the Web suggests, it not only prevents heart disease, Alzheimer's disease and cancer but also relieves symptoms of depression and arthritis. Most of the claims that support the glorious potential of curcumin are mainly based on *in vitro* studies.[19] On the counter side a few scientists have revealed a different story of turmeric, highlighting a major obstacle of bioavailability. Vareed and colleagues in 2008 examined the pharmacokinetics of curcumin in 12 healthy human volunteers, among them only one subject had detectable free curcumin.[19] Garg and colleagues, through their extensive study on 600 patients, concluded that oral curcumin was no more effective than placebos in ameliorating inflammatory complication of aortic aneurysm repair.[60] Similarly, patients with advanced colorectal cancer kept on a daily dose of 3.6 g for up to four months showed no remarkable response and a decrease in tumor markers was not observed.[19] It is strongly recommended that turmeric should be subjected to a high standard of scientific testing and journals should publish and promote these high quality studies in the interests of the public.

13.7 Limitations and Future Perspective

Current approaches used by researchers from both academia and industry don't give enough focus on assessing the negative markers during herbal drug development and standardization. Even the Drugs and Cosmetics Act needs to have more clarity. For instance, according to new amendments for phytopharmaceuticals, qualitative as well as quantitative analysis of a minimum of four phytoconstituents from herbal drugs or formulations is required. But, the criteria and basis of selection of these phytoconstituents is yet to be clarified.[61–63] It is necessary to check whether all of the marketed formulations have passed all the requirements of the D & C Act. Along with these four phytoconstituents, the study of the negative biomarker is also necessary, especially to ensure the safety and minimize any chance of toxicity. In the due course of enrichment of major phytoconstituents one eye should be kept on the negative biomarker. Negative biomarkers can be toxic phytoconstituents and adulterants such as metanil. These biomarkers need to be identified using a standardized method that has been developed for their qualitative as well as quantitative analysis. Herb–drug interactions are a major issue but there is no proper information available along with a product for co-administration with therapeutic drugs that need to be addressed. In detail, metabolite studies also need to be focused. A clear understanding about the standardization of major phytoconstituents and negative markers, herb–drug interaction studies and metabolite analysis will help to build faith about the safety and efficacy of herbal formulation.

Rhizomes, oil and enriched extracts of turmeric should be considered as novel formulations on a case by case basis. The details focusing on herb–drug–food–disease interactions have to be studied. Interaction studies should include pharmacokinetic as well as pharmacodynamic study.[21] In depth *in silico*, *in vitro* as well as *in vivo* studies will play a key role in the development of integrative medicine based prescription.[53,64]

13.8 Discussion and Conclusion

Turmeric and curcumin are often considered as interchangeable, which is not the case. As curcumin is one of the pharmacologically active components of turmeric, Aggarwal *et al.* discussed a case study where they showed that curcumin free extract also gave almost similar actions to that of curcumin and curcumin alone.[20] This indicates that the actions are mediated *via* a complex mixture of phytoconstituents present in the turmeric. Therefore, the misconceptions associated with turmeric and curcumin need to be avoided and their benefit *versus* risk analysis should be checked on a case by case basis. Here, case by case basis means toxicity/safety assessment should consider the concomitantly consumed drugs, herbs or foods that may affect the turmeric actions (PK-PD and TK-TD: pharmacokinetics–pharmacodynamics and toxicokinetics–toxicodynamics). The dose and duration play a significant role in developing toxicity instead of giving desired pharmacotherapeutic actions. Table 13.3 describes the putative toxicological mechanism of

Table 13.3 Toxicology of curcumin from turmeric.

Phytoconstituent	Approximate amount	Toxicology		Toxicology mechanism	Reference
		P	C		
Curcumin I, Curcumin II (demethoxy-curcumin), Curcumin III (bis-demthoxycurcumin)	Human: 32.28 g turmeric/day Mice: 0.2% dietary curcumin for six months	Curcumin iron deficiency	A man with osteoarthritis consumed too high a dose (three times more than a safe dose) for a long time leading to a decrease in Hb up to 12 mg dL^{-1}	Curcumin chelated iron but not zinc and copper *in vivo*. Liver hepcidin and ferritin expression was strongly suppressed in curcumin-fed mice. This too high a dose of curcumin can induce expression of hepatic iron transporters DMT1 and TfR1.	83 and 84

curcumin, which is a major phytoconstituent of turmeric. The disease condition or altered homeodynamics of an individual may alter the PK-PD and TK-TD of the administered drug. Due to the presence of multiple phytoconstituents as a complex mixture, predicting toxicity and increasing safety is a major challenge. Also, the use of novel integrative approaches such as chemoinformatics, *Ayurnization*, Ayur-informatics, may offer great help.[50,65–67] In conclusion, even the safest food, herb or drug may also lead to detrimental, harmful, and/or toxic effect when a pharmacist/physician forgets or ignores taking these confounding factors into consideration. Therefore, we feel that it is the responsibility of all the stakeholders of the healthcare system, starting from the patient to physician/pharmacist to family members to share complete information about herbal medicines such as turmeric (kitchen queen, golden spice[68]) to ensure safety and minimize toxicity.

Conflict of Interest

None to declare.

Acknowledgements

The authors are thankful to B. V. Patel PERD Centre and AYUSH-Centre of Excellence for providing all the necessary facilities to carry out this work. Inputs from Dr Angad Shirpat, Dr Girish Tillu, Dr Supriya Zende, Dr Kamlesh Mishra and Ms Vedika Bhat are also acknowledged.

References

1. S. Prasad and B. B. Aggarwal, Turmeric, the golden spice: from traditional medicine to modern medicine, in *Herbal Medicine: Biomolecular and Clinical Aspects*, ed. I. F. F. Benzie and S. Wachtel-Galor, CRC Press/Taylor & Francis, Boca Raton, 2011, vol. 2, pp. 263–268.
2. *The Ayurvedic Pharmacopoeia of India Part-1*, Government of India, Ministry of Health and Family Welfare, Department of AYUSH, 1989, Sr. No. 30, vol. 1, p. 60.
3. *The Ayurvedic Pharmacopoeia of India Part-1*, Government of India, Ministry of Health and Family Welfare, Department of AYUSH, 1989, Sr. No. 30, vol. 1, p. 61.
4. S. Parasuraman, *J. Pharmacol. Pharmacother.*, 2011, **2**, 74.
5. C.-C. Hsueh, C.-C. Wu and B.-Y. Chen, *Biotechnol. Biofuels*, 2019, **12**, 271.
6. S. P. Borse, *Ancient Sci. Life*, 2016, **36**, 56.
7. E. Vetrova, E. Maksimenko, S. Borisenko, A. Lekar, N. Borisenko and V. Minkin, *Russ. J. Phys. Chem. B*, 2017, **11**, 1202.
8. I. Sandeep, N. Sanghamitra and M. Sujata, *Indian J. Exp. Biol.*, 2015, **6**, 406.
9. V. Govindarajan and W. H. Stahl, *Crit. Rev. Food Sci. Nutr.*, 1980, **12**, 199.

10. S. Kar, B. Tudu, A. K. Bag and R. Bandyopadhyay, *Food Anal. Methods*, 2018, **11**, 1291.
11. B. Amel, *Int. J. Pharmacogn.*, 2015, **2**, 173.
12. L. Sabatino, M. Scordino, M. Gargano, A. Belligno, P. Traulo and G. Gagliano, *Nat. Prod. Commun.*, 2011, **6**, 193.
13. K. Dhanya and B. Sasikumar, *Curr. Trends Biotechnol. Pharm.*, 2010, **4**, 454.
14. V. Parvathy, V. Swetha, T. Sheeja and B. Sasikumar, *Pharm. Microbiol.*, 2015, **53**, 1774.
15. B. Sasikumar, S. Syamkumar, R. Remya and T. John Zachariah, *Food Biotechnol.*, 2004, **18**, 299.
16. E. A. Petrakis, L. R. Cagliani, M. G. Polissiou and R. Consonni, *Food Chem.*, 2015, **173**, 890.
17. S. P. Borse, D. P. Singh and M. Nivsarkar, *Porto Biomed. J.*, 2019, **4**, e15.
18. S. Balaji and B. Chempakam, *Food Chem. Toxicol.*, 2010, **48**, 2951.
19. E. Burgos-Morón, J. M. Calderón-Montaño, J. Salvador, A. Robles and M. López-Lázaro, *Int. J. Cancer*, 2010, **126**, 1771.
20. B. B. Aggarwal, W. Yuan, S. Li and S. C. Gupta, *Mol. Nutr. Food Res.*, 2013, **57**, 1529–1542.
21. S. P. Borse, D. P. Singh and M. Nivsarkar, *Porto Biomed. J.*, 2019, **4**, e15.
22. I. Chattopadhyay, K. Biswas, U. Bandyopadhyay and R. K. Banerjee, *Curr. Sci.*, 2004, **87**, 44.
23. A. Alok, I. D. Singh, S. Singh, M. Kishore and P. C. Jha, *J. Clin. Diagn. Res.*, 2015, **9**, ZE01.
24. D. Yadav, S. K. Yadav, R. K. Khar, M. Mujeeb and M. Akhtar, *Int. J. Green Pharm.*, 2013, **7**, 85.
25. P. Prehm, *Food Chem. Toxicol.*, 2013, **62**, 76.
26. M. L. van Iersel, J.-P. H. Ploemen, M. L. Bello, G. Federici and P. J. van Bladeren, *Chem.-Biol. Interact.*, 1997, **108**, 67.
27. L. P. Volak, S. Ghirmai and J. R. Cashman, *Drug Metab. Dispos.*, 2008, **36**, 1594.
28. V. Law, C. Knox, Y. Djoumbou, T. Jewison, A. C. Guo, Y. Liu, A. Maciejewski, D. Arndt, M. Wilson and V. Neveu, *Nucleic Acids Res.*, 2013, **42**, D1091.
29. Drug Bank, https://www.drugbank.ca/drugs/DB11672 (assesed on January 2020).
30. W. Zhang, T. M. C. Tan and L.-Y. Lim, *Drug Metab. Dispos.*, 2007, **35**, 110.
31. J. Adiwidjaja, A. J. McLachlan and A. V. Boddy, *Expert Opin. Drug Metab. Toxicol.*, 2017, **13**, 953.
32. C. Mancuso, T. E. Bates, D. A. Butterfield, S. Calafato, C. Cornelius, A. D. Lorenzo, A. T. Dinkova Kostova and V. Calabrese, *Expert Opin. Invest. Drugs*, 2007, **16**, 1921.
33. R. Hayeshi, I. Mutingwende, W. Mavengere, V. Masiyanise and S. Mukanganyama, *Food Chem. Toxicol.*, 2007, **45**, 286.
34. N. K. Basu, M. Ciotti, M. S. Hwang, L. Kole, P. S. Mitra, J. W. Cho and I. S. Owens, *J. Biol. Chem.*, 2004, **279**, 1429.

35. S. Oetari, M. Sudibyo, J. N. Commandeur, R. Samhoedi and N. P. Vermeulen, *Biochem. Pharmacol.*, 1996, **51**, 39.
36. R. Appiah-Opong, J. N. Commandeur, B. van Vugt-Lussenburg and N. P. Vermeulen, *Toxicology*, 2007, **235**, 83.
37. C. Mancuso and E. Barone, *Trends Pharmacol. Sci.*, 2009, **30**, 333.
38. S. Zhou, S. Y. Chan, B. C. Goh, E. Chan, W. Duan, M. Huang and H. L. McLeod, *Clin. Pharmacokinet.*, 2005, **44**, 279.
39. L. Hagvall, J. M. Baron, A. Börje, L. Weidolf, H. Merk and A.-T. Karlberg, *Toxicol. Appl. Pharmacol.*, 2008, **233**, 308.
40. A. Chadha and K. M. Madyastha, *Xenobiotica*, 1984, **14**, 365.
41. F. I. Al-Jenoobi, A. A. Al-Thukair, M. A. Alam, F. A. Abbas, A. M. Al-Mohizea, K. M. Alkharfy and S. A. Al-Suwayeh, *Eur. J. Drug Metab. Pharmacokinet.*, 2015, **40**, 61.
42. A. Jain, S. Doppalapudi, A. J. Domb and W. Khan, *J. Controlled Release*, 2016, **243**, 132.
43. S. Somasundaram, N. A. Edmund, D. T. Moore, G. W. Small, Y. Y. Shi and R. Z. Orlowski, *Cancer Res.*, 2002, **62**, 3868.
44. M. T. M. Lee and T. E. Klein, *J. Hum. Genet.*, 2013, **58**, 334.
45. C. Goodpasture and F. Arrighi, *Food Cosmet. Toxicol.*, 1976, **14**, 9.
46. J. Cao, L. Jia, H.-M. Zhou, Y. Liu and L.-F. Zhong, *Toxicol. Sci.*, 2006, **91**, 476.
47. N. T. Program, *Natl. Toxicol. Program Tech. Rep. Ser.*, 1993, **427**, 1.
48. S. T. Dance-Barnes, N. D. Kock, J. E. Moore, E. Y. Lin, L. J. Mosley, R. B. D'Agostino Jr, T. P. McCoy, A. J. Townsend and M. S. Miller, *Carcinogenesis*, 2010, **31**, 1903.
49. Y. Jiao, J. Wilkinson, X. Di, W. Wang, H. Hatcher, N. D. Kock, R. D'Agostino, M. A. Knovich, F. M. Torti and S. V. Torti, *Blood*, 2009, **113**, 462–469.
50. J. Xu and A. Hagler, *Molecules*, 2002, **7**, 566–600.
51. B. Patwardhan and U. Chandran, *Indian J. Tradit. Knowl.*, 2015, **14**, 574.
52. U. Chandran, N. Mehendale, G. Tillu and B. Patwardhan, *Comb. Chem. High Throughput Screening*, 2015, **18**, 846.
53. S. B. Shekarappa, S. Kandagalla and M. Hanumanthappa, *J. Proteins Proteomics*, 2019, 1.
54. Z. M. Buch, J. Joshi, A. Amonkar and A. B. Vaidya, *Clin. Cancer Invest. J.*, 2012, **1**, 45.
55. P. M. Reddi, *Adv. Anthropol.*, 2013, **3**, 91.
56. S. Dey and P. Pahwa, *J. Ayurveda Integr. Med.*, 2014, **5**, 15.
57. P. Govindaraj, S. Nizamuddin, A. Sharath, V. Jyothi, H. Rotti, R. Raval, J. Nayak, B. K. Bhat, B. Prasanna and P. Shintre, *Sci. Rep.*, 2015, **5**, 15786.
58. R. C. Juyal, S. Negi, P. Wakhode, S. Bhat, B. Bhat and B. Thelma, *PLoS One*, 2012, **7**, e45752.
59. Y. Ghodke, K. Joshi and B. Patwardhan, *Evid. base Compl. Alternative Med.*, 2011, **2011**, 1.
60. K. Patrick and M. B. Stanbrook, *Can. Med. Assoc.*, 2018, E1270.
61. D. A. Narayana and C. Katiyar, *J. Ayurveda Integr. Med.*, 2013, **4**, 245.
62. A. Bhatt, *Perspect. Clin. Res.*, 2016, **7**, 59.

63. Government of India, Ministry of Health and Family Welfare Gazette Notification G.S.R. 918(E).
64. G. Javir and K. Joshi, *3 Biotech*, 2019, **9**, 428.
65. P. Bagchi, M. Anuradha and A. Kar, *Int. J. Adv. Chem. Eng. Biol. Sci.*, 2017, **4**, 21.
66. M. Gore, P. Bagchi, N. Desai and A. Kar, *Int. J. Bioinf. Res.*, 2010, **2**, 33–37.
67. S. Borse, Trademark: Ayurnization (Application No.: 3281488-92; Class 3,5,9,16,42). 2016.
68. P. Rathaur, W. Raja, P. Ramteke and S. A. John, *Int. J. Pharm. Sci. Res.*, 2012, **3**, 1987.
69. S. Pal, Do-At-Home Tests to Check If Your Kitchen Ingredients Are Adulterated, Food-Information 12 December 2019, Available from: https://www.thebetterindia.com/114412/simple-home-tests-food-adulteration-kitchen-ingredients/.
70. FSSAI, *Quick Test for Some Adulterants in Food, Instructional Manual Part-I (Common Methods for Detection at Households)*, FSSAI, Goverment of India, New Dhelhi, 2012, pp. 01–23.
71. J. García-Lestón, J. Méndez, E. Pásaro and B. Laffon, *Environ. Int.*, 2010, **36**, 623.
72. S. Sen, P. S. Mohanty and V. Suneetha, *Res. J. Pharm. Technol.*, 2017, **10**, 3057.
73. J. Singh, D. E. Pritchard, D. L. Carlisle, J. A. Mclean, A. Montaser, J. M. Orenstein and S. R. Patierno, *Toxicol. Appl. Pharmacol.*, 1999, **161**, 240.
74. Q. Liu, D.-S. Dang, Y.-F. Chen, M. Yan, G.-B. Shi and Q.-C. Zhao, *Genet. Test. Mol. Biomarkers*, 2012, **16**, 1293.
75. A.-C. Liu, L.-X. Zhao, J. Xing, T. Liu, F.-Y. Du and H.-X. Lou, *Biol. Pharm. Bull.*, 2012, **35**, 145.
76. H. Juan, T. Jing, Y. Wan-Hua, S. Juan, L. Xiao-Lei and P. Wen-Xing, *J. Bioequivalence Bioavailability*, 2013, **5**, 236.
77. S. Ge, T. Yin, B. Xu, S. Gao and M. Hu, *Pharm. Res.*, 2016, **33**, 590.
78. R. Bahramsoltani, R. Rahimi and M. H. Farzaei, *J. Ethnopharmacol.*, 2017, **209**, 1.
79. X. Zhou, F. Zhang, C. Chen, Z. Guo, J. Liu, J. Yu, Y. Xu, D. Zhong and H. Jiang, *Xenobiotica*, 2017, **47**, 267.
80. S. Ganta, H. Devalapally and M. Amiji, *J. Pharm. Sci.*, 2010, **99**, 4630.
81. X. Sun, J. Li, C. Guo, H. Xing, J. Xu, Y. Wen, Z. Qiu, Q. Zhang, Y. Zheng and X. Chen, *Drug Metab. Pharmacokinet.*, 2016, **31**, 269.
82. A.-C. Liu, L.-X. Zhao and H.-X. Lou, *Planta Med.*, 2013, **79**, 971.
83. D. Chin, P. Huebbe, J. Frank, G. Rimbach and K. Pallauf, *Redox Biol.*, 2014, **2**, 563.
84. T. J. Smith and B. H. Ashar, *Cureus*, 2019, **11**, 1.

CHAPTER 14

Production, Economics and Marketing of Turmeric

KARTHIK VARMA*[a] AND SREERAJ GOPI*[a]

[a]R&D Centre, Aurea Biolabs (P) Ltd, Kolenchery, Cochin–682 311, Kerala, India
*E-mail: karthik.varma@plantlipids.com, sreerajgopi@yahoo.com

14.1 Introduction

Turmeric (*Curcuma longa* L.), belonging to the Zingiberaceae family, is regarded as a golden spice and is one of the most important commercial crops grown in India, Asia and many other countries. Turmeric is also referred to as 'Indian Saffron' as its history in India dates back nearly 5000 years.[1] This miraculous herb finds its application in many fields such as an ingredient in culinary, food stuffs, pharmaceuticals, nutraceuticals, cosmetic applications, *etc.* Turmeric is mostly cultivated in the tropical and subtropical regions around the world.[2] The origin of turmeric is reported to be of South East Asia. Other Asian countries that produce turmeric include Sri Lanka, Pakistan, Myanmar, Bangladesh, Taiwan, China and Indonesia.

Turmeric is also cultivated in countries like Vietnam, Nigeria and Latin American countries like Jamaica, Costa Rica, Haiti, Peru and Brazil, *etc.* Even though there are reports indicating the global scenario of turmeric production, many of them are contradictory. India accounts for about 80% of the global production of turmeric. The major importing countries include

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

the United States of America, Malaysia, United Kingdom, Germany, Japan, United Arab Emirates, Netherlands, Sri Lanka and Spain. The major products of economic importance include the dried rhizome, oleoresin, turmeric oil, curcuminoids powder and other nutraceutical formulations. Turmeric is one of the most potent herbs with a wide range of pharmacological activities evident in both traditional and most modern technologies. It has antioxidant,[3] anti-inflammatory,[4] anti-tumor,[5] anti-aging,[6] antimicrobial,[7] anticancer[8] properties *etc.* Turmeric products are used in food,[9] textiles,[10] drug manufacturing,[11] and medical[12,13] and cosmetic industries.[14]

This chapter presents an overview of turmeric production, marketing and economics by collecting the data available from the Spices Board, The Agricultural and Processed Food Products Export Development Authority (APEDA), Agri Exchange, Indian Council of Agricultural Research (ICAR) and other statistical data.

14.2 Production of Turmeric

The global and Indian scenario of production and marketing of turmeric is unsteady because of the seasonal variation in the crop and the price fluctuation in the market.

14.2.1 Global Scenario

The global market of turmeric is controlled by India. According to the present data available, India accounts for about 82% of the global production. Indian turmeric is known for its quality and medicinal properties. Other countries that produce turmeric include Pakistan, China, Myanmar, Indonesia, Nigeria, Ethiopia, Haiti, Jamaica, Peru, Taiwan and Thailand. Figure 14.1 illustrates the global production of turmeric.

Turmeric is also cultivated in Pakistan. The major area of cultivation includes the Kasur district of the Punjab province, where 80% of the country's production is concentrated. The other areas of production include Sindh and North Western Frontiers of Pakistan.

14.2.2 Indian Scenario

Turmeric is the most traded spice commodity from the Indian market. India holds about 80% of the turmeric production throughout the world. Indian turmeric, especially Alleppey turmeric, is of high economic importance in the US and EU markets on account of it having the highest curcuminoid content and volatile oil content. Turmeric is cultivated in India across 200 districts, which is spread across 18 districts. The top ten turmeric producing states in India, shown in Figure 14.2, include Telangana, Maharashtra, Andhra Pradesh, Karnataka, Gujarat, Tamil Nadu, West Bengal, Mizoram, Haryana and Assam. Telangana is the highest producing turmeric state in India.

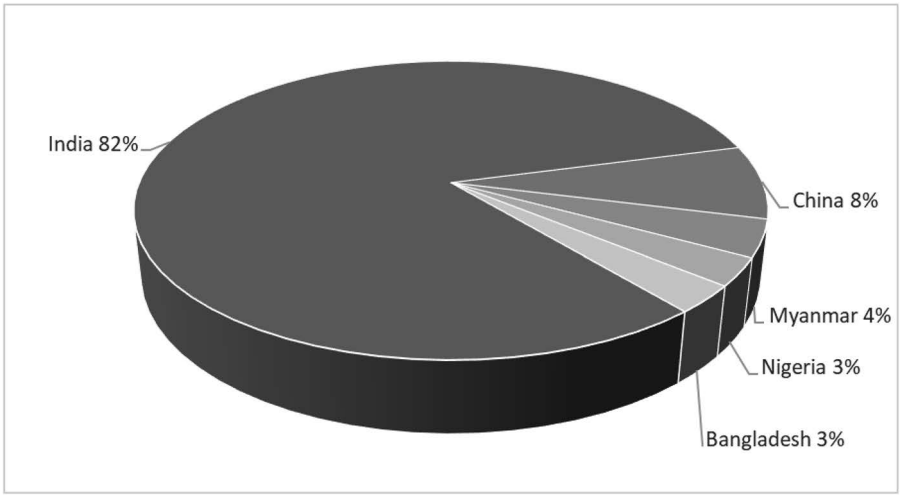


Figure 14.1 Global turmeric production.

14.3 Varieties of Turmeric

There are almost 30 plus varieties of turmeric, which are cultivated in India. But most of them, either because of low curcumin content or low yield, are not viable for commercial cultivation. The mean yield is mentioned in Ton/hectare (t ha^{-1}). Some of the improved varieties are given in Table 14.1. The yield of the fresh crops is 20–40 t ha^{-1} and the average maturity is around 7–9 months.[15]

14.4 State-wise Production

14.4.1 Telangana

Telangana holds the number one position for turmeric cultivation in India. The major cultivation areas in Telangana include Karimnagar, Nizamabad, Adilabad, Khammam, Warangal, Mahabooba Nagar, Rangareddy, Medak and Nalgonda. Of the different states, the majority of the cultivation is from Karim Nagar and Nizamabad. The total production of turmeric in Telangana contributes almost 27% of the country's production.

14.4.2 Maharashtra

The Sangli district in Maharashtra is well known for turmeric cultivation. The most commonly cultivated turmeric in Maharashtra is Rajapuri turmeric, which contributes 85% of the total. For the other 15%, Kadappa is 5%, Salem 5%, and rest are other varieties cultivated in India.

Figure 14.2 Spatial distribution of turmeric production in India across the states.

14.4.3 Other States

Turmeric is cultivated in most of the Indian states, increasing the annual production of the country's total production. Andhra Pradesh and Tamil Nadu also hold key positions among the top cultivators of turmeric in India. Erode, a district in Tamil Nadu, is the major turmeric cultivation area in the state. The other areas include Karur, Coimbatore, Salem, Dharmapuri and Krishangiri. The major areas of cultivation of turmeric in Karnataka include

Table 14.1 Different varieties of turmeric and their production yield.

Varieties of turmeric	Mean yield (fresh) t ha^{-1} (%)	Dry recovery (%)	Curcumin (%)	Oleoresin (%)	Oil (%)
Suvarna	17.4	20.00	4.3	13.50	7.00
Suguna	29.3	12.00	7.3	13.50	6.00
Sudarsana	28.8	12.00	5.3	15.00	7.00
IISR Prabha	37.5	19.50	6.5	15.00	6.50
IISR Prathibha	39.1	18.50	6.2	16.20	6.20
Roma	20.7	31.00	9.3	13.20	4.20
Suroma	20	26.00	9.3	13.10	4.40
Rajendra Sonia	4.8	18.00	8.4	—	5.00
Ranga	29	24.80	6.3	13.50	4.40
Rasmi	31.3	23.00	6.4	13.40	4.40
Alleppey	35.4	19.30	6	16.00	4.00
IISR Kedaram	34.5	18.90	5.5	13.6	3.00
Duggirala Red	32	17.6	4.5	14.6	4.00

Table 14.2 State-wise production of turmeric in India. Source: State Agri/Hort. Departments/DASS Kozhikode/Spices Board.

	2015–2016		2016–2017		2017–2018	
State	Area (hectares)	Production (tonnes)	Area (hectares)	Production (tonnes)	Area (hectares)	Production (tonnes)
Telangana	42 540	184 290	51 000	294 000	51 000	294 000
Maharashtra	10 710	177 850	14 050	224 680	14 050	224 680
Andhra Pradesh	17 020	121 120	19 180	79 730	19 180	79 730
Karnataka	14 990	76 490	19 330	114 510	14 990	76 490
Gujarat	3550	69 250	3710	73 150	3710	73 150
Tamil Nadu	29 880	115 350	35 800	129 560	16 190	57 150
Orissa	2480	30 000	27 860	54 500	27 860	54 500
West Bengal	16 710	42 410	18 000	45 500	18 000	45 500
Mizoram	7200	27 820	7480	28 890	7740	29 820
Haryana	1330	23 840	1500	22 000	1500	22 000
Assam	16 270	16 180	16 805	16 750	17 110	19 170
Total	183 480	967 060	1 215 520	224 260	224 260	1 107 920

Charmaraja Nagar, Mysore, Bagalkott, Belgaum and Bidar. Out of the 29 states in India, 18 of the states, even though in minor amounts, cultivate turmeric contributing to the dominant position of the country in turmeric cultivation. There has been an increasing trend in state-wise production. There are mainly two factors contributing to this, which include increase in the area of production and advanced production techniques. Data representing the state-wise production of turmeric are given in Table 14.2. In the past five years the trend for turmeric production is unsteady as is evident from the figures represented in Table 14.3.

Table 14.3 Year-wise production of turmeric in India. Source: State Agri/Hort. Departments/DASS Kozhikode/Spices Board.

Area in hectares, production in tonnes									
2013–2014		2014–2015		2015–2016		2016–2017		2017–2018[a]	
Area	Production	Area	Production	Area	Production	Area	Production	Area	Production
207 570	1 092 630	178 470	846 250	183 480	967 060	248 050	1 215 520	224 260	1 107 920

[a]Estimated Production.

Kumaran and Sankaran studied the factors responsible for the instability in turmeric production in India for the period 1970–1990. Yield instability was the dominant factor responsible for the instability in the production throughout this period.[16] In another study, data on the instability in the production of turmeric for south Indian states Andhra Pradesh, Tamil Nadu and Kerala were studied. The study was done on the basis of Hazel's decomposition model for analysis. In the study, the dominating factor responsible for the instability in production was an instability in the yield.[17]

14.5 Economics, Factors and Trends in Turmeric Production

14.5.1 Economics of Turmeric Production

The cost of production can be divided into two parts, which include total variable cost and total fixed cost. There are several parameters that account for the total variable cost, which include seed cost, farm yard manure, chemical fertilizers, plant protection chemicals, hired human labor and interest on working capital. These parameters mainly contribute to per kilogram cost of the dried rhizome. All of these parameters are included in the head of variable cost, since these parameters may change from year to year. Table 14.4 represents the contributing factors and their percentage to the cultivation price and pattern.

14.5.2 Constraints for Turmeric Production

From ancient times onwards, turmeric has played a predominant role in the spice domain of India and this is evident from the global percentage contribution of the crop from India. India always dominates the turmeric market in terms of quality and quantity. The increase in the area of production

Table 14.4 Factors contributing the production of turmeric.

Item	Price (rs/ha)	Contribution to percentage (%)
Seed/Rhizome (kg)	82 800.00	26.78
Hired human labor (individual/days)	29 450.00	9.53
Bullock pair (days)	7605.00	2.46
Machine labor (h)	4008.00	1.30
Fertilizer/manure (kg)	13 500.00	2.67
Plant protection chemicals	2870.00	0.93
Water/irrigation (h)	7510.00	0.04
Incidental expenditure	280.85	0.09
Interest on working capital (@13%)	19 285.00	6.24
Depreciation on assets	418.34	0.13
Land value	123 074.69	39.81
Fixed Capita interest	460.18	0.15
Family Labor	17 698.00	5.73

and use of new productive technologies has led to the increased productivity of turmeric. There are various factors that affect production including climatic conditions, harvesting technologies, crop diseases including pests and insects and labor shortage. Inadequate and increased rainfall can lead to substantial yield loss to the crop. Crop diseases also play a predominant role in affecting the crop yield. Crop diseases that kill the whole plant include rhizome rot and those affecting the aerial root include leaf blotch and leaf spots. Rhizome rot disease was first reported in Ceylon park[18] and in India the disease was first reported from Andhra Pradesh.[19] The organism responsible for the disease is *Pytium aphandermatum*. The organism is susceptible to all soil conditions irrespective of the pH and fertility of the soil. The disease can be controlled by using different good agricultural practices like the use of healthy rhizomes, phyto sanitation, crop rotation, chemical control, host resistance, *etc.*[20–22] Leaf blotch disease is characterized by the appearance of small yellow spots with a diameter 1 mm to 2 mm and on subsequent propagation leading to death of the crop.[23] Another disease affecting the crop is leaf spot, which occurs when there is high humidity in the atmosphere. But the diseases are also reported in normal conditions, though in a small amount. The pathogen responsible for the disease in leaf spot disease is *C. capsici.* The pathogen initially attacks the leaves from where the disease is further confined to the leaf blades and leaf sheaths also. The disease can be controlled by using proper sanitation techniques, host resistance and proper shade for turmeric plantations. Nematode infestations also affect the production yield of turmeric cultivation. Pest insects also lead to crop yield reduction. The major insect pests include shoot borer and rhizome scale. Shoot borer infection was first reported in India in 1914.[24] The pathogen scrapes the newly formed leaf and consequently spreads, which results in yellowing and drying of the central shoot. The infection can be controlled by using biopesticides,[25] plant-based products[26–28] and sex pheromones.[29,30] Turmeric cultivations in tropical regions in Asia, Africa, Central America and Caribbean Islands are mostly affected by rhizome scale disease. Even though there are reports for the disease for turmeric cultivations in India, the data are limited. The infection affects turmeric under cultivation and harvested materials also. The disease is most commonly seen in crops in the final stages and, depending on the severity, the stored buds and rhizomes are greatly affected.

Minor insects can also lead to crop damage, which can result in the poor yield/quality of the turmeric rhizome. The SAP feeder insects, which attack the turmeric crop, include *Stepanitis Typica, Panchaetothrips Indicus, Anapothrips Sudanensis, Haplothrips spp, Planococus spp, Pseudococus spp, Howardia biclavus and Hemiberlesia palmae*.

The common leaf feeding insects that attack the turmeric plant include grass hoppers, leaf feeding caterpillars *viz*, turmeric skipper.[31] There are many species of leaf feeding caterpillars that have the ability to attack the turmeric crop in a minor and severe way resulting in crop damage.[32] Madan *et al.* (2002) studied the production and post-harvest problems for the cultivation of important crops such as chilies and turmeric across the state of Andhra Pradesh. The result of the study concluded that a lack of scientific

knowledge on post-harvesting technologies and fluctuations in the price was reported as the major constraint for turmeric production.[33]

14.5.3 Trend in Turmeric Production

With the increase in production areas and improvement in production techniques there has been an increase in turmeric production in India. Indian turmeric always has a market demand on account of its high curcuminoid content and other quality parameters.

14.6 Marketing

14.6.1 Products of Commercial Importance

The value of Indian turmeric is always on the high side owing to the quality and quantity of the herb. India is the key producer and major exporter throughout the world. Indian turmeric is known for its high curcuminoid and volatile content. The products of marketing importance include fresh rhizomes, dried rhizomes, oleoresins, essential oil and nutraceutical supplements. In addition, turmeric powder is also included in cosmetic products, beverage products and curry powder blends. The demand for turmeric rhizomes and other value-added products based on organic cultivation is increasing on account of the absence of pesticides and other heavy metal residues in the products. The marketing structure of turmeric and turmeric-based products is described in Figure 14.3.

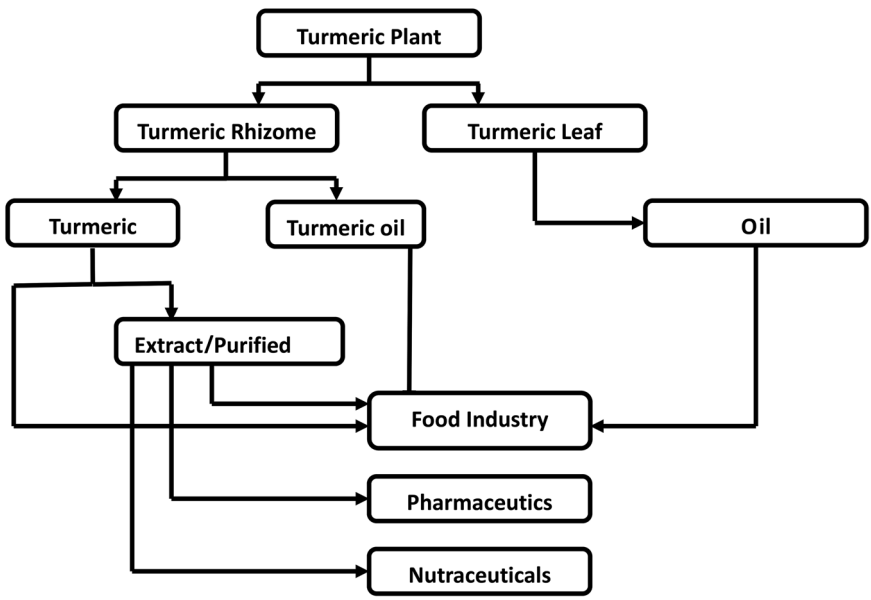


Figure 14.3 Marketing structure of turmeric products in industry.

14.6.2 Organic Turmeric

Turmeric rhizomes cultivated purely based on organic cultural practices are fast gaining importance in the export markets. The stringent regulations from the governing regulators have led customers to procure organic turmeric. Organic turmeric is supposed to be free from contaminants such as pesticide residues and heavy metals.

14.6.3 Fresh Rhizome

Fresh rhizomes are also exported to many countries for taking turmeric juice. Turmeric juice finds its application in many nutraceutical beverages.

14.6.4 Dried Rhizome

The end usage application for dried turmeric rhizomes varies from extraction of oleoresin, distillation of volatile oils and even as turmeric powders. Dried turmeric powder is normally sieved through 60–80 mesh to be of uniform particle size. Turmeric powder finds its use in the food industry as a coloring and flavoring agent. Dried turmeric powder is also used in various curry powder blends.

14.6.5 Turmeric Oleoresin/Oil

One of the most important traded products from turmeric includes oleoresin, which is a combination of both oil and resin. Turmeric oleoresin is obtained by solvent extraction of dried rhizomes.[34] Normal oleoresin contains 30–35% curcuminoids. Based on the end use application, the curcuminoid content can be adjusted to different concentrations and different forms by adding permitted food additives. Usually, for commercial and end use application nonvolatile additives such as edible oils, propylene glycol, polysorbate, glyceryl mono esters, *etc.* are added to make the product either water soluble or oil soluble depending upon the consumer requirement.[35] Food additives are added in permitted levels either to adjust the curcuminoid content or to increase the water/oil solubility.

14.6.6 Turmeric Oil

The dried rhizome of turmeric contains 3–7% volatile oil depending upon the variety. The leaves also yield volatile oil. Turmeric oil finds its use in aromatherapy, perfumery and cosmetic applications, *etc.* The major constituents of turmeric oil include sesquiterpenes such as turmerone, bisabolene and curdione, *etc.* Turmeric oil is also used in mosquito repellant applications.[36]

14.6.7 Curcumin 95%

Curcumin 95% is obtained by the purification of turmeric oleoresin. Curcumin 95% occupies a dominant position in the traded commodities from turmeric. It is also traded by other names such as curcumin crystals, curcumin dye and curcuminoids powder. Curcumin 95% is used in cosmetic, nutraceutical, food and beverage applications. Traders also focus on exporting curcumin to blend with other phytochemicals and additives for delivery in various forms.

14.6.8 Bioavailable Curcuminoids

The trend for natural products has always triggered scientists to deliver products that come under the category of nutraceuticals with attributed functional properties. The low bioavailability of curcuminoids 95% has led researchers to deliver bioavailable curcuminoids.[37] Most of the bioavailable curcumin brands have been well studied and characterized.[38] There are different innovative formulas developed by various organizations to deliver bioavailable curcuminoids in different forms. Most of the bioavailable curcuminoid brands are marketed with enormous clinical studies such as bioavailability,[39] comparisons with competitors[40] and clinical indications, *etc.*[41–47]

14.6.9 Encapsulated Products

With the growing demand in the consumer market, there are several products that are encapsulated with specific carriers for delivery in powder form. Turmeric oil is usually encapsulated with starch-based carriers for delivery in powder form thereby extending the umbrella of application.

14.6.10 Value-added Products

On account of the miraculous pharmacological activities of turmeric, the trend of research concentrates in the field of delivering value-added nutraceutical products with biological activities. Dietary fiber isolates and composites are also traded in nutraceutical categories as natural drug delivery carriers.[48,49] Curcumin finds its application in many of the dietary supplements traded from India. Curcumin capsules are also traded from India and are supplied to many countries and also domestically.

14.6.11 Turmeric and Ayurveda

India is always regarded as the pioneer in the field of traditional systems of medicines like Ayurveda, where turmeric is known as ‘haridra’. In Ayurveda, haridra is referred as rasayana. There are four different varieties

of haridra mentioned in Bhavaprakasa *viz*; haridra, wana haridra, karpoora haridra and daru haridra. In Ayurvedic practices of medicine, the various forms of turmeric used are dried rhizome, raw juice and combination drugs. The group of drugs that use turmeric in Ayurveda include churnam, gritham, kashayam, lehyam and thailam; all of these find a wide variety of pharmacological activities. The boom of good ayurvedic practitioners and the efficacy of ayurvedic drugs will always draw the attention of consumers who seek traditional systems of medicine for the treatment of various diseases.

14.7 Export and Import Scenario

Indian turmeric demand in the export market does play a significant role in turmeric trading. There has been always an increase in demand for Indian turmeric owing to its features of superior quality. The high curcuminoid content is the dominant factor that contributes to the supremacy of Indian turmeric throughout the world.

14.7.1 Factors Dominating the Trade/export

Spices have always dominated the Indian economy from ancient times onwards. Turmeric occupies a dominant role in the spices exported from India. Indian turmeric has always been known for its quality. There is an increasing demand for turmeric and turmeric-based products in the global market. The global turmeric market is always controlled by India. Eighty percent of the global production is controlled by India on account of the quality and quantity of the turmeric. Reports from the Spices Board indicate that throughout the years, the demand for turmeric is always on the high side. The market price of any raw material that is used for trading is governed by the quality of the raw material.

The National Commodity Stock Exchange Ltd. has set up some quality parameters for procuring and trading turmeric. The phytochemical constituents play a predominant role in determining the price of turmeric. The important phytochemical constituents include curcuminoids and volatile oil. The phytoconstituents have a dominating role in determining the price of the raw material. Curcuminoid content in the raw material varies from 2–8% depending on the variety. The other quality parameters include:

(1) Length – at least 75% turmeric should be more than 3 cm in length.
(2) Moisture should be not more than 12%.
(3) Turmeric bulbs should be more than 3%.
(4) Should be free from fungus and not be artificially colored with dyes or chemicals.

Exporting countries have also started fixing stringent quality standards regarding the maximum residual limits of impurities such as heavy metals, mycotoxins, plasticizers, pesticides and other contaminants that have an adverse effect on health.

14.7.2 Export Market

Turmeric and turmeric-based products find a top position among the list of spices exported from India. As per the data available from the Spices Board of India, the major exporting destination of Indian turmeric is Iran followed by other destinations such as the US and EU markets (see Table 14.5).

14.7.3 Import

Even though turmeric is exported from India, the country also trades turmeric from different locations. The major countries of import of turmeric to India include Myanmar, Ethiopia and Indonesia. The demand for turmeric and turmeric-based products from industries have led suppliers to import turmeric from different locations. The spice industries always look for the quality parameters of the traded turmeric, which include curcuminoids, microbial contaminations and mycotoxins. There is a steady increase in the import market scenario also, as the demand for the curcumin products is high.

Table 14.5 Country-wise export of turmeric (QTY in metric tonnes, value in US $). Source: Spices Board of India: http://Indianspices.com/sites/default/files/MajorItemCountrywiseexportofSpices2016web1412018_new.pdf.

	2015–16		2016–17		2017–18	
Country	Quantity (metric tonnes)	Value ($)	Quantity (metric tonnes)	Value ($)	Quantity (metric tonnes)	Value ($)
USA	5543.95	9388.53	6830.39	11 410.39	6434.75	11 158.83
Iran	13 141.09	11 975.44	14 862.4	13 575.5	13 431.4	10 290.83
UAE	5905.65	5298.46	8195.76	7645.26	7952.3	6548.91
Malaysia	6375.79	6593.1	6249.37	6408.74	6562.12	6104.92
Morocco	2294.14	2027.27	5271.3	4655.87	6220.14	4911.81
UK	3935	4410.65	3892.94	5473	4417.28	4878.4
Sri Lanka	4634.09	4337.24	4758.18	4482.59	5252.54	4462.32
Germany	2450.9	3145.06	2838.15	4231.07	2872.23	3644.75
Japan	2531.13	3428.39	2421.28	3734.44	2835.11	3392.81
Saudi Arabia	4105.28	4196.04	5257.16	5036.65	4006.5	3379.36
Netherlands	2605.61	3200.18	2337.78	3087.44	2897.17	3336.95
Bangladesh	4802.8	2478.43	12 772.51	10 889.55	4276.8	3190.27
South Africa	2228.69	2444.97	2617.43	3172.24	2735.78	2812.19

14.8 Market Structure

The market structure from the farmer to consumers is shown in Figure 14.4. The root of the market structure is the farmer and finally leads to the consumer through different channels.

The price of turmeric before reaching consumers is predominantly controlled by the mediators in the chain. In India there are many assembling and trade centers for the marketing of turmeric crops. The major collection centers and trading points for turmeric in India are given in Table 14.6.

14.9 Marketing Prospects

The demand for natural products is always high. The low risk factor when compared to synthetic counterparts is the main reason for the increasing demand. Turmeric with a solid scientific and clinical background dominates among the spices/natural products of interest. Turmeric and turmeric-based products have always been studied by scientific communities for different clinical conditions. Innovative products from the scientific communities

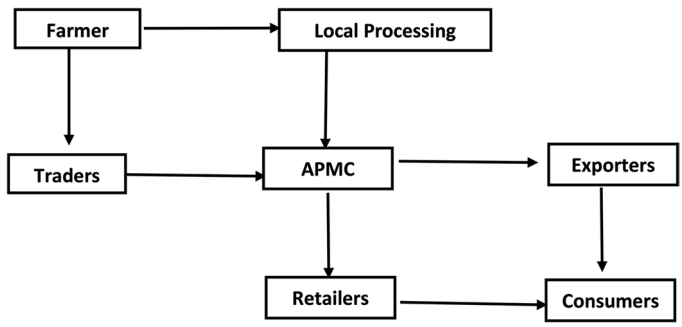


Figure 14.4 Market structure of the turmeric supply chain in India.

Table 14.6 Collection points of turmeric across different states in India. Source: Agricultural Marketing (AGMARK).

States	District
Andhra Pradesh and Telangana	East Godavari, Guntur, Karimnagar, Medak, Rangareddy, Srikakulam, Vizianagaram, Vishakhapatnam, Warangal, West Godavari
Assam	Cachar, Kamrup
Bihar	Samastipur
Gujarat	Surat, Kheda, Anand, Sabarkantha
Maharashtra	Chandrapur, Hingoli, Sangli, Satara,
Kerala	Wayanad, Palakkad, Idukki, Kollam, Kozhikode
Tamil Nadu	Karur, Villupuram, Coimbatore, Salem, Dharmapuri, Krishangiri, Erode
Orissa	Kalahandi, Koraput, Mayurbhanj

have led marketing and company manufacturers to invest more into research-based activities. If supplied with a solid scientific background, consumers will be drawn to the natural product market.

Medicines that make use of traditional systems of medicines like Ayurveda use turmeric as such and as a combination of different drugs to deliver value-added products. Ayurvedic drugs are gaining more market value on account of their efficacy and attributed health benefits. Indian turmeric and turmeric-based products always occupy a dominant position in the world on account of both quality and quantity.

14.10 Risks and Uncertainty

The production of natural products always faces the risk of low yield, susceptibility of crops to plant diseases, climatic conditions, *etc.* The vulnerability of natural products to plant diseases greatly contributes to the low yield/quality of turmeric. If a one-year crop results in a loss to the farmer, the chance of investment on a high level is always difficult. This uneven trend can lead to low production. Climatic conditions are also one of the risk factors that contribute to the production of outcome and price index of turmeric. High rainfall can lead to crop damage, which will affect the current season and the upcoming season. A recent study conducted by the Indian Institute of Spices Research has found that there was nearly a loss of 976 tons of turmeric because of the flood in the state of Kerala in 2018.

The price of the turmeric market highly fluctuates on account of the demand for products. The US and EU market trend is always on the procurement of new, value-added products, which may lead to the low procurement rate of conventional products such as dried rhizome and volatile oils. Companies are always focusing on the development of new and new value-added products from turmeric.

14.11 Conclusion

Turmeric is the most important commercial crop cultivated in India. Most modern farming techniques needs to be adopted for increasing the yield and recovery of active components. Continuous research activities have to be focused to deliver more and more value-added products from the herb. Price fluctuation needs to be controlled to deliver a marginal amount to the customer to promote good farming techniques.

References

1. T. Esatbeyoglu, P. Huebbe, M. A. Insa, E. Dawn Chin, A. E. Wagner and G. Rimbach, *Angew. Chem., Int. Ed.*, 2012, **51**, 5308.
2. M. Parmasivam, R. Poi, H. Banerjee and A. Bandyopadhyay, *Food Chem.*, 2009, **113**, 640.

3. R. W. Kalpravidh, N. Siritanaratkul, P. Insain, R. Charoensakdi, N. Panichkul, S. Hatairakhtam, S. Srichairatanakool, C. Phisalaphong, E. Rachmilewitz and S. Fucharoen, *Clin. Biochem.*, 2010, **43**, 424.
4. L. Peret-Almeida, A. P. F. Cherubino, R. J. Alves, L. Dufosse and M. B. A. Gloria, *Food Res. Int.*, 2005, **38**, 1039.
5. M. A. Khan, R. El-Khatib, K. D. Rainsford and M. W. Whitehouse, *Bioorg. Chem.*, 2012, **40**, 30.
6. Y. Panahi, A. Saadat, F. Beiraghdar, S. M. H. Nouzari, H. R. Jalalian and A. Sahebkar, *J. Funct. Foods*, 2014, **6**, 615.
7. P. Y. Zhan, X. H Zeng, H. M. Zhang and H. H. Li, *Food Chem.*, 2011, **129**, 700.
8. V. Basile, E. Ferrari, S. Lazzari, S. Belluti, F. Pignedoli and C. Imbriano, *Biochem. Pharmacol.*, 2009, **78**, 1305.
9. A. M. Serpa Guerra, C. Gomez Hoyos, J. A. Velasquez-Cock, L. Velez Acosta, P. Ganan Rojo, A. M. Velasquez Girdalo and R. Zuluaga Gallego, *Crit. Rev. Food Sci. Nutr.*, 2019, **24**, 1.
10. S. Umbreen, S. Ali and T. Hussain, *Res. J. Text. Apparel*, 2008, **12**, 1.
11. P. Lu, Q. Tong, F. Jiang, L. Zheng, F. Chen, F. Zeng, J. Dong and Y. Du, *J. Huazhong Univ. Sci. Technol., Med. Sci.*, 2005, **25**, 668.
12. A. Mukerjee and J. K. Vishwanatha, *Anticancer Res.*, 2009, **29**, 3867.
13. T. Perko, M. Ravber, Z. Knez and M. Skerget, *J. Supercrit. Fluids*, 2015, **103**, 48.
14. B. Sasikumar, J. K. George, T. J. Zachariah, M. J. Ratnambal, K. N. Babu and P. N. Ravindran, *J. Spices Aromat. Crops*, 1996, **5**, 41.
15. D. Chanchal and S. Swarnalata, *J. Cosmet., Dermatol.*, 2008, **7**, 89.
16. N. A. Kumar and P. G. Sankaran, *J. Spices Aromat. Crops*, 1998, **7**, 19.
17. S. Angels and S. B. Hosamani, *Madras Agric. J.*, 2005, **92**, 271.
18. M. Park, Ceylon Admn. *Rep. Dir. Agric.*, 1934, p. D124.
19. T. S. Ramakrishnan and C. R. Soumini, *Indian Phytopathol.*, 1954, **7**, 152.
20. S. P. Geetha, In Vitro Technology for the Genetic Conservation of Some Genera of Zingiberaceae, PhD, thesis, Calicut University, Calicut, India, 2006, pp. 169–194.
21. K. Nirmal Babu, D. Minoo, S. P. Geetha and V. N. Jayakumar, *Advances in Spices Research,* ed. P. N. Ravindran, K. Nirmal Babu, K. N. Shiva and J. A. Kallupurackal, Agrobios Publications, Jodhpur, India, 2006, vol. 6.
22. R. J. Anandan, A. S. Rao and K. V. Babu, Studies on rhizome rot of turmeric (*Curcuma longa* L.), *Indian Cocoa Arecanut Spices J.*, 1996, **20**, 17–20.
23. T. G. N. Rao, *J. Spices Aromat. Crops*, 1995, **4**, 49–56.
24. S. Devasahayam, *Biological Control in Spices,* ed. M. Anandaraj and K. V. Peter, Indian Institute of Spices Research, Calicut, India, 1996, pp. 34–35.
25. IISR, *Annual Report: 2002–03*, Indian Institute of Spices Research, Calicut, Kerala, 2003.
26. IISR, *Research Highlights: 2002–03*, Indian Institute of Spices Research, Calicut, Kerala, 2003.
27. IISR, *Annual Report, 2003–2004*, Indian Institute of Spices Research, Calicut, Kerala, 2004.
28. T. Kimura and H. Honda, *Appl. Entomol. Zool.*, 1999, **34**, 147.
29. R. X. Cai and Z. L. Mu, *China Citrus*, 1993, **22**, 33.

30. A. K. Chakravarthy and N. E. Thyagaraj, *Pest Manage. Trop. Ecosyst.*, 1998, **4**, 78.
31. Y. K. Kotikal and K. A. Kulkarni, *J. Agric. Sci.*, 2000, **13**, 858.
32. M. S. Madan, Y. R. Sarma and J. Nagendra, Spices production and prospects in Andhra Pradesh, *Indian J. Arecanut, Spices Med. Plants*, 2002, **4**(1), 42–50.
33. N. Krishnamurthy, A. G. Mathew, E. S. Nambudiri, S. Shivashankar, Y. S. Lewis and C. P. Natarajan, *Trop. Sci.*, 1976, **18**, 37.
34. J. W. Purseglove, E. G. Brown, C. L. Green and S. R. J. Robbins, *Turmeric-The Genus Curcuma*, ed. P. N. Ravindran, K. Nirmal Babu and K. Sivaraman, CRC Press, Finland, 1st edn, 2007, vol. 1, ch. 2, pp. 532–580.
35. K. A. Saju, M. N. Venugopal and M. J. Mathew, *Curr. Sci.*, 1998, **75**, 650.
36. S. Prasad, A. K. Tyagi and B. B. Aggarwal, *Cancer Res. Treat.*, 2014, **46**, 2.
37. A. Amalraj, A. Pius, S. Gopi and S. Gopi, *J. Tradit. Complementary Med.*, 2017, **7**, 205.
38. S. Gopi, R. George, M. Thomas and S. Jude, *Asian J. Pharm. Technol. Innovation*, 2015, **3**, 92.
39. S. Gopi, R. George and V. T. Sriram, *Asian J. Pharm. Technol. Innovation*, 2014, **2**, 123.
40. S. Gopi, R. George, S. Jude and V. T. Sriraam, *J. Chem. Pharm. Res.*, 2014, **6**, 96.
41. S. Gopi, R. George and V. T. Sriraam, *Br. Biomed. Bull.*, 2014, **2**, 545.
42. S. Gopi, R. George and V. T. Sriraam, *Int. J. Curr. Res.*, 2014, **6**, 8473.
43. A. Amalraj, K. Varma, J. Jacob, C. Divya, A. B. Kunnumakkara, S. J. Stohs and S. Gopi, *J. Med. Food*, 2017, **20**, 1022.
44. A. Amalraj, S. Jude, K. Varma, J. Jacob, S. Gopi, O. S. Oluwafemi and S. Thomas, *Mater. Sci. Eng., C*, 2017, **75**, 359.
45. S. Gopi, J. Jacob, K. Varma, S. Jude, A. Amalraj, C. A. Arundathy, R. George, T. R. Sreeraj, C. Divya, A. B. Kunnumakkara and S. J. Stohs, *Phytother. Res.*, 2017, **31**, 1883.
46. S. Jude, A. Amalraj, A. B. Kunnumakkara, C. Divya, B. M. Löffler and S. Gopi, *Molecules*, 2018, **23**, 2415.
47. S. Gopi, A. Amalraj, S. Jude, K. Varma, T. R. Sreeraj, J. T. Haponiuk and S. Thomas, *Mater. Sci. Eng., C*, 2017, **81**, 20.
48. S. Gopi, A. Amalraj, K. Varma, S. Jude, P. B. Reddy, C. Divya, J. T. Haponiuk and S. Thomas, *Int. J. Polym. Mater. Polym. Biomater.*, 2019, **67**, 581.
49. S. Gopi, A. Amalraj, J. Jacob, N. Kalarikkal, S. Thomas and Q. Guo, *New J. Chem.*, 2018, **42**, 5117.

CHAPTER 15

Nanodrug Delivery Formulations for Curcumin Absorption

YASAMIN DAVATGARAN TAGHIPOUR[a], HADI SAMADIAN[b] AND MOHAMMAD HOSEIN FARZAEI*[b]

[a]Department of Medical Nanotechnology, School of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran; [b]Pharmaceutical Sciences Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
*E-mail: mh.farzaei@gmail.com

15.1 Introduction

Nanotechnology is defined as the study of phenomena and fine-tuning of materials at the atomic, molecular and macromolecular scale, where properties differ significantly from those at a larger scale. Nanotechnology as an enabling technology offers a great number of advantages such as manipulation of materials and structures at molecular and submolecular levels with atomic precision, and materials and structures with improved mechanical, physical, chemical and optical properties. Moreover, nanotechnology and nanomaterials are able to improve current and conventional procedures, technologies and materials. There are many consumer products based on nanotechnology on the market and these are increasing at the rate of three to four per week.[1–3]

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

From the material sciences point of view, materials exhibit different properties than bulk states at the nanoscale. Once the dimensions of specific materials decrease, subsequently the specific surface area and the number of surface atoms increase exponentially. As the various properties of materials such as thermal, optical, electrical, mechanical and chemical depend on these surface atoms, this change in the dimensions would affect the characteristics of the materials. This phenomenon is prominent when the dimensions reach the nanoscale area, around 100 nm.[4–6] From the biomedical point of view, due to the nanometric scales of cellular and subcellular structures, nanomaterials can effectively interact with these structures resulting in the desired effects. In this regard, different kinds of nanostructures have been developed for various biomedical applications, for instance, polymeric, metallic, lipid-based, and carbon-based nanoparticles, nanorods, nanofibers and porous structures.[7]

Moreover, nanomaterials can effectively encapsulate sensitive bioactive molecules, low soluble active agents, and even perilous anticancer drugs to protect the sensitive bioactive molecules, improve the solubility, and protect the healthy cells from anticancer drugs, respectively. There are several nanomaterials-based products on the market developed for drug delivery systems. When it comes to curcumin, its low solubility and instability in biological fluid have to be considered. As a genius solution, nanotechnology comes into play to improve its low solubility, biodistribution, pharmacokinetics and stability in the body. Moreover, curcumin-loaded nanomaterials have the additional advantage of being targeted toward various cells, tissues and organs.

15.2 Nanotechnology Approaches to Overcome Curcumin's Inherent Constraints

Curcumin is a miracle from nature due to its brilliant biological properties such as antioxidant, anti-arthritic, antibacterial, antidiabetic, anticancer, antiviral, anti-inflammatory and antiprotozoal activities. Curcumin has been found to be effective for treating heart attacks, gastrointestinal problems, ocular perceiver infections, Alzheimer's disease, hypoglycemia, liver cirrhosis, depression, menstrual problems, worms, swelling, leprosy, headaches, cancers, kidney problems and fibromyalgia. Although it has outstanding biological properties and promising potential applications, it suffers from poor bioavailability due to its poor absorption, rapid excretion from the body, short half-life, antithrombotic activity that can interfere with blood clotting and poor solubility in aqueous solutions.[8–10]

The recent and ongoing trends in curcumin research have aimed to surmount the inherent constraints of the native curcumin and improve its solubility, stability and bioavailability. Curcumin encapsulation into the proper nanostructures will protect it from hydrolytic and enzymatic degradation. Moreover, nanostructures with suitable surface properties can enhance the

circulation time in the body, which improve bioavailability and the biodistribution of the formulation. Nanoformulations as a 'magic bullet' not only fulfill the aforementioned requirements but also provide the targeting ability of curcumin to the desired biological targets. Nanoformulations can reach the target site *via* passive and active targeting approaches. Passive targeting is based on either the tailoring of the nanoformulation size to localize into the tissues, surface coating to escape from the immune system, and provide long circulation time. On the other hand, active targeting utilizes the targeting ligands to orient the nanoformulation toward the receptors or other surface membrane proteins overexpressed on the target cells.[11–14]

Nanomaterials have a high specific surface area with plenty of functional groups that provide a platform to apply a proper surface coating such as polyethylene glycol (PEG) and an adequate amount of the desired targeting moieties such as small molecules, antibodies and aptamers. Many different kinds of nanomaterials have been developed to overcome the drawbacks of native curcumin; for example, polymer-based, lipid-based, carbon-based and metal-based nanostructures.[15,16]

15.3 Curcumin Nanoformulations

15.3.1 Lipid-based Nanoformulations

Lipidic systems are attractive for drug delivery applications due to their ability and affinity to fuse with cell membrane lipids and to release their encapsulated drugs into the cells. Moreover, the nanometric dimension of lipid-based nanoformulations is another parameter that favors cell internalization. This class of nanomaterials has the ability to be specifically targeted toward various organs/tissues/systems such as the brain, kidney, liver, skin and lung. Generally, lipid-based nanoformulations are composed of phospholipids, cholesterol, triglycerides and sometimes free fatty acids and bile salts.[15,17] A number of these lipids, such as glycerol monooleate, phosphatidylcholine, cholesterol and stearic acid have well-established safety profiles with appropriate toxicological data and have been used in FDA-approved pharmaceutical applications. Lipid-based nanomaterials are widely used to improve the bioavailability of low soluble, sensitive, and even dangerous substances such as anticancer drugs, antibiotics and natural extracts. Accordingly, various types of lipid-based nanomaterials have been studied for drug delivery applications but liposomes and solid lipid nanoparticles (SLNs) are extensively evaluated for curcumin delivery applications.[18,19]

15.3.1.1 Liposomes

Liposomes are bilayers or multilayer vesicles consisting of phospholipids and cholesterol, which form a lipid bilayer that surrounds an aqueous compartment. The hydrophilic drug is trapped in the core and aqueous part of

the liposome while the lipophilic drug is entrapped within the phospholipid bilayer of the liposome. The drug release from the liposome occurs at the intestinal membrane surface. Liposomes exhibit various advantages such as enhanced efficacy, therapeutic index, stability, low toxicity, biocompatibility and non-immunogenicity. On the other hand, liposomes have a few disadvantages including short half-life and low stability in the body, which can be eliminated with liposomes' surface functionalization.[20]

Various curcumin-loaded liposomes have been developed in recent years (Table 15.1) and a few studies are highlighted. In a study, Chen *et al.* synthesized pH-sensitive calcium carbonate ($CaCO_3$) encapsulated liposomes *via* water in oil (W/O) emulsion for efficient delivery of curcumin to a colon tumor. $CaCO_3$ possesses innate pH sensitivity that has been used for drug delivery systems. This component incorporates into the nanostructures and once it reaches the endosome/lysosome breaks down, which can result in the dissociation of nanoparticles and release of the encapsulated drugs. Also, dissociation of $CaCO_3$ releases a large quantity of Ca^{2+} that can enhance osmotic pressure, which results in the release of the cargo. The efficacy of the prepared liposomes was evaluated *in vitro* and *in vivo* on an HCT-116 human colon carcinoma cell line and azoxymethane (AOM) dextran sodium sulfate (DSS)-induced colorectal cancer, respectively. The results showed that the fabricated liposomes have an average size of 155 nm. The cellular uptake and the lysosome escape of the liposomes were observed by confocal microscopy, which confirmed the efficacy of the nanoformulation as an efficient curcumin delivery system. This study proposed an efficient curcumin delivery system stable in the neutral pH of the blood that releases the drug into the cytosol at the acidic pH of the endosome/lysosome.[21]

In another study, Fernàndez-Busquets *et al.* developed liposomes-incorporated curcumin for the treatment of malaria disease. Curcumin possesses antimalarial activity and inhibits *in vitro* growth of *Plasmodium falciparum* in a dose-dependent manner. The surface of the liposomes was functionalized with methacrylic acid copolymer Eudragit S100 and hyaluronan for protection against gastric alkaline pH. Furthermore, the liposomes were combined with dextrin Nutriose FM06 instead of hyaluronan to compare the efficacy of the prepared liposomes. Eudragit-hyaluronan and Eudragit-nutriosomes liposomes were administered orally on mice that were infected with the murine malaria *parasites Plasmodium yoelii yoelii* 17XL. The results demonstrated that only Eudragit-nutriosomes liposomes loaded with curcumin-enhanced antimalarial activity improved the survival of the infected animals. On the other hand, the Eudragit-hyaluronan liposomes did not prolong the survival of mice compared with the control group due to low stability after administration.[22]

In another research program by Xu *et al.*, arginine, glycine, aspartic acid peptide (RGD)-modified liposomes loaded with paclitaxel and curcumin were developed for drug delivery to lung cancer. They used a solvent evaporation method to fabricate the liposomes and the resulting nanoparticles had an average size of 130 nm with spherical morphology and long stability

Table 15.1 Currently developed curcumin-loaded liposomes formulation.

Formulation	Size (nm)	Entrapment efficiency (%)	Release kinetics	Model	Findings
Curcumin-loaded liposomes coupled with the ApoE peptide	≈132	NA		*In vitro*	Increased accumulation of curcumin in RBE4 cell brain capillary endothelial cells.
Curcumin-loaded liposomes coated with *N*-dodecyl chitosan-HPTMA chloride	≈73	NA	>80% in 10 h	*In vitro*	Biocompatible with murine fibroblasts (NIH3T3) whereas toxic for murine melanoma (B16F10) cells
Curcumin-loaded lipoe PEGePEI complex	≈269	45	90% in 120 h	*In vitro*/*vivo*	More toxic than free curcumin against both curcumin-sensitive cells and curcumin-resistant cells. 60–90% inhibition of tumor growth in mice inoculated with CT-26 or B16F10 cells.
Curcumin-conjugated nanoliposomes	≈207	NA	NA	*In vivo*	Reduced the secretion of amyloid peptide (Ab) and partially prevented Ab-induced toxicity in a mouse model of Alzheimer's disease
Curcumin-loaded silica-coated flexible liposomes	≈157	91	NA	*In vivo*	Increased 3.3-fold bioavailability compared with curcumin-loaded liposomes in mice through oral administration
Curcumin-loaded, $CaCO_3$-encapsulated pH sensitive liposome	≈155.3	NA	NA	*In vitro*/*vivo*	Increase in cellular uptake and lysosome scape

Curcumin-loaded liposomes-incorporated Eudragit S100	≈150	79.3 ± 5.6%	NA	*In vivo*	Increase in antimalarial activity with the oral administration Increase stability of curcumin
Curcumin-loaded (RGD)-modified liposomes	120.6 ± 10.8	82.3 ± 7.31%	60% in 12 h	*In vitro*/*vivo*	Decrease in cell proliferation Increase in therapeutic efficiency
Curcumin-loaded egg phosphatidyl-choline liposomes	NA	NA	NA	*In vivo*	Exhibited cytoprotection for renal ischemic reperfusion injury.
Curcumin-loaded liposomes and PVP-capped gold NPs loaded and unloaded curcumin	350 ± 94	83% ± 4.5%	38% and 30% in 4 h for liposomes and GNPs, respectively	*In vivo*	Biocompatible and safe drug delivery carrier.
Curcumin-loaded soybean phosphate dylcholine liposomes	≈176	NA	37% in 48 h	*In vivo*	Decreased parasitemia and increased survival of plasmodium berghei infected mice (anti-malarial therapy).
Curcumin-conjugated nanoliposomes	≈207	NA	NA	*In vivo*	Downregulated the secretion of amyloid peptide (Ab) and partially prevented Ab-induced toxicity in mouse model of Alzheimer's disease.

over three months. BALB/c nude mice bearing human lung adenocarcinoma (A549) cells were used for *in vivo* studies and the results showed a remarkable inhibitory effect on tumor growth and progression.[23] Karewicz *et al.* used the film evaporation technique to prepare curcumin-loaded liposomes composed of dihexyl phosphate (DHP), egg yolk phosphatidyl choline (EYPC) and cholesterol. The fluorescent probe's results revealed that curcumin positioned closely to the glycerol groups and located at the hydrophobic acyl side of liposomes. They further evaluated the effect of coating and incorporated the cationic lipid/polymer conjugate *N*-dodecyl chitosan-*N*-[(2-hydroxy-3-trimethylamine) propyl] (HPTMA) chloride as coating materials. They reported that the prepared liposomes have an average size of 73 nm, exhibited a sustained release over 10 h, and have the ability to penetrate cells due to their cationic nature. Cell toxicity assessment showed higher cell toxicity than free curcumin.[24]

15.3.1.2 Solid Lipids Nanoparticles (SLNs)

Solid lipid nanoparticles (SLNs) are a fascinating class of the lipid-based drug carrier family that possesses special properties (Table 15.2) including biocompatibility, high drug loading, good tolerability and ease of scale up. The lipids contributing to the synthesis of SLNs are generally triglycerides (tri-stearin), partial glycerides (Imwitor), fatty acids (stearic acid, palmitic acid), steroids (cholesterol) and waxes (cetyl palmitate).

Drugs can be incorporated into the SNLs with two different configurations, a core–shell structure or solid solution form. In the first, the drugs are entrapped in the matrix of the solid core or in the bilayer shell of the structure, and in the latter, a drug disperses in the lipid matrix and directly interacts with the lipid without the aid of any surfactant.[25]

In a study by Hamidi *et al.*, nanostructured lipid carriers (NLCs) and SLNs loaded with curcumin were developed for enhancement of drug concentration in the brain. The entrapment efficiency of the prepared NLCs and SLNs were 94 ± 0.74% and 820.49% ±, respectively. Moreover, X-ray diffraction and differential scanning calorimetry (DSC) indicated that curcumin

Table 15.2 Advantages of solid lipid nanoparticles.

Advantages of solid lipid nanoparticles
Control and/or target drug release.
Improve stability of pharmaceuticals.
High and enhanced drug content (compared to other carriers).
Feasibilities of carrying both lipophilic and hydrophilic drugs.
Most lipids being biodegradable, SLNs have excellent biocompatibility.
Water-based technology (avoid organic solvents).
Easy to scale up and sterilize.
More affordable (less expensive than polymeric/surfactant-based carriers).
Easier to validate and gain regulatory approval.

had been dispersed as amorphous in the nanocarriers. The *in vivo* studies on rats indicated that the plasma concentration and the half-life of curcumin were enhanced using SLNs and NLCs nanoformulations. The cell viability assay demonstrated that the nanoformulations had proliferative effects and enhanced cells growth more than 80% compared with the controls. The authors proposed that the prepared curcumin-loaded SLNs and NLCs can be an effective alternative to cure neurodegenerative diseases such as Alzheimer's.[26] In another study by Chen *et al.*, SLNs loaded with curcumin were developed and their anticancer efficiency was evaluated on breast cancer. The results showed that the prepared SLNs had an average size of 40 nm, with spherical morphology and negative charge. Moreover, the encapsulation efficiency and drug loading were 72.47% and 23.38%, respectively. Cell uptake and toxicity studies showed that the formulation was efficiently internalized into the SKBR3 cells, enhanced production of ROS, decreased the expression of CDK4 and cyclin D1, promoted the ratio of Bax/Bcl-2, and consequently induced cell apoptosis.[27] In another research by Mackenzie *et al.*, SLNs and D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) were prepared and loaded with curcumin for investigation of the nanoformulation efficiency in Hodgkin's lymphoma. *In vitro* and *in vivo* studies were performed on L-540 cells and BALB/c mice, respectively. The results demonstrated that the SLNs-Cur reduced the levels of TNF-α and IL-6. The effectiveness of SLNs-Cur formulation was greater than TPGS-Cur. Furthermore, the Cur-SLNs formulation shrunk the tumor size and remarkably decreased the XIAP and McI-1 levels and enhanced p21 levels, which are the vital cell cycle regulators. These results strengthen the hypothesis of using curcumin-based formulations to cure Hodgkin's lymphoma.[28] In another study, an emulsification and low-temperature solidification method was used to fabricate SLNs loaded with curcumin and dexanabinol (SLNs/Cur-HU-211). The anti-depression effects of the prepared SLNs were evaluated on corticosterone-induced cell and mice depression models. Cannabinoid receptor type 1 (CBR1) is implicated to be the major cause of depression because of a lack of endocannabinoids in the central and peripheral nervous system. These SLNs/Cur-HU-211 nanoparticles are assumed to have anti-depressant impact *via* targeting CBR1 on depression. *In vitro* and *in vivo* studies were concocted on PC12 cells and C57BL/6 mice, respectively. The results indicated that treatment with the Cur/SLNs-HU-211 nanoformulation led to more release of DA/5-HU with decreasing CORT-induced apoptotic cell death. The animal studies showed the increased protein expression of *p*-MEK1 and *p*-ERK1/2, along with an increased level of DA/5-HT, CB1 mRNA and CB1 in the hippocampus and striatum. Moreover, the nanoformulation treatment enhanced CB1 expression and induced the proliferation of astrocytes in the hippocampus and striatum. These results indicated that the prepared nanoformulation had anti-depression effects due to the neuroprotective effects of curcumin.[29]

15.3.2 Polymeric Nanostructures

Polymeric nanomaterials have attracted great attention in drug delivery, formulation and development due to their adjustable and promising bio–physico–chemical properties. Polymeric nanostructures can effectively encapsulate sensitive and also low solvable biomolecules to protect against destructive agents and improve solubility as well as bioavailability. Various kinds of natural and synthetic polymers with a variety of bio-physico–chemical properties have been used to prepare polymeric nanocarriers. Amongst them, biodegradable polymers such as poly (ε-caprolactone) (PCL), poly (D, L-lactic-*co*-glycolic acid) (PLGA) and poly (D, L-lactic acid) (PLA) and their copolymers with poly(ethylene glycol) (PEG) have been extensively used to produce biodegradable polymeric nanocarriers. The nanocarriers can also be used as targeted drug delivery systems along with solubilizing agents. The multifunctionality of polymeric nanocarriers make them ideal vehicles to improve curcumin circulation time and may improve its accumulation at the desired site. Different kinds of polymeric nanostructures including nanoparticles, nanofibers and micelles have been exploited for curcumin drug deliveries which have their own unique characteristics.

15.3.2.1 Polymeric Nanoparticles

Various kinds of polymeric nanoparticles have been formulated to enhance aqueous solubility, stability and bioavailability of native curcumin. PLGA is widely used for drug delivery purposes not only due to its biocompatibility but also due to its biodegradability. Encapsulation of curcumin in PLGA nanoparticles can elicit its effect properly on the target site. Doggui *et al.*[30] showed that encapsulation of curcumin in PLGA nanoparticles could improve the release profile, cellular uptake and also induce its neuroprotective effects against oxidative damage in the human SK-N-SH cell line. They suggested that curcumin-loaded PLGA could be a promising drug delivery strategy toward Alzheimer's disease. Another research team tried to improve the oral bioavailability of curcumin using PLGA nanoparticles. They fabricated curcumin-loaded PLGA nanoparticles using an emulsification/solvent evaporation technique and found that the oral bioavailability of the nanoformulation was 22-fold higher than free curcumin.[31]

In another study, Chereddy and colleagues reported that the encapsulation of curcumin in PLGA nanoparticles can protect curcumin from light degradation, provide a sustained release over a period of eight days, and improve its water solubility. Their animal studies confirmed that the nanoformulation had a significant anti-inflammatory effect, granulation tissue formation and re-epithelialization induction property.[32] Nanoprecipitation is another useful method to fabricate curcumin-encapsulated PLGA nanoparticles. This method is based on the interfacial deposition of a polymer and subsequent displacement of the lipophilic solution with a water

miscible semi-polar solvent. Yallapu *et al.* fabricated curcumin-encapsulated PLGA nanoparticles using a nanoprecipitation technique and utilized PVA and poly (L-lysine) as the stabilizers. They reported that PVA concentration had an inverse correlation with the size of the prepared nanoparticles and increase of the stabilizer concentration resulted in smaller nanoparticles. The release profile of the prepared nanoformulation included a small burst of around 20% of the loading followed by a sustained release of curcumin for 25 days. Moreover, the results showed that PVA can control the release of loaded curcumin from the nanoparticle. This study showed that polymeric materials are not only encapsulating materials but also the stabilizer and surface tailoring agents.[33] In another study, Ghosh *et al.* used didodecyldimethylammonium bromide as the stabilizer in an emulsion diffusion-evaporation method to fabricate curcumin-loaded PLGA nanoparticles and applied the prepared nanoformulation for the treatment of diethylnitrosamine (DEN). Atomic force microscopy (AFM) revealed that the particles had a very small average diameter of 14 nm which can be attributed to the strength of the surfactant and high-speed homogenizer that was used to produce the nanoformulation.[34]

Polyethylene glycol (PEG) is a fascinating polymer with promising properties which has tremendous application in nanomedicine. From the drug delivery point of view, polyethylene glycol modification (PEGylation) is widely used to prolong the circulation half-life of drug carriers in the body and reduce immunogenicity. PEG is a highly biocompatible and hydrophilic polymer which adsorbs water molecules on to its chains in aqueous environments. The high degree of hydration along with an intense movement of the PEG chains provide a steric barrier against plasma protein adsorption and subsequently drug-carrier clearance from the body. Farnia *et al.* used PEG as a co-solvent of curcumin-encapsulated chitosan–gelatin nanoparticles as the carrier. The results showed that the solubility rate increased up to 2000-fold with a sustained and controlled release.[35] Thadakapally *et al.* used the desolvation technique to prepare novel long circulating serum stable polymeric nanoparticles for curcumin delivery based on serum albumin and PEG. The results showed that the entrapment efficiency was above 95% with a small initial burst followed by slower and controlled release over 35 days. The biodistribution assay revealed that the PEGylated particles had lower liver and Kupffer cell uptake than curcumin–albumin nanoparticles without PEGylation, which confirmed the immune system evading ability of PEG. They also examined the antiproliferative activity of the nanoformulation against the breast cancer cell line MD-MB-231 and observed more potent antiproliferative activity than native curcumin. They suggest that PEGylated curcumin–albumin nanoparticles could be an alternative to conventional drugs for breast cancer treatment.[36]

The non-specificity of the drug curcumin is a vital concern in cancer therapy, which can be eliminated by passive and active targeting approaches. Passive targeting is based on the coordination between size and physico–chemical properties of the nanoformulation with the microenvironment of

the tumor tissue such as the enhanced permeability and retention (EPR) effect and prolonged blood circulation time. Active targeting is based on the strong interaction between a specific marker overexpressed on cancer cells and its targeting ligand on the nanoparticles, which facilitates the active uptake of nanoparticles by the tumor cells themselves.[37,38] Hua Jin *et al.* tried to develop a strategy to overcome poor bioavailability and non-specificity of curcumin using the combination of PEGylated PLGA as the carrier and GE11 peptides as an active targeting agent in antitumor therapy. Their results implied that the treatment with the nanoformulation decreased cancer cell viability, enhanced blood circulation time and suppressed tumor burden compared with non-EGFR targeting nanoparticles or free curcumin.[39] Phuong *et al.* used folate, an essential molecule for proper DNA synthesis, to target curcumin-encapsulated *O*-carboxymethyl chitosan (OCMCs) toward the Hep-G2 cell line.[40] They observed significant cellular uptake and cytotoxicity effect on the incubated cells compared with non-targeted nanoparticles. These studies revealed the necessity of targeting approaches.

15.3.2.2 Polymeric Micelles

Polymeric micelles are nanoscale core–shell structures in the size range of 20 to 100 nm composed of amphiphilic block or graft copolymer. The amphiphilic polymer chains spontaneously form micelles above the critical aggregation concentration (CAC) or critical micellar concentration (CMC) in aqueous solution. The hydrophobic segments of polymers start to adhere to each other to minimize their free energy and avoid contact with water, therefore forming a vesicular or core–shell micellar structure. The isolation of the hydrophobic segment from the aqueous environment and further rearrangement of the hydrogen bond network in water lead to the formation of stable micelles. A wide variety of methods can be used to encapsulate the hydrophobic substance into the micelles, for instance, the oil-in-water emulsion solvent evaporation method, direct dissolution, chemical conjugation, dialysis method, solid dispersion method, various solvent evaporation procedures and complexation.[41–46] Hydrophobic drugs such as curcumin can be accommodated in the core structure, whereas the hydrophilic shell can interact with aqueous media, which makes curcumin water soluble. Polymeric micelles offer a great number of advantages such as high stability in aqueous media, the size range suitable for drug delivery applications, and ability to encapsulate various hydrophobic and low soluble substances.

Song *et al.* used a solid dispersion method to load curcumin into micelles of amphiphilic poly (ε-caprolactone-*co*-*p*-dioxanone)-*b*-methoxy poly (ethylene glycol). The results showed that the prepared micelles have an average diameter of 30 nm with a narrow size distribution along with an entrapment efficiency of more than 95%. Interestingly, the release assay showed that 80% of the curcumin released over 300 h without any burst effect.[47] In another research, the ring-opening polymerization of the D, L-lactide method and

PEG as macroinitiator were used to synthesize a PLGA-PEG-PLGA triblock copolymer and subsequently curcumin-loaded triblock copolymer micelle fabricated by a dialysis method. The results showed that the micelles have a spherical morphology with an average diameter size of 26 nm, moreover, entrapment efficiency and drug loading capacity were 70% and 4%, respectively.[48]

From a different point of view, simulation approaches can be exploited along with experimental studies to optimize the formulation. Simulation methods are promising ways to reduce the optimizing cost and definitely save a lot of time *via* conducting trial and error process *in silico*. In this regard, Zhao *et al.* applied a central composite design (CCD), an experimental design useful in response surface methodology (RSM), to optimize the formulation of Pluronics P123 and F68 mixed micelles. The average size of the mixed micelles, loading capacity and encapsulation efficiency for curcumin were 68 nm, 7% and 78%, respectively. The release profile assessment confirmed the sustained release of 50% of the loaded curcumin over 72 h.[49] In another study, Samanta *et al.* simulated the interactions between curcumin with pluronic block copolymers using molecular dynamics simulation. Their simulation demonstrated the formation of curcumin-loaded polymeric micelles under interaction of hydrophobic PPO chains with curcumin and hydrophilic PEO chains with aqueous media.[50] These studies clearly confirmed the promising potential of the simulation approaches in polymeric nanofiber structures' development.

15.3.2.3 Polymeric Nanofibers

Nanofibers are a unique class of nanomaterials identified with a very high aspect ratio, nanometric diameter, and micro or even millimetric length. Nanofibers have attracted a great deal of attention for local drug delivery applications due to their promising properties.[51] Nanofibers act not only as a carrier of the drug but also as physical support; these factors are applicable for drug delivery, tissue engineering, wound dressing and anti-adhesive applications.[52] Nanofibers with various morphologies, geometry and composition can be fabricated by different methods, but the most sophisticated method is the electrospinning technique, which utilizes a high-voltage electric field to draw micro and nanofibers from a polymer solution.[53] The electrospinning technique offers a great number of advantages such as relatively simple procedure, flexibility to fabricate a wide range of polymeric nanofibers, low cost and ability to scale up.

Numerous researchers have fabricated curcumin-loaded electrospun nanofibers for the given applications. Based on the proposed applications, curcumin can be incorporated into the nanofibers *via* simply blending with the polymer solution or applying a coaxial electrospinning set up to fabricate core–shell nanofibers. In the first, curcumin accommodates in the matrix of the carrier polymer and releases with the degradation of the polymer.

The core–shell nanofibers consist of two immiscible polymer solutions extruded through a coaxial nozzle; the curcumin compatible polymer is considered as the core and the other polymer as the shell. The core–shell nanofibers provide a more controlled release over the blending approach.

Mutlu *et al.* fabricated curcumin-loaded poly (3-hydroxy butyric acid-*co*-3-hydroxy valeric acid) (PHBV) nanofibers by electrospinning methods as wound dressing materials. The results showed that the fabricated nanofibers have a diameter of 207 ± 56 to 519 ± 15 nm, depending on the curcumin concentration. The release profile of the curcumin-loaded PHBV nanofibers includes an initial burst effect at 30 min followed by a sustained release. Moreover, a cell proliferation assay showed that the fabricated nanofibers have positive effects on the cells' proliferation. The authors proposed the prepared curcumin-loaded PHBV nanofibers as a proper wound dressing for various wound healing applications.[54] The curcumin-loaded nanofibrous wound dressings provide a barrier against microorganism penetration to the wound, adjust the moisture of the wound, absorb the exudate and provide a topical drug delivery system to the wound.

In another study, Mei *et al.* further evaluated the wound healing efficacy of curcumin-encapsulated electrospun nanofibers *in vitro* and *in vivo*. They fabricated nanofibers consisting of a grafted chitosan and poly (propylene carbonate) by an electrospinning technique. They reported that the prepared wound dressings have a sustained release over 288 h, eminent free radical scavenging capacities and enhanced wound healing efficacy. The animal study showed 100% wound closure ratio at day 21 post surgery, higher collagen contents and higher granulation scores compared with the control groups.[55] The curcumin-incorporated nanofibers were applied as wound dressings in the healing of diabetic foot ulcers. Electrospun PCL nanofibers were fabricated as the delivery vehicle of curcumin for diabetic wound healing applications. The *in vitro* results showed a sustained release of curcumin over 72 h, cytocompatibility against human foreskin fibroblast cells (HFF-1) and also high antioxidant activity. Moreover, *in vivo* demonstrated an increased rate of wound closure in a diabetic mice model.[56]

Fallah *et al.* assessed the antibacterial activity of curcumin-loaded PCL/gelatin nanofibers fabricated using the electrospinning technique. They reported that the nanofibers were 99.9% bactericidal against *extended-spectrum beta-lactamase (ESBL)* and *methicillin-resistant Staphylococcus aureus (MRSA)*. They proposed the fabricated nanofibers for antibacterial applications.[57] Curcumin-incorporated PCL nanofibers have also been applied as an anticancer agent. Sridhar *et al.* applied curcumin-encapsulated electrospun PCL nanofibers against lung cancer (A459) and human breast cancer (MCF7) cell lines *in vitro*. They reported that the prepared nanofibers exhibited 15% more cytotoxicity than a commercial anticancer drug (1% *cis*-Platin-loaded PCL nanofiber) after 24 h incubation.[58]

15.3.3 Conjugates

The attachments of curcumin to synthetic and natural hydrophilic polymers as well as small molecules (such as amino acids) have been exploited to improve curcumin stability, aqueous solubility and bioavailability. Various types of polymers and amino acids (isoleucine, phenyl glycine, proline, alanine, valine, cysteine, leucine, phenylalanine, serine and glycine) have been conjugated to curcumin and their properties tested. In a study, hyaluronic acid was coupled with curcumin using 4-dimethylaminopyridine (DMAP) as the catalyzer and N,N_0 dicyclohexylcarbodiimide (DCC) as the coupling agent. The conjugation turned hydrophilic hyaluronic acid into an amphiphilic moiety, which formed a micellar structure with a size between 300 and 600 nm and a negative zeta-potential (−25 to −75 mV). Further evaluation revealed the stability of the conjugated curcumin once incubated in aqueous solution at pH 7.4 for 8 h.[59] In another study, Tang *et al.* developed a temporal targeting approach to release curcumin within the cells. They used glutathione and esterases sensitive bond (*b*-thioester bonds) to conjugate curcumin to two short oligo (ethylene glycol) chains. They reported that the conjugates self-assembled into a micellar structure with an average size of 37 nm. The stability studies showed that the conjugates have acceptable stability in pure PBS (at pH 7.4 and 5.0). And, on the other hand, 35 and 80% of the conjugated curcumin released in the PBS contained reduced glutathione (GSH) and esterase, respectively. This curcumin conjugate can be assumed to be an interesting delivery system which is stable in the blood circulation and will release conjugated curcumin under the interaction with intracellular GSH and esterase once entering the cells.[60] In a recent study, amino acids of isoleucine, phenyl glycine, proline, alanine, valine, cysteine, leucine, phenylalanine, serine and glycine were conjugated to curcumin in dry dioxane using DCC as the coupling agent and triethylamine (TEA) and DMAP as catalysts and finally, the products were purified by column chromatography. The results showed that the coupling significantly increased the aqueous solubility of curcumin to 1–10 mg mL^{-1}.[61]

15.3.4 Peptide/Protein Carriers

Proteins are a group of natural molecules that possess unique performances and powerful applications in biomedical sciences. Proteins serve as ideal materials for the fabrication of nanoparticles due to the amphiphilic properties of this structure that can interact well with both solvent and drug. A wide variety of proteins have been exploited for drug delivery applications such as albumin, gelatin, elastin, gliadin and legumin, zein, soy proteins, milk proteins and whey proteins. Albumin can be acquired from different sources such as egg white (ovalbumin), human serum albumin (HAS) and bovine serum albumin (BSA). Albumin plays an important role in the binding and

transportation of drugs within the circulation system. Albumin has been used in the preparation of different nanocarriers and is easy to synthesize, is biodegradable and possesses functional groups such as thiol, carboxyl and amino on the surface that can be attached to ligands and other surface functional groups. Albumin is the most commonly applied protein but there are a lot of proteins that are being used as carriers or ligands for better targeting of the organ and targeted delivery.[62] In a study by Fan *et al.*, PLGA-PEG nanoparticles loaded with curcumin and modified with B6 peptide were developed and this nanostructure was evaluated in the treatment of Alzheimer's disease. The *in vitro* study was done in HT22 cells and the *in vivo* part was performed in APP/PS1-induced plaques transgenic mice. The synthesis of PLGA-PEG copolymers was performed *via* a ring opening method and then modified with B6 peptide. The cytotoxicity assay proved that PLGA-PEG-B6/Cur nanoparticles possessed biocompatibility and low toxicity profiles. The silver staining and IF/IHC of Aβ in the brain sections of mice were done and the results demonstrated that curcumin reduced Aβ levels and the phosphorylation level of tau in APP/PS1 mouse brains. This nanoformulation could ameliorate AD pathogenesis by diminishing tau phosphorylation and Aβ deposition.[63] In another research by Han *et al.*, R3V6 peptide micelles composed of a 3-arginine stretch and 6-valine stretch, were prepared and loaded with curcumin and applied to INS-1 insulinoma cells for protection of islet β-cells under hypoxia. The cellular internalization assay was conducted on INS-1 cells and the results indicated that curcumin was efficiently delivered to the INS-1 cells. The pancreatic β-cells experience apoptosis under hypoxia and the TUNEL assay results in INS-1 cells indicated that cells treated with R3V6-curcumin possessed anti-apoptotic effects. The results imply that curcumin-based formulations can be applied as a protector after imputation of the β-cell islets.[64] Le *et al.* used egg ovalbumin (OVA) to improve the solubility, bioavailability and photostability of curcumin. The characterization using a thermodynamic titration technique in combination with molecular dynamics simulation revealed that curcumin was entrapped into the hydrophobic core of ovalbumin *via* hydrophobic interaction and then the interactions further increased *via* hydrogen bonds. The authors proposed the polyphenol–protein binding by the 'hands-gloves' model as the interaction mechanism.[65]

15.3.5 Cyclodextrins

Cyclodextrins (CD) are cyclic oligosaccharides composed of glucopyranose units linked by α-(1–4) bonds which are considered as excellent molecular chelating agents. These cyclic structures have been widely used to enhance the stability of labile substances and increase aqueous solubility and bioavailability of poorly soluble drugs due to their lipophilic central cavity and the hydrophilic outer surface. CDs are classified to α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin consisting of 6, 7 and 8 glucopyranose units, respectively. DCs are physically and chemically stable macromolecules which are generated

via enzymatically hydrolyzing starch using DC glucosyltransferase. DCs have various attractive properties including low toxicity, low immunogenicity, water-solubility and stability, which have been made them an ideal class of drug delivery vehicle.[66–68]

They are not completely cylindrical in form and are a rather truncated cone-shape because of the chair structure of the glucopyranose unit. They are homogeneous, crystalline and nonhygroscopic agents. Among the three mentioned cyclodextrins, β-CD is a good choice for complexation because of ultimate cavity size, efficient drug loading and complexation, low cost and availability. Diverse hydrophobic, hydrophilic and ionic derivatives have been structured and applied to modify the biopharmaceutical and physicochemical characteristics of the drug. Hydroxypropyl-β-cyclodextrin (HP-β-CD), randomly methylated-β-cyclodextrin (RM-β-CD), and sulfobutylether-β-cyclodextrin (SBE-β-CD) are preferred for complexation. Due to great solubilizing and complexing properties which are embedded in these particles, they are mostly selected for complexation nowadays.[69–71] In a research by Yu *et al.*, a thermosensitive hydrogel based on chitosan/β-glycerophosphate loaded with curcumin- β-CD was prepared and the effect of this structure was evaluated for the treatment of a cutaneous wound infection. *In situ* gel forming hydrogels are used in a way that can effectively deliver the drugs *via* the porous structure of the hydrogel in a minimal invasive administration to the wound site. The results showed that CDs in combination with curcumin can enhance cellular uptake, improve curcumin half-life and increase skin permeability which lead to prominent biological effects. *In vivo* studies demonstrated that chitosan/glycerophophate/curcumin-treated wounds exhibited faster wound closure and modified histological consequences. Furthermore, antimicrobial, antioxidant and anti–NF–κB signaling effects of this structure were assessed. The integration of cyclodextrin/curcumin into a chitosan/glycerophosphate hydrogel led to pro-healing and anti-infection effects and generated a location for epithelial migration, granulation and revascularization resulting in tissue formation. Moreover, this nanostructure decreased the level of SOD and CAT enzyme which contributed to the production of excessive ROS in the wound region and postponed the healing process of wounds.[72] In another study by Akbari Javar *et al.*, a magnetic nanocomposite based on Fe_3O_4 NPs loaded with doxorubicin and curcumin was developed and surface functionalized with hydroxyapatite and cross-linked with β-CD. The effect of this nanoformulation was applied to mitigate the chemoresistance in breast cancer. An *In vitro* study was done in MCF-7 (DOX sensitive) and MCF-7/adr (DOX resistant) cells and treated with DOX-CUR loaded NCs. Also, an *in vivo* study was investigated in MALB/c mice. The results demonstrated that coating the surface of NCs with β-CD can minimize absorption of proteins on the surface of NPs. Also, β-CD chunked scavenger receptors on phagocytes. Furthermore, DOX concentration in the tumor region increased DOX-CUR loaded NCs plus the magnetic field compared to other treatment groups. DOX-CUR loaded NCs resulted in the prohibition of P-gp expression. The magnetic field application led to an increased concentration

of DOX in the tumor tissue.[73] In a study by Yamamoto *et al.*, various cyclodextrins including α, β, γ, hydroxypropyl (HP)-β-CD and dimethyl (DM)-β-CD loaded with curcumin were developed for evaluating intestinal absorption of this formulation. The intestinal absorption of curcumin was examined *via* an *in situ* closed-loop experiment. Also, for clarifying the mechanism of increasing absorption of CDs *via* a paracellular pathway, cellular transport of 5(6)-carboxyfluorescein (CF) in Caco-2 cell monolayers were examined and the expression of claudin-4 in brush border membrane vesicles (BBMVs) was elucidated. The results of this study demonstrated that amongst the used CDs, α-CD and DM-β-CD remarkably boosted the intestinal absorption of curcumin in a rat loop experiment ($p < 0.05$). Furthermore, the results revealed that one possible mechanism for the modified intestinal absorption of curcumin by CDs was the modified solubility of curcumin in the existence of CDs. Also, 50 Mm α-CD was the optimum enhancer of absorption and did not cause any toxicity to the intestinal membrane. In the end, α-CD was introduced as a bright absorption enhancer with less toxicity for increasing the intestinal absorption of poorly absorbable drugs like curcumin.[74]

15.3.6 Metallic Nanoparticles

Metallic nanoparticles are one of the most fabricated, evaluated, modified and commercialized parts of nanomaterials due to their brilliant physico–chemical properties. A wide variety of metallic nanoparticles such as gold nanoparticles (GNPs), silver nanoparticles (AgNPs), iron oxide, zinc oxide and titanium dioxide (TiO_2) nanoparticles have been applied in the field of nanomedicine, especially for drug delivery applications. The size, charge, shape, protein binding, dose and surface coating can influence the pharmacokinetics of metallic nanoparticles. The application of gold, silver and iron oxide nanoparticles for delivery of curcumin will be discussed in the following section.[75–77]

15.3.6.1 Gold Nanoparticles

Gold nanoparticles (GNPs) are broadly employed as novel drug delivery, sensing, photodynamic therapy and imaging approaches due to their unique properties. GNPs possess unique characteristics that are derived from surface plasmon resonance described as absorption and interaction with incident light in diverse wavelengths and illustration of an absorption peak in the visible region.[78] Surface modification of AuNPs including size, core–shell type and morphology can alter the wavelength of absorption light. Therefore, GNPs are a good candidate for photothermal therapy to eliminate cancer cells in a strategy called 'theranostics' which is a combination of diagnostic and therapy.[79] In a study by Adlia *et al.*, gold and curcumin were conjugated for delivery of curcumin to the liver and the effect of this formulation was tested in fibrosis. Gold-curcumin NPs (cAuNPs) were developed *via* varying the concentration and pH of the curcumin solvent and combining with the green

synthesis of AuNPs. Also, *in vitro* analysis of this synthesized formulation was evaluated in NIH/3T3 cells. The cAuNPs demonstrated a narrow peak and highest intensity at pH 9.3, which indicated a homogenous size and abundance of nanoparticles. Furthermore, an *in vivo* study was done to validate the non-toxicity of the nanoformulation and also to test the cytotoxicity of the nanostructure. The results indicated that the nanoformulation was non-toxic up to 300 $\mu g\ mL^{-1}$ and did not damage the chicken embryo. The results further indicated that the antifibrotic activity of curcumin could be because of the anti-inflammatory and antioxidant activity of curcumin. The nanoformulation preserved the cell viability of NIH/3T3 cells and was a non-toxic nanostructure.[80] In another study by Nasr *et al.*, two different nanocarrier systems were prepared for delivery of curcumin including PVP and nanoliposome capped gold nanoparticles. The effect of this nanoformulation was tested in photodynamic therapy while curcumin acted as a photosensitizer to eliminate the HepG2 cancer cell line. An *in vivo* study was performed on albino mice that were injected with Ehrlich ascites's carcinoma cells. The encapsulation efficiency of liposomes loaded with curcumin was approximately 83%, which was a high degree of loading. The results demonstrated that liposomes loaded with curcumin possessed superior effects in the elimination of tumor volume rather than CURAu NPs. This study indicated liposomes to be a biocompatible and safe drug delivery carrier for curcumin.[81] In a study by Fu *et al.*, curcumin nanocapsules modified with BSA-AuNPs as a core–shell structure were developed and the effect of this nanostructure was evaluated in tumor cell growth. The *in vitro* part was done in human neuroblastoma cells (SH-SY5Y). The results indicated that this synthesized nanohybrid formulation possessed greater apoptotic rate in tumor cells and it can be used as a drug delivery system along with its synergistic effects on inhibiting the growth of tumor cells.[82] In another study by Ozeki *et al.*, polyethylene glycol grafted gold nanoparticles loaded with curcumin were synthesized and PEG-grafted AuNPs were conjugated with three types of α, β and γ CDs and the cytotoxic effects of this nanoformulation was evaluated in A549 cells. The overall diameter of the CUR-CD-GNPs was calculated to be less than 10 nm. The encapsulation efficiency of the synthesized nanoparticles was different and the results demonstrated that curcumin was efficiently incorporated into β-CDs. Further results in A549 cells showed that CD-GNPs without curcumin did not have cytotoxicity on cells, therefore, GNPs were a safe carrier for drug delivery. Also, cur-CD-GNPs demonstrated cytotoxic effects in cells. The *in vivo* section should be included in the study for a complete investigation.[79] In a study by Sattarahmady *et al.*, curcumin-polyethylene glycol gold nanoparticles were developed as a novel nanoparticle for photothermal therapy, and the impact of this nanoformulation was tested in mouse malignant melanoma cell line C540 (B16/F10). An *in vivo* study of photothermal therapy of this formulation was performed in male C57/inbred mice which were injected with B16/F10 cells. The results demonstrated that the Cur-PEG-Au NPs possessed a spherical shape with an average diameter of 15.6 ± 3.2 nm. Photothermal therapy was performed with this nanoformulation and

demonstrated strong photothermal impact under 808 nm wavelength light irradiation. Further results indicated that the nanoformulation had cytotoxic effects and induced apoptosis in human melanoma cell lines. The nanoformulation was entered into the cells of animals and had great photodynamic and photothermal effects for the treatment of melanoma.[83] In another study by Soo Han *et al.*, halloysite nanotubes (HNTs) loaded with curcumin and AuNPs and modified with chitosan (CS) were developed and the anticancer potential of this nanoformulation was tested in the MCF-7 breast cancer cell line. HNTs are aluminosilicates with a tubular structure which is hollow like a carbon nanotube. They occur naturally at a low cost and can be used as a drug delivery carrier. This developed nanoformulation possessed NIR and pH-sensitive characteristics and the curcumin was released according to the acidic pH (5.5) of the cell line. The results demonstrated that the maximum loading of curcumin in the nanoformulation was 3.4%. This nanostructure showed cytotoxic and apoptotic effects on the MCF-7 cell line in a dose-dependent manner.[84]

15.3.6.2 Silver Nanoparticles

Silver nanoparticles (AgNPs) have been extensively used in consumer products such as household, medical and personal products due to their exceptional antibacterial effects on a broad spectrum of bacteria along with optical, magnetic and chemical properties. AgNPs can also be employed as catalysts and drug delivery carriers. AgNPs result in the induction of apoptosis and enhancement in the production of ROS leading to DNA damage and necrosis.[85] In a study by Baydoun *et al.*, AgNPs were incorporated with curcumin as a probe molecule to determine the effects of AgNPs in cell membrane characteristics including phase transition temperature and permeability. 1,2-dimyr istoyl-*sn*-glycero-3-phosphocholine (DMPC) liposomes were used as a pattern for simulation of the phospholipid bilayers membrane. The results demonstrated that a low concentration of AgNPs diminished the partition of curcumin in the membrane. Furthermore, curcumin was successfully used for the investigation of the phase transition of membranes and AgNPs changed the phase transition temperature (T_m) and pre-transition temperature of DMPC liposomes. The results showed that AgNPs might have toxic effects on membrane integrity.[86] In another research by Bajpai *et al.*, nanocrystals of cellulose loaded with chitosan films and also embedded with AgNPs/curcumin were prepared and the antimicrobial impacts of this nanoformulation as a wound dressing was tested in albino rats. The preparation method of cellulose nanocrystals containing chitosan was based on ultra-sonication of cellulose microparticles and then the synthesized film was loaded with silver NPs and curcumin for enhancement of the wound healing process. The albino rats treated with this synthesized dressing demonstrated an exceptional healing process up to 98%. This nano dressing remarkably modified the tensile characteristics of the films. The Ch/CNC (Ag NP_x/Cur_y) films possessed powerful potential to be employed as a wound healing dressing.[87]

Polymeric micelles loaded with curcumin and decorated with AgNPs were developed by Liu and his colleagues for eliciting antibacterial effects. Curcumin was surrounded with poly (ε-caprolactone), which created the core of the polymeric micelles. This nanoformulation was prepared to overcome multidrug resistance, which becomes a world health-threatening problem and was evaluated in gram-positive *S. aureus* and gram-negative *P. aeroginosa* bacteria. Further results illustrated that this dressing possessed considerable cytocompatibility toward mammalian cells, especially red blood cells.[88] In another study by Schwartz *et al.*, curcumin nanoparticles stabilized with AgNPs were synthesized and the antiretroviral activity of this nanoformulation was evaluated in an ACH-2 cell, which is a cell line infected with an HIV-1 and THP-1 cell line. The results demonstrated that both silver and curcumin NPs possessed remarkable antiviral and anti-inflammatory effects. Also, curcumin had anti-HIV activity *via* inhibition of HIV-1 integrase and protease. Furthermore, curcumin inhibited the transcription of pro-inflammatory cytokines including TNF-α, IL-6 and IL-1β and prevented the degradation and phosphorylation of IκBα.[89]

15.3.6.3 Iron Oxide Nanoparticles

Magnetic nanoparticles (MNPs), particularly iron oxide MNPs, are extensively employed as magnetic resonance imaging (MRI) agents and drug carriers, which are employed as diagnostic and therapeutic carriers and possess stimuli-sensitive characteristics that can enhance biocompatibility along with increasing correct and specific targeting. In a study by Khoobi *et al.*, Fe_3O_4 magnetic NPs were synthesized and coated with polyethyleneimine conjugated with glutathione and the impact of this nanoformulation was tested as a theranostic approach for delivery of curcumin in cancer therapy. Coating of the surface with polymer-modified glutathione enhanced blood compatibility and reduced toxicity. The *in vitro* study was performed in a SK-N-MC cell line and the MRI was performed to evaluate the MRI characteristics. Curcumin was used as an anticancer drug, PEI was used as passive targeting, GSH was employed as active targeting, and the theranostic approach was performed by utilizing MRI and application of curcumin. The curcumin encapsulation into Fe_3O_4 MNPs enhanced cellular uptake and magnetically directed drug delivery to the tumor site.[90] In a study by Keiri *et al.*, gold-iron oxide nanoparticles modified with PEG were prepared and evaluated for delivery of curcumin and sulforaphane and the impact of this nanoformulation was tested in a human breast adenocarcinoma cell line (SK-BR-3). The synthesized nanoformulation induced necrosis and apoptosis in the cell line. The average size of the nanoparticles was 20 nm with good monodispersity and loading capacity of approximately 16.32%. This nanoformulation enhanced Bcl-2, MMP-9 and BAK genes expression to induce apoptosis.[91] Fe_3O_4–Au nanocomposites were prepared and coated with curcumin-lipoic acid and glutathione was employed as the targeting ligand for drug delivery to the brain by Khoobi *et al.* The size of the nanoparticle

was found to be less than 50 nm with loading efficiency of more than 70%. The prepared nanocomposite had biocompatibility with low adsorption of protein. Fe_3O_4@Au-LA-CUR/GSH NCs possessed less toxicity under an *in vitro* study. The MRI was performed and the results demonstrated that an adequate amount of nanoparticles had been delivered to the brain tumor cells.[92] In another study by Samrot *et al.*, superparamagnetic iron oxide nanoparticles (SPIONs) were synthesized for delivery of curcumin and functionalized with SDS to become a core–shell structure coated with chitosan. The impact of this nanoformulation was evaluated in a HeLa cell line. The surface charge of the core–shell structure was cationic with a size of approximately 40–45 nm. The results demonstrated that this nanostructure induced apoptosis and expression of caspase 3 and killed the tumor cells.[93]

15.4 Conclusions and Prospects

Curcumin is a miracle from nature due to its astonishing properties such as antioxidant, anti-inflammatory, antibacterial, anticancer, antidiabetic, and anti-Alzheimer activities. However, its translation to the clinic is hampered by its poor aqueous solubility and bioavailability. Various approaches have been conducted to overcome these shortcomings but the most promising are nanotechnology methods. Nanotechnology as an enabling technology acts in the submicron and the molecular level effectively manipulating curcumin molecules in ways which not only mitigate curcumin limitation but also maintain its physiological functions. Various studies have shown the promising potential of nanomaterials and nanotechnology to enhance the aqueous solubility and bioavailability of curcumin. In spite of the good safety profiles of curcumin-loaded nanomaterials in both humans and animals, it is vital to monitor the toxicity of the long-term and repeated administration of high-dose nanoformulations on the body. High concentrations of curcumin may induce production of reactive oxygen species (ROS) in cells which have various adverse effects such as lipid peroxidation, proteins denaturation and DNA damage, which in turn might result in cytotoxicity, genotoxicity and carcinogenesis.[94] These concerns imply that there is still a pressing need to understand the detailed effects of curcumin-based nanoformulations on human bodies and strict preclinical and clinical studies must be conducted.

References

1. V. Karunaratne and A. De Alwis, *The Nanotechnology and its Contributions to Economic Development*, 2017, http://dl.lib.mrt.ac.lk/handle/123/12242.
2. M. Eleftheriadou, G. Pyrgiotakis and P. Demokritou, *Curr. Opin. Biotechnol.*, 2017, **44**, 87.
3. J. P. Dopazo and F. Zivic, *Supporting University Ventures in Nanotechnology, Biomaterials and Magnetic Sensing Applications: Policies, Practices, and Future*, Springer, Switzerland, 2018.

4. M. R. Wiesner, G. V. Lowry, P. Alvarez, D. Dionysiou and P. Biswas, *Environ. Sci. Technol.*, 2006, **40**, 4336.
5. C. N. Rao, G. U. Kulkarni, P. J. Thomas and P. P. Edwards, *Chemistry*, 2002, **8**, 28.
6. G. Hodes, *Adv. Mater.*, 2007, **19**, 639.
7. B. V. Chikkaveeraiah, S. G. Malghan and K. Girish, *Physico–Chemical Properties of Nanomaterials*, ed. R. C. Pleus and V. Murashov, Jenny Stanford Publishing, New York, 2018, vol. 9, pp. 295–320.
8. H. Mirzaei, A. Shakeri, B. Rashidi, A. Jalili, Z. Banikazemi and A. Sahebkar, *Biomed. Pharmacother.*, 2017, **85**, 102.
9. S. Hewlings and D. Kalman, *Foods*, 2017, **6**, 92.
10. Z. Stanić, *Plant Foods Hum. Nutr.*, 2017, **72**, 1.
11. K. Doello, R. Ortiz, P. J. Alvarez, C. Melguizo, L. Cabeza and J. Prados, *Nutr. Cancer*, 2018, **70**, 569.
12. A. C. da Silva, P. D. de Freitas Santos, J. T. do Prado Silva, F. V. Leimann, L. Bracht and O. H. Gonçalves, *Trends Food Sci. Technol.*, 2018, **72**, 74.
13. R. M. Di Martino, B. Luppi, A. Bisi, S. Gobbi, A. Rampa, A. Abruzzo and F. Belluti, *Expert Opin. Ther. Pat.*, 2017, **27**, 579.
14. M. Gera, N. Sharma, M. Ghosh, D. L. Huynh, S. J. Lee, T. Min, T. Kwon and D. K. Jeong, *Oncotarget*, 2017, **8**, 66680.
15. L. Miao, S. Guo, C. M. Lin and Q. Liu, *Adv. Drug Delivery Rev.*, 2017, **115**, 3.
16. U. Chitgupi, Y. Qin and J. F. Lovell, *Nanotheranostics*, 2017, **1**, 38.
17. V. Girdhar, S. Patil, S. Banerjee and G. Singhvi, *Curr. Nanomed.*, 2018, **8**, 88.
18. R. M. Pinto, D. Lopes, C. Nunes, B. Sarmento and S. Reis, *Nanoparticles in Life Sciences and Biomedicine*, ed. A. R. Neves and S. Reis, Jenny Stanford Publishing, New York, 2018, vol. 4, pp. 75–110.
19. M. Lucia, *Curr. Drug Metab.*, 2017, **18**, 469.
20. D. Sharma, A. A. E. Ali and L. R. Trivedi, *PharmaTutor*, 2018, **6**, 50.
21. Y. Chen, Q. Du, Q. Guo, J. Huang, L. Liu, X. Shen and J. Peng, *Drug Dev. Ind. Pharm.*, 2019, **45**, 282.
22. E. Martí Coma-Cros, A. Biosca, E. Lantero, M. L. Manca, C. Caddeo, L. Gutiérrez, M. Ramírez, L. N. Borgheti-Cardoso, M. Manconi and X. Fernàndez-Busquets, *Int. J. Mol. Sci.*, 2018, **19**, 1361.
23. K. Jiang, M. Shen and W. Xu, *Int. J. Nanomed.*, 2018, **13**, 2561.
24. A. Karewicz, D. Bielska, A. Loboda, B. Gzyl-Malcher, J. Bednar, A. Jozkowicz, J. Dulak and M. Nowakowska, *Colloids Surf., B*, 2013, **109**, 307.
25. J. E. N. Dolatabadi, H. Valizadeh and H. Hamishehkar, *Adv. Pharm. Bull.*, 2015, **5**, 151.
26. S. Sadegh Malvajerd, A. Azadi, Z. Izadi, M. Kurd, T. Dara, M. Dibaei, M. Sharif Zadeh, H. Akbari Javar and M. Hamidi, *ACS Chem. Neurosci.*, 2019, **10**, 728–739.
27. W. Wang, T. Chen, H. Xu, B. Ren, X. Cheng, R. Qi, H. Liu, Y. Wang, L. Yan, S. Chen, Q. Yang and C. Chen, *Molecules*, 2018, **23**, 1578.
28. J. Guorgui, R. Wang, G. Mattheolabakis and G. G. Mackenzie, *Arch. Biochem. Biophys.*, 2018, **648**, 12.

29. X. He, L. Yang, M. Wang, X. Zhuang, R. Huang, R. Zhu and S. Wang, *Cell. Physiol. Biochem.*, 2017, **42**, 2281.
30. S. Doggui, J. K. Sahni, M. Arseneault, L. Dao and C. Ramassamy, *J. Alzheimer's Dis.*, 2012, **30**, 377.
31. Y. M. Tsai, C. F. Chien, L. C. Lin and T. H. Tsai, *Int. J. Pharm.*, 2011, **416**, 331.
32. K. K. Chereddy, R. Coco, P. B. Memvanga, B. Ucakar, A. des Rieux, G. Vandermeulen and V. Préat, *J. Controlled Release*, 2013, **171**, 208.
33. M. M. Yallapu, B. K. Gupta, M. Jaggi and S. C. Chauhan, *J. Colloid Interface Sci.*, 2010, **351**, 19.
34. D. Ghosh, S. T. Choudhury, S. Ghosh, A. K. Mandal, S. Sarkar, A. Ghosh, K. D. Saha and N. Das, *Chem.-Biol. Interact.*, 2012, **195**, 206.
35. P. Farnia, S. Mollaei, A. Bahrami, A. Ghassempour, A. A. Velayati and J. Ghanav, *Biomed. Res.*, 2016, **27**, 659.
36. R. Thadakapally, A. Aafreen, J. Aukunuru, M. Habibuddin and S. Jogala, *Indian J. Pharm. Sci.*, 2016, **78**, 65.
37. R. Bazak, M. Houri, S. El Achy, S. Kamel and T. Refaat, *J. Cancer Res. Clin. Oncol.*, 2015, **141**, 769.
38. V. P. Torchilin, *Handb. Exp. Pharmacol.*, 2010, **197**, 3.
39. H. Jin, J. Pi, Y. Zhao, J. Jiang, T. Li, X. Zeng, P. Yang, C. E. Evans and J. Cai, *Nanoscale*, 2017, **9**, 16365.
40. P. T. Ha, M. H. Le, T. M. N. Hoang, T. T. H. Le, T. Q. Duong, T. H. H. Tran, D. L. Tran and X. P. Nguyen, *Adv. Nat. Sci.: Nanosci. Nanotechnol.*, 2012, **3**, 035002.
41. K. Van Butsele, P. Sibret, C. A. Fustin, J. F. Gohy, C. Passirani, J.-P. Benoit, R. Jérôme and C. Jérôme, *J. Colloid Interface Sci.*, 2009, **329**, 235.
42. J. Taillefer, M. C. Jones, N. Brasseur, J. E. van Lier and J. C. Leroux, *J. Pharm. Sci.*, 2000, **89**, 52.
43. V. Y. Alakhov, E. Y. Moskaleva, E. V. Batrakova and A. V. Kabanov, *Bioconjugate Chem.*, 1996, **7**, 209.
44. N. Nishiyama and K. Kataoka, *J. Controlled Release*, 2001, **74**, 83.
45. Y. Li and G. S. Kwon, *Pharm. Res.*, 2000, **17**, 607.
46. A. Lavasanifar, J. Samuel and G. S. Kwon, *J. Controlled Release*, 2001, **77**, 155.
47. L. Song, Y. Shen, J. Hou, L. Lei, S. Guo and C. Qian, *Colloids Surf., A*, 2011, **390**, 25.
48. Z. Song, R. Feng, M. Sun, C. Guo, Y. Gao, L. Li and G. Zhai, *J. Colloid Interface Sci.*, 2011, **354**, 116.
49. L. Zhao, J. Du, Y. Duan, Y. Zang, H. Zhang, C. Yang, F. Cao and G. Zhai, *Colloids Surf., B*, 2012, **97**, 101.
50. S. Samanta and D. Roccatano, *J. Phys. Chem. B*, 2013, **117**, 3250.
51. H. Samadian, H. Mobasheri, S. Hasanpour and R. Faridi-Majid, *J. Nano Res.*, 2017, **50**, 78.
52. K. Khoshnevisan, H. Maleki, H. Samadian, S. Shahsavari, M. H. Sarrafzadeh, B. Larijani, F. A. Dorkoosh, V. Haghpanah and M. R. Khorramizadeh, *Carbohydr. Polym.*, 2018, **198**, 131.

53. S. Farzamfar, M. Naseri-Nosar, A. Vaez, F. Esmaeilpour, A. Ehterami, H. Sahrapeyma, H. Samadian, A.-A. Hamidieh, S. Ghorbani, A. Goodarzi, A. Azimi and M. Salehi, *Cellulose*, 2018, **25**, 1229.
54. G. Mutlu, S. Calamak, K. Ulubayram and E. Guven, *J. Drug Delivery Sci. Technol.*, 2018, **43**, 185.
55. L. Mei, R. Fan, X. Li, Y. Wang, B. Han, Y. Gu, L. Zhou, Y. Zheng, A. Tong and G. Guo, *Polym. Chem.*, 2017, **8**, 1664.
56. J. G. Merrell, S. W. McLaughlin, L. Tie, C. T. Laurencin, A. F. Chen and L. S. Nair, *Clin. Exp. Pharmacol. Physiol.*, 2009, **36**, 1149.
57. M. Fallah, S. H. Bahrami and M. Ranjbar-Mohammadi, *J. Ind. Text.*, 2016, **46**, 562.
58. R. Sridhar, S. Ravanan, J. R. Venugopal, S. Sundarrajan, D. Pliszka, S. Sivasubramanian, P. Gunasekaran, M. Prabhakaran, K. Madhaiyan, A. Sahayaraj, K. H. Lim and S. Ramakrishna, *J. Biomater. Sci., Polym. Ed.*, 2014, **25**, 985.
59. R. Yang, S. Zhang, D. Kong, X. Gao, Y. Zhao and Z. Wang, *Pharm. Res.*, 2012, **29**, 3512.
60. H. Tang, C. J. Murphy, B. Zhang, Y. Shen, M. Sui, E. A. Van Kirk, X. Feng and W. J. Murdoch, *Nanomedicine*, 2010, **5**, 855.
61. K. Parvathy, P. Negi and P. Srinivas, *Food Chem.*, 2010, **120**, 523.
62. W. Lohcharoenkal, L. Wang, Y. C. Chen and Y. Rojanasakul, *BioMed Res. Int.*, 2014, 180549.
63. S. Fan, Y. Zheng, X. Liu, W. Fang, X. Chen, W. Liao, X. Jing, M. Lei, E. Tao, Q. Ma, X. Zhang, R. Guo and J. Liu, *Drug Delivery*, 2018, **25**, 1091.
64. J. Han, J. Oh, S. H. Ihm and M. Lee, *J. Drug Targeting*, 2016, **24**, 618.
65. Y. Liu, Y. Cai, D. Ying, Y. Fu, Y. Xiong and X. Le, *Int. J. Biol. Macromol.*, 2018, **116**, 893.
66. E. M. Del Valle, *Process Biochem.*, 2004, **39**, 1033.
67. Y. Shen, Z. Yu, X. Yang, F. Wang, J. Luo and M. Wang, *J. Ind. Microbiol. Biotechnol.*, 2017, **44**, 1.
68. J. Conceicao, O. Adeoye, H. M. Cabral-Marques and J. M. S. Lobo, *Curr. Pharm. Des.*, 2018, **24**, 1405.
69. B. Gidwani and A. Vyas, *BioMed Res. Int.*, 2015, **2015**, 198268.
70. N. Ono, H. Arima, F. Hirayama and K. Uekama, *Biol. Pharm. Bull.*, 2001, **24**, 395.
71. A. Vyas, S. Saraf and S. Saraf, Cyclodextrin based novel drug delivery systems, *J. Inclusion Phenom. Macrocyclic Chem.*, 2008, **62**, 23.
72. Y. Zhao, J. G. Liu, W. M. Chen and A. X. Yu, *Exp. Ther. Med.*, 2018, **15**, 1304.
73. R. Rastegar, H. A. Javar, M. Khoobi, P. D. Kelishadi, G. H. Yousefi, M. Doosti, M. H. Ghahremani, A. Shariftabrizi, F. Imanparast, E. Gholibeglu and M. Gholami, *Artif. Cells, Nanomed., Biotechnol.*, 2018, **46**, 207.
74. X. Li, S. Uehara, K. Sawangrat, M. Morishita, K. Kusamori, H. Katsumi, T. Sakane and A. Yamamoto, *Int. J. Pharm.*, 2018, **535**, 340.
75. A. Schröfel, G. Kratošová, I. Šafařík, M. Šafaříková, I. Raška and L. M. Shor, *Acta Biomater.*, 2014, **10**, 4023.

76. P. Sanderson, J. M. Delgado-Saborit and R. M. Harrison, *Atmos. Environ.*, 2014, **94**, 353.
77. Z. Jiang, N. D. B. Le, A. Gupta and V. M. Rotello, *Chem. Soc. Rev.*, 2015, **44**, 4264.
78. Y. D. Taghipour, S. Kharrazi and S. M. Amini, *Nanomed. Res. J.*, 2018, **3**, 102.
79. A. Hoshikawa, M. Nagira, M. Tane, K. Fukushige, T. Tagami and T. Ozeki, *Biol. Pharm. Bull.*, 2018, **41**, 908.
80. A. Adlia, I. Tomagola, S. Damayanti, A. Mulya and H. Rachmawati, *Sci. Pharm.*, 2018, **86**, 41.
81. M. Fadel, K. Kassab, A. E. Fadeel, M. Nasr and N. M. E. Ghoubary, *Drug Dev. Ind. Pharm.*, 2018, **44**, 1809.
82. C. Fu, C. Ding, X. Sun and A. Fu, *Mater. Sci. Eng., C*, 2018, **87**, 149.
83. F. Rahimi-Moghaddam, N. Azarpira and N. Sattarahmady, *Laser Med. Sci.*, 2018, **33**, 1769.
84. K. M. Rao, A. Kumar, M. Suneetha and S. S. Han, *Int. J. Biol. Macromol.*, 2018, **112**, 119.
85. R. de Lima, A. B. Seabra and N. Durán, *J. Appl. Toxicol.*, 2012, **32**, 867.
86. N. Wehbe, D. Patra, R. M. Abdel-Massih and E. Baydoun, *Colloids Surf., B*, 2019, **173**, 94.
87. S. K. Bajpai, S. Ahuja, N. Chand and M. Bajpai, *Int. J. Biol. Macromol.*, 2017, **104**, 1012.
88. F. Huang, Y. Gao, Y. Zhang, T. Cheng, H. Ou, L. Yang, J. Liu, L. Shi and J. Liu, *ACS Appl. Mater. Interfaces*, 2017, **9**, 16880.
89. R. K. Sharma, K. Cwiklinski, R. Aalinkeel, J. L. Reynolds, D. E. Sykes, E. Quaye, J. Oh, S. D. Mahajan and S. A. Schwartz, *Immunol. Invest.*, 2017, **46**, 833.
90. N. Aeineh, F. Salehi, M. Akrami, F. Nemati, M. Alipour, M. Ghorbani, B. Nikfar, F. Salehian, N. R. Alam, S. E. S. Ebrahimi, A. Foroumadi, M. Khoobi, M. Rouini, M. Dibaei, I. Haririan, M. R. Ganjali and S. Safaei, *J. Biomater. Sci., Polym. Ed.*, 2018, **29**, 1109.
91. H. Danafar, A. Sharafi, S. Askarlou and H. K. Manjili, *Drug Res.*, 2017, **67**, 698.
92. M. Ghorbani, B. Bigdeli, L. Jalili-Baleh, H. Baharifar, M. Akrami, S. Dehghani, B. Goliaei, A. Amani, A. Lotfabadi, H. Rashedi, I. Haririan, N. R. Alam, M. P. Hamedani and M. Khoobi, *Eur. J. Pharm. Sci.*, 2018, **114**, 175.
93. C. Justin, A. V. Samrot, P. D. Sruthi, C. S. Sahithya, K. S. Bhavya and C. Saipriya, *PLoS One*, 2018, **13**, e0200440.
94. M. López-Lázaro, *Mol. Nutr. Food Res.*, 2008, **52**, S103.

CHAPTER 16

Curcumin as Dietary Supplements Against Various Diseases: An Insight into the New Trends and Future Perspectives

AKHILA NAIR[a] AND SREERAJ GOPI*[a]

[a]R&D Centre, Aurea Biolabs (P) Ltd, Kolenchery, Cochin–682 311, Kerala, India
*E-mail: sreerajgopi@yahoo.com

16.1 Introduction

Unprecedented technological progress has enslaved both the lifestyle and diet of individuals and led to many lifestyle-associated diseases including diabetes, cardiovascular diseases, obesity and so on. The solution to these problems is often sought in nutritive remedies.[1] The superior functionality of natural bioactive compounds is well established in research and the food industry.[2] Among these compounds, turmeric, an ancient dietary spice, that belongs to the Zingiberaceae family, is acknowledged as both culinary and as a nutraceutical. Turmeric, termed 'multi-antispice', is a perennial herb widely found in tropical countries, namely China and India.[3] The major bioactive

Food Chemistry, Function and Analysis No. 25
The Chemistry and Bioactive Components of Turmeric
Edited by Sreeraj Gopi, Sabu Thomas, Ajaikumar B. Kunnumakkara, Bharat B. Aggarwal and Augustine Amalraj

Published by the Royal Society of Chemistry, www.rsc.org

compound of turmeric is curcumin or diferuloylmethane, with the chemical name 1, 7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, a yellow colored polyphenolic compound isolated from the rhizome of *Curcuma longa* L. by Vogel *et al.*[4] It is known as the 'yellow gold' and even called 'curecumin' because of its voluminous therapeutic benefits.[5] The pleiotropic therapeutic activities of curcumin include anti-asthmatic, antioxidant, anti-inflammatory, antivenom, antiprotozoal, antimicrobial, antiproliferative, anti-angiogenic, anti-aging, antitumor, antimalarial, antifungal, antiviral, neurodegenerative, antihypertensive, anti-arrthymic, immunosuppresants, antihistamines, antiretroviral, antidiabetic, hypoglycemic and radical scavenger.[6,7] Curcumin exhibits higher bioavailability when used as a food colorant and additive because of the cooking method as well as excellent dissolution property in oil. These enormous benefits attained from curcumin have been very well documented in the past and updated frequently. The studies in the literature reveal that the safe maximum intake of curcumin is up to 12 gm per day with negligible side effects. Therefore, curcumin as a dietary supplement is marketed extensively around the globe in the form of various formulations.[8] Statistically, the demand for curcumin supplements is increasing and about 80–85% sales increase is witnessed through each passing year in the US Mainstream Multi-Outlet Channel.[9] This tremendous rise in sales is considered to be because of the emerging new nano/micro formulations of curcumin involving new technologies that promise to solve the solubility and bioavailability problems of curcumin that pose problems in clinical trials.[8] Numerous preparations to amplify the bioavailability of curcumin are continuously researched that involve encapsulated formulations such as liposomes, nanoparticles and so on. These formulations offer many beneficial regimens such as (1) greater aggregation stability with particle size reduction: as the attractive forces between the colloidal particles decrease at a higher rate than repulsive interactions; (2) delivers high optical clarity: as the dimensions of nanoparticles get lower than the wavelength of light; and (3) greater sedimentation or creaming stability: as the gravitational forces that act upon them are relatively weak, and considered to be balanced by Brownian motion.[9] Furthermore, non-food grade excipients, high cost, hydrophobicity and regulatory noncompliance limits further applications of curcumin. Therefore, successive methods to utilize cost-effective, natural, abundantly found ingredients or carrier agents, GRAS (generally regarded as safe) have been initiated.[10] Various natural food-derived excipients are utilized in the form of surfactants, phospholipids, natural proteins and carbohydrate. Selection of the most favorable ingredients for the food or beverage to be encapsulated and the encapsulating techniques are important parameters as they enhance the functional, physicochemical properties and gastrointestinal fate because small particles are hydrolyzed more easily by digestive enzymes in the gastrointestinal tract (GT) than larger ones, hence, successfully enhancing the bioavailability.[9,11]

The present review specifically focuses on the oral drug delivery of curcumin supplements and highlights their cardinal virtues in curing various lifestyle-associated diseases such as cardiovascular diseases, diabetes,

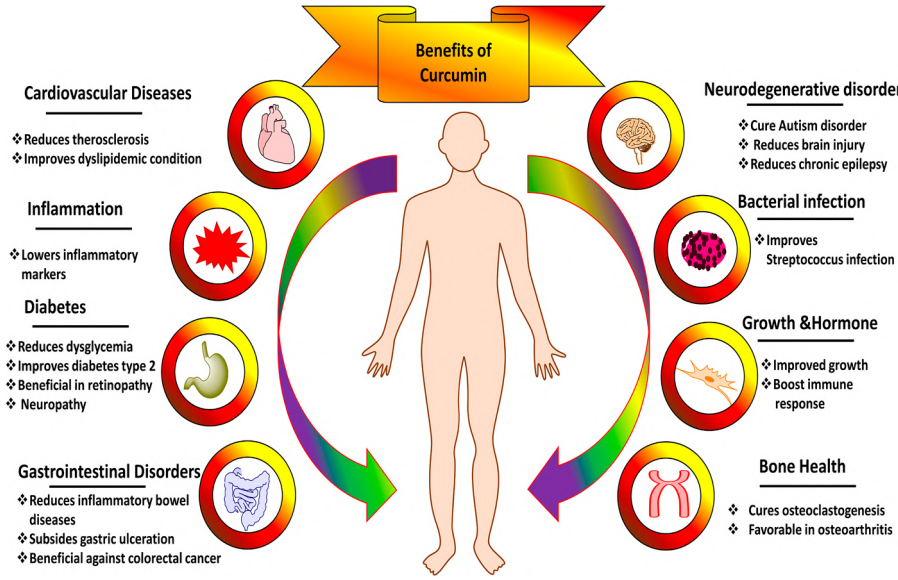


Figure 16.1 Benefits of curcumin supplements.

neurological disorders, bacterial as well as viral infections, gastrointestinal diseases, growth disorders, bone diseases, hormonal disorders and so on (Figure 16.1). Precisely, solubility and bioavailability, the most pressing concern in the case of curcumin, are addressed by focusing on novel encapsulating solutions such as food-grade carrier agents, encapsulation techniques and *de novo* encapsulates. This review reports the successful impregnation of curcumin supplements with these food-derived carrier agents and new encapsulation techniques, in order to reduce their limitations as well as clinically prove them to be of great solace in this technologically driven century, where diet and lifestyle are major victims. This review is not only beneficial in the field of food and nutrition but also useful to medicine and research at laboratory and industrial levels. Finally, the requirement of thorough and advanced clinical trials for these curcumin supplements are suggested.

16.2 Oral Delivery of Curcumin Supplements – An 'Achilles' Heel'

There are various forms in which curcumin can be delivered such as oral, subcutaneous, intraperitoneal, intravenous, topical and nasal so that it can be utilized for several biological activities. The major therapeutic activities of curcumin being antioxidant, anti-inflammatory, anticancer, antidiabetic, *etc.* These activities are limited in crude curcumin due to poor GT absorption and fast metabolic activity in hepatic and intestinal sections, which possess an adverse effect on the bioavailability of curcumin. As the water solubility is 11 ng mL^{-1} when administered orally, curcumin exhibits only a minimal

amount in the blood with a maximum amount excreted through feces, because the rate of passive diffusion is reasonably high.[11] The active metabolites in plasma succeeding the oral route are namely curcumin glucuronides and curcumin sulfates. In kidney, liver and intestinal mucosa, the coupling of enzymatic activities is established for glucuronidation and sulfation of curcumin. Although, only a certain portion of curcumin in the form of the conjugates of glucuronide and sulfate after metabolism are available in the blood circulation, clinical trials prove curcumin supplements are safe and superbly tolerated at higher doses.[12] Curcumin supplement impaired oxidative stress, when 3 mg of curcumin was orally administered in mice through declension inducing muscle damage. Also, in humans, 90 mg of curcumin supplement, when taken two hours before and after exercise, diminish exercise-induced oxidative stress thereby increasing the antioxidant capacity.[13] A study explained that the anti-inflammatory activity of curcumin when administered orally in mice inhibited inflammatory cytokines such as cyclooxygenase (COX)-2, tumor necrosis factor (TNF) and inducible nitric oxide synthase.

Therefore, oral administration of curcumin supplement is beneficial for cancers such as lung, skin, oral, head and neck. It also proves worthwhile for cardiovascular, neurodegenerative diseases, growth and hormone, bacterial infection, diabetic conditions, bone and gastrointestinal diseases (Table 16.1).

16.2.1 Cardiovascular Diseases

It has been seen through the years that curcumin supplements are important in the treatment of various diseases. Curcumin exerts its therapeutic properties by regulation of various cellular and molecular targets. It is considered to act by blocking nuclear factor-κB (NF-κB) upstream *via* IKK inhibition, which results in a lowering of the tumor necrosis factor-α (TNF-α) thereby improving the endothelial functions. Besides, the inhibition of the toll-free receptor-4 also contributes to the reduced activation of NF-κB to produce a protective effect against atherogenesis. Curcumin has potentially improved atherosclerosis by reducing intestinal cholesterol absorption and accumulation by downregulating scavenger receptor class A (SR-A) through activation of proteasome as well as ATP-binding cassette transporter (ABC) A1 upregulation through the liver X receptor α pathway (LXRα) in macrophages.[14] Recently, this was seen through a study, which demonstrated that when a curcumin supplement was fed to apolipoprotein E-knockout (ApoE) mice, which were being fed on a diet with high fat content, it reduced the atherosclerotic lesions and cholesterol levels by 45% and 56%, respectively (observed by aortic sections). This reduction in cholesterol level was the result of downregulation of Neimann-Pick C1-like 1(NPC1L1), an intestinal expression which is principally found in jejunal and duodenal segments.[15] In addition, with the reduction of atherosclerotic lesions, improvement in the increase of Th2 and Th17 cells inhibited the pro-inflammatory mediators' expression and

Table 16.1 Summary of studies involving curcumin supplementation.

S. No	Sample/study	Dose/intervention	Test	Observation	Result	Reference
Cardiovascular diseases						
1	ApoE Mice	0.1% w/w, 16 weeks	Atherosclerotic lesions measurement, quantitative RT-PCR, Western blotting	Reduction in atherosclerotic lesions-45% and cholesterol levels-56%	Improved atherosclerosis	14
2	Patients	36 mg, 180 mg, 8 g	Safety and efficacy measurement	Well tolerated, radiological disease stabilization	Colon, pancreatic and biliary cancers	15
3	Patients	4 weeks, 200 mg per day, 2 g per day	Serum lipid analysis	Total cholesterol-11%, LDL cholestrol-14%	Improved cardio protective property	18
4	Patients	Curcumin-250 mg, sterol esters & stanols-425, fermented red rice-166 mg, olives-25 mg, 3 months	Tolerability assessment, plasma biochemistry, endothelial damage markers under basal condition	Enhance lipid profile	Cardiovascular diseases	19
Neurodegenerative disorders						
5	Epileptic rats	100 mg kg^{-1}	Active & passive avoidance test, cresyl violet staining, electron microscopy, glutathione, mitochondrial swelling, PTZ kindling score	Increase memory, ROS generation reduction and rejuvenate mitochondrial complexes, oxidation stress prevention, protein carbonyls reduction and lipid peroxide reduction	Chronic epilepsy	20
6	Male Sprague-Dawley Rats	500 ppm, 4 weeks	Western blotting, immuno histochemical analysis	Increase AMP activated protein kinase (AMPK), cytochrome c oxidase II (COX-II) and ubiquitous mitochondrial creatine kinase (uMtCK) levels	Brain injury conditions	21

(*continued*)

Table 16.1 (*continued*)

S. No	Sample/study	Dose/intervention	Test	Observation	Result	Reference
7	Male Sprague-Dawley Rats	500 ppm	Elevated plus maze test, immunoblotting, lipid analysis, *in vitro* cell culture blotting	Anxiety behaviour decreased	DHA deficient	22
8	Adult male rat model	75 mg kg^{-1}, 8 day	Generalization of fear	↑Hippocampal neurogenesis, ↑fear discrimination learning	Fear discrimination contextual learning pattern and neurogenesis dependent learning	23
9	Cerebral ischemia (BCCAO model) rats	25–50 mg kg^{-1}	Biodistribution studies	↑Neurological scoring-79%, ↑Brain bioavailability (AUC)-16.4 times	Alzheimer's disorder	24
9	Male Sprague Dawley Rats	50, 100, 200 mg kg^{-1} 28 day	Neurobehavioral tests, Elisa, mitochondrial respiratory chain enzyme assessment, biochemical assessment graphic	↓ mitochondrial dysfunction, oxidative nitrosative stress, matrix metalloproteinases-9(MMP-9), tumor necrosis factor alpha (TNF-α) expressions in propanoic acid	Autism spectrum disorders	25
Growth and hormone						
10	Crucian carp fish	105 day, 5 g kg^{-1}, 1 g kg^{-1}, 0	Enzyme activity analysis, real-time quantitative, PCR analysis of trypsin lipase, amylase in hepatopancreas	↓in protein carbonyl and malondialdehyde, ↑ in intestinal parameters such as hydroxyl radical scavenging ability, superoxide anion, glutathione reductase (GR), total superoxide dismutase (T-SOD), glutathione, glutathione-S-transferase (GST), glutathione peroxide (GPx)	Improved growth of fish and promising antioxidant, absorptive and digestive effects	27

11	Nile tilapia fishes	0, 50, 100, 150, 200 mg CUR kg^{-1} 84 day	Immunological assay	↑in lysosomal activity and immunoglobulin concentration boosting immune response	Growth, oxidative status, disease resistance immune response	28
			Oxidative stress level test	↓ glutathione content, ↑ in malondialdehyde (MDA) level		
			Microbiology	↓ mortality		
12	Patient/BALB-c mice/rat/	10, 30 or 50 μm	Cell culture studies/ *in vivo*	↓ inflammation *via* inhibition of inflammatory cytokines expression, lesions	Endometriosis	29
Bacterial infection						
13	Silver catfish	150 mg kg^{-1}, 30 days	Bacterial culture treatment efficiency	↓ corneal opacity, appetite loss, skin lesions in tail and fin, erratic swimming	Streptococcus infection	30
Diabetes						
14	Patients	500 mg, 3 per day	Meta-analysis	↓ plasma MDA values and excretion of urinary microalbumin, ↑ antioxidant enzyme such as NAD(P)H quinone oxidoreductase 1 (NQO-1), Nrf2 regulated protein	Diabetes	37
15	Patients	0.07–4 g, 60 day	Fasting blood glucose, HbA1c, HOHA-IR, meta regression analysis	↓ adult fasting blood glucose level, decreased dysglycemia, HbA 1c values	Diabetes	39
Bone health						
16	Diabetes mellitus induced rats	120 mg per day	Histochemical analysis	↓ in osteoclast numbers and bone resorptive activity resulted in ↑ in cathepsin K and tartrate resistant acid phosphatase (TRAP) mRNA levels	Osteoclastogenesis	40

(*continued*)

Table 16.1 (*continued*)

S. No	Sample/study	Dose/intervention	Test	Observation	Result	Reference
17	Patients	500 mg per day 42 mg^{-1} 4 g^{-1}	Observational cohort study/ WOMAC subscale/disease activity score (DAS) score/ American college of rheumatology (ACR) score	↑bioavailability, ↑ pharmaco-kinetic and clinical efficacy	Osteoarthritis	41
	Patients	250 mg per day & 500 mg per day, 3 months	Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), visual analog scale (VAS), rheumatoid factor (RF), disease activity score (DAS)-28, American College of Rheumatology (ACR)	↓inflammation, well tolerated, safe	Rheumatoid arthritis	42
Gastrointestinal diseases						
18	Patients	Therapeutic dose	*In vitro*/*in vivo* studies	↓ inflammation in colon	Human inflammatory bowel diseases	43
19	Mice (Mdr1a)	0.2% CUR	Microarrays 2D gel electrophores LCMS protein Ingenuity pathway analysis	Activation of α-catechin, extra-cellular signal regulated kinase (ERK), tumor necrosis factor (ligand) superfamily member 12 (TNFSF12), fibronectin (FN1), phosphatidylinositide 3-kinase complex (PI3K) and main transcription factors	Human inflammatory bowel diseases	44
20	Patients	10 day standard triple therapy	Cytokine, chemokine, toll-like receptor (TLR) PCR assay and C13 urea breath test	↓ inflammation in gastric mucosa	*Helicobacter pylori*	45

facilitated the amelioration of regulatory T cells in macrophages. Hence, curcumin supplements were essential for cholesterol management and improving atherosclerosis.[16] Vernieri *et al.* reviewed that curcumin supplements alone or in combination are beneficial in colon, pancreatic and biliary cancers. Also, two different daily doses that were considered, namely: 1) 36–180 mg per day and 2) 8 g per day, prove that curcumin incurred radiological disease stabilization and satisfactorily tolerated to about 9 to 33% in patients, however, the author suggested more clinical studies to be conducted to establish their excellent safety profile.[17]

Moreover, curcumin as a dietary supplement is beneficial for preventing atherosclerosis. In this context, a study conducted on mice with ApoE high fat fed diet, compared the activities of mice supplemented with and without curcumin. Analyses of the triglyceride, low- and high-density cholesterol levels (LDL and HDL) through serum lipid assay showed significant reduction. The mRNA levels downregulated, which was quantified by a reverse transcription polymerase chain reaction (RT-PCR). The curcumin-supplemented ApoE mice exhibited reduced absorption of intestinal cholesterol as observed by western blotting and reduced atherosclerotic lesions in the aortic sinus which facilitated an immense decrease of cholesterol. Altogether these findings display the anti-atherogenic effects of curcumin when taken as a dietary supplement.[18]

Recently, a study explained that the combination of curcumin with sterol esters, stanols, fermented red rice and olives elucidates the potential scope of using this combination of polyphenols to treat cardiovascular diseases as this combination of supplements enhance the lipid profile and is effective for endothelial damage markers in dyslipidemic patients.[19] Hence, although numerous studies have been performed to learn the efficiency of curcumin supplements to cure various cardiovascular diseases, these recent studies form a firm accretion to establish the potency of curcumin as a dietary supplement.

16.2.2 Neurodegenerative Disorders

Curcumin supplements have the potency to fight against chronic epilepsy as seen by clinical studies on epilepsy-induced rats. These epileptic rats were given curcumin supplements of 100 mg kg^{-1}, which facilitated an increase in memory, reactive oxygen species (ROS) generation reduction and rejuvenated mitochondrial complexes, oxidation stress prevention, protein carbonyls reduction and lipid peroxide reduction.[20] These results are promising to develop new therapeutic regimens for patients with traumatic brain injury by endogenous upregulation of molecules that are crucial for functional recovery. Trials were carried out on rats induced with a mild injury of fluid percussion which lowered adenosine monophosphate activated protein kinase (AMPK), cytochrome c oxidase II (COX-II) and ubiquitous mitochondrial creatine kinase (uMtCK) levels. However, it was observed through western blot that as these rats were fed curcumin supplements these parameters

exhibited increased levels.[21] In addition, a study found that curcumin is beneficial for patients who are vegans and docosahexaenoic acid (DHA) deficient. This study demonstrated the superlative power of curcumin in enriching the DHA synthesis from α-linolenic acid (ALA or C18:3*n*−3) precursor as well as raising enzyme levels such as elongase 2 and fatty acid desaturase (FADS)2 that initiate this synthesis. It was seen that the anxiety behavior of rodents was decreased when treated with ALA and curcumin in combination, which denoted a rise in brain DHA content. Thus, the use of curcumin as a dietary supplement is promising in the prevention of cognitive diseases.[22] There is also recent evidence that dietary supplements enrich fear discrimination contextual learning patterns and neurogenesis dependent learning in mice.[23] In the hippocampus, curcumin supplements improved the brain-derived neurotrophic factor in a dose-related manner in a Wistar Kyoto male rat model. Also, bilateral carotid artery occlusion provoked cerebral ischemia enhanced neurological factors in the brain by solid lipid nanoparticles of curcumin through oral administration with a significant increase in AUC.[24] Another study researched the efficacies of curcumin (*via* oral administration) as potent neuropsychopharmacotherapeutics to treat autism spectrum disorders. The rats were examined at different doses such as 50, 100, 200 mg kg^{-1} for a 28 day period. It was seen that curcumin revitalizes autistic phenotype symptoms by reducing mitochondrial dysfunction, oxidative nitrosative stress, matrix metalloproteinases-9(MMP-9), tumor necrosis factor alpha (TNF-α) expressions in propanoic acid induced autism in rats when compared to the control group. Hence, curcumin supplements are beneficial for autism patients.[25] Taken together, preclinical trials confirm that supplements of curcumin are beneficial in improving brain conditions; this fact is also elaborately reviewed by Amalraj *et al.*, by highlighting the capability of curcumin in binding with cadmium and copper metal ions and modulating as well as interacting with various inflammatory cytokines, transcription factors and enzymes to serve as a potent neuroprotective agent.[26]

16.2.3 Growth and Hormones

There were other clinical trials which explained the benevolence of curcumin supplements for growth, antioxidant property in intestine, immunity activities concerning digestive enzymes and hepatic growth factors. In this series, Jiang *et al.* performed a 105 day feed trial of curcumin supplement on crucian carp fish. For this, various parameters of intestine and hepatopancreas were checked and compared to a control or basal group. These parameters, such as the content of hepatopancreas protein, hepatopancreas weight, intestinal weight, content of intestinal protein, intestinal somatic index, lipase and trypsin of intestine as well as hepatopancreas, alkaline phosphatase (AKP), creatine kinase (CK), gamma-glutamyl transpeptidase (γ-GT), Na+, K+-ATPase (NKA) along with their relative mRNA expressions, showed a significant increase with a dose of 5 g kg^{-1} than 1 g kg^{-1} and 0. It was even observed

that at 5 g kg^{-1}, contents of protein carbonyl and malondialdehyde (MDA) were decreased and there was an increase in intestinal parameters such as hydroxyl radical scavenging ability, superoxide anion, glutathione reductase (GR), total superoxide dismutase (T-SOD), glutathione, glutathione-S-transferase (GST), glutathione peroxide (GPx). Taken together, this showed the improved growth of fish and the promising performance of antioxidant, absorptive and digestive effects of curcumin when taken as a dietary supplement.[27] In the same way, another study demonstrated an improved growth and antioxidant property by performing an 84 day curcumin supplement fed trial on Nile tilapia fish. It was noticed from an immunological assay that the lysosomal activity (innate immune defense index of fish) increased and immunoglobulin concentration also increased and the boosting immune response of fish catalase activity increased. The oxidative stress level test explained that glutathione content decreased with an increase in the MDA level demonstrating the antioxidant property of curcumin. Even tests conducted on *Aeromonas hydrophila* exhibited reduced mortality when compared to control groups. These studies reflected that the curcumin supplement is indeed beneficial in growth oxidative status and disease resistance immune response.[28] Furthermore, research conducted to examine the growth of tilapia fish, fed with a curcumin supplement, confirms curcumin potency as a dietary supplement.

Dietary curcumin is beneficial for endometriosis, an inflammatory disorder which is estrogen dependent, mostly developed in females in their reproduction period. The *in vitro* animal studies are reviewed by Arablou *et al.* (2018), suggesting the potency of curcumin to reduce inflammation *via* inhibition of inflammatory cytokines expression as well as the fact that lesions in endometriosis are unable to invade, attach and form angiogenesis. This disease benefits by the characteristic property of curcumin to facilitate cell cycle arrest and apoptosis.[29] These dietary curcumin supplements support growth, immunity and antioxidant properties immensely.

16.2.4 Bacterial and Viral Infection

The increasing complexities of bacterial infection and failure of monotherapy have urged the need for strong bacterial agents or hybrid antibacterial agents which are safe and efficient. Silver nanoparticles are excellent promotors of antibacterial agents, however, the solvents used in their synthesis possess a certain amount of toxicities, are highly reactive as well as possessing biological and environmental hazards especially reducing agents such as surfactants, *N*, *N*-dimethyl formamide, sodium borohydride and hydrazine.[30] Therefore, utilizing natural products especially for synthesizing silver nanoparticles in aqueous solution, the use of polysaccharides such as amylose, chitosan, xanthan, starch are focused because they are biocompatible, ameliorate stability and are environmentally friendly.[31] For example, encapsulating polyphenolic hydrophobic drugs,

namely curcumin, with polysaccharides (oxidized amylose), to form a curcumin and oxidized amylose inclusion complex have provided excellent results as hybrid antibacterial agents especially against gram positive *S. aureus* and gram negative *P. aeruginosa*.[32] Furthermore, the effects of a curcumin supplement on silver catfish induced with streptococcus infection were studied. The successful outcome of the study highlighted the bactericidal potential of curcumin as noticed by the dilapidated symptoms such as corneal opacity, appetite loss, skin lesions in tail and fin, and erratic swimming. Thus, these studies suggested that curcumin can be utilized as a potent dietary supplement that could escalate resistance to disease and can be used as an alternative to antibiotics, which can result in immense side effects.[33]

Curcumin is considered as a potential candidate for numerous viral infections because of the minimal side effects. The virus loses infectivity if curcumin is incubated with it.[34] Besides, it should act at an early stage in viral infection through PI3K/Akt and NF-κB pathways to avoid viral replication and produce beneficial effects. Curcumin in a dose-dependent manner is reported to interfere with the binding of these contagious viruses to the cell. The antiviral effects of curcumin against various viruses such as parainfluenza virus type 3 (PIV-3), herpes simplex virus (HSV), feline infectious peritonitis virus (FIPV), flock house virus (FHV), vesicular stomatitis virus (VSV), zika, chikangunya and respiratory syncytial virus (RSV) have been already observed to be effective. Following these studies it can be suggested that curcumin supplements may be beneficial against coronavirus (COVID 19).[35,36] However, no evidence-based studies have been conducted until now to establish their effects on this virus and more studies are recommended which may prove some solace to many in need.

16.2.5 Diabetes

Evidence from clinical trials reflects a clear mechanism which could improve oxidative stress demonstrating the strong antioxidant property of curcumin and anti-inflammatory pathways which benefit diabetes (type 2).[37] Diabetic neuropathy is due to the consequences of lipid accumulation in kidneys and the lipid metabolism regulator known as sterol regulatory element-binding protein (SREBP)-1c that facilitates lipid accumulation in diabetic kidneys. AMP-activated protein kinase (AMPK) is stimulated by curcumin to suppress SREBP-1c as well as decrease gene expression. This contributes to the synthesis and assembling of fatty acid synthase and acetyl-Co-A carboxylase (triglycerides) to exert beneficial effects on diabetic neuropathy.[38] Parsamanesh *et al.* also reviewed the ability of curcumin to defeat complications related to diabetes such as neuropathy and retinopathy. Although there were only a few clinical trials, all of them provide promising results in favor of curcumin. Trials contemplate that curcumin supplements were excellently tolerated in patients with neuropathy declining the plasma MDA values and excretion of

urinary microalbumin as well as increasing the antioxidant enzyme such as NAD(P)H quinone oxidoreductase 1 (NQO-1), Nrf2 regulated protein and so on. Even the inflammation and diabetes related kidney diseases were significantly reduced when compared to control group patients who hadn't taken the supplement.[37] The vascular endothelial growth factor (VEGF), which is responsible for the stimulation of neovascularization, plays a crucial role in diabetic retinopathy. It is reported that curcumin inhibits induction of hyperglycemia-mediated VEGF to have a beneficial effect in diabetic retinopathy.[17] Furthermore, the phospholipid complex of curcumin well managed degeneration and inflammation related eye diseases, proving it to be beneficial for retinopathy.[37] Besides this, a meta-analysis conveyed that curcumin, curcuminoids, or turmeric extract are beneficial in lowering adult-fasting blood glucose levels by consequently lowering dysglycemia. These supplements when compared to placebos show decreased glycated hemoglobin (HbA 1c) values.[39] Hence, these clinical studies provide data that support dietary curcumin for the efficient treatment of diabetes and other complications related to the same.

16.2.6 Bone Health

It is proved that curcumin supplements promote bone health. In addition, a recent report suggests that osteoclastogenesis can be cured with curcumin supplements. The inhibitory effects of these supplements can very well accelerate osteoclastic activity for those patients who are diabetes mellitus prone insulin dependent. For this study, rats induced with diabetes mellitus by streptozotocin were utilized, which inhibited the transcription factor activator protein-1 diabetes mellitus. Reduction in osteoclast numbers and bone resorptive activity resulted in an increase in cathepsin K and tartrate resistant acid phosphatase (TRAP) mRNA levels of diabetic rats when compared to a control group, which was observed with the help of histochemical analysis. Also, observations from this study strengthen the case that supplements of curcumin can enhance the resorptive activity of bone marrow when cultured with NF-κB ligand (RANKL) receptor activator and macrophage colony stimulated factor, as these cultured cells of diabetes induced rats supplemented with curcumin showed declination of mRNA levels, protein c-jun and c-fos and multinucleated TRAP-positive cells.[40] It has been reported through preclinical studies that supplements of curcumin are favorable to those patients who suffer from osteoarthritis.[41] Lately, a randomized placebo-controlled study on 24 patients revealed that hydrogenated curcuminoids marketed as 'Curo white' prove optimistic for the treatment of rheumatoid arthritis (RA), as this formulation drastically reduced RA related inflammation.[42] Thus, these recent investigations support the case related to curcumin for targeting and improving bone health as it is safe, well tolerated, with minimal side effects.

16.2.7 Gastrointestinal Diseases

Yang *et al.* (2017) reviewed that nutraceuticals such as curcumin can effectively reduce the severity of gastrointestinal diseases such as inflammatory bowel diseases (IBD), gastric ulceration and colorectal cancer.[43] Beside this, a study revealed that curcumin is beneficial for human IBD as it reduces inflammation in the colon through various mechanisms. For this purpose, clinical trials on mice were performed and microarrays were utilized for measuring colon mRNA transcript levels and 2D gel electrophoresis as well as LCMS protein identification for measuring colon protein expression. Supplementation with curcumin demonstrated a reduced colonic histological injury score (HIS) of mice (Mdr1a). The author also utilized ingenuity pathway regulator effect analysis (IPA) to clearly show that α-catechin activation controlled by actin cytoskeleton are responsible for enriching barrier integrity and other regulator molecules are also activated such as extracellular signal regulated kinase (ERK), tumor necrosis factor (ligand) superfamily member 12 (TNFSF12), fibronectin (FN1), phosphatidylinositide 3-kinase complex (PI3K) and the main transcription factors leading to anti-inflammatory activity in mice.[44] Curcumin as a supplement can be utilized as an adjunct to various infectious diseases distorting the gastrointestinal condition for which vaccines are still undiscovered. An infectious disease, namely *helicobacter pylori*, that causes inflammation in gastric mucosa eventually leading to cancer is successfully eradicated when curcumin is supplemented with clarithromycin and amoxicillin as demonstrated by 10 day standard triple therapy on 150 patients.[45] Thus, curcumin as a pleiotropic compound has the capability to rectify gastrointestinal diseases with minimal side effects.

16.3 Encapsulation – A Benignant Revelation for Curcumin Supplements

Extensive studies and clinical trials provide enough evidence that curcumin can be reasonably tolerated up to 12 g per day. Even the USFDA has listed turmeric as generally regarded as GRAS and in 1996, the World Health Organization (WHO) jointly with the Food and Agriculture Organization (FAO) vouchsafed curcumin with an acceptable daily intake (ADI) level of about 0.1–3 mg kg^{-1}-body weight. Therefore, it is marketed around the globe in the form of dietary supplements to cure various disease conditions.[46] However, conventional curcumin has very low plasma curcumin concentration. In addition, its log *p* value is about 3.2, displaying its hydrophobic nature: insolubility in water with a half-life of about 10 minutes in phosphate buffer 7.4. Other pressing factors are poor absorption, rapid metabolism and systemic elimination.[3,8] As it is sparingly soluble in aqueous medium, severe issues in GT subdue the bioavailability of curcumin.[47] Therefore, different formulations are continuously researched to ameliorate the solubility, bioavailability and stability of curcumin. Indeed, the incessant efforts of researchers find encapsulation to be a solution for preserving the functionality of curcumin.

This process involves the capping or coating of carrier material over active material where the active or coated material may be either fill, core or internal phase and the carrier or coated material may be a capsule, matrix, membrane or material.[11] In this context, multitudinous encapsulation methods have emerged, which promise to enhance the bioavailability of curcumin by increasing the water solubility and reducing the particle size. Food-grade excipients are favorable carrier agents that overcome the solubility and bioavailability problems because of their composition and microstructure.[48] These excipients or carrier agents might be in the form of surfactants, phospholipids, proteins, carbohydrates, *etc.* and influence the physicochemical properties, functional attributes and gastrointestinal fate of the formulation of curcumin.[9] In addition, continuous research unveiled novel encapsulating techniques such as supercritical antisolvent technology (SAS), atomized rapid injection solvent extraction system (ARISE), nanofiber weaving technology, electrospinning and polar-nonpolar sandwich technology (PNS) that are devoid of harsh chemicals, temperature and pressure.[49] Moreover, upcoming and promising formulations such as marinosomes, nanotubes, nanomesh, phytosomes and theranostic liposomes are found to be extremely favorable.[11]

16.3.1 Food-grade Carrier Agent

Curcumin, the hydrophobic biomolecule, has enormous health benefits along with low toxicity. However, the challenges faced by this golden component is poor solubility, low bioavailability and higher susceptibility to chemical/biochemical degradation.[9] It is thought that the strenuousness in the bioavailability encountered in various formulations of curcumin might be because of the ingredients used, as these excipients are either not licensed as food-grade or subject to severe repercussions in the market such as polysorbate.[47]

Many food-derived excipients have been continuously researched in the food industry such as phytosterols, polyphenols, vitamins, functional lipids, bioactive peptides, minerals, polysaccharides and probiotic bacteria. However, all these excipients encounter problems such as storage conditions (temperature, light, oxygen), GT (pH, enzymes, nutrient) or modification in chemical and physical properties. Hence, to protect and facilitate controlled release, food-grade delivery systems covering a wide range of natural biomaterials have granted GRAS, which are being successfully utilized.[50] These bioactive ingredients are seen to amplify the solubility and bioavailability of curcumin, such as biopolymers, and low molecular weight surfactants or copolymers were utilized.[9] However, it is important to select an appropriate encapsulant from the proteins, lipids or phospholipid when the bioavailability of curcumin is to be increased. Therefore, it is important to know some GRAS certified natural excipients or carrier agents suitable for curcumin encapsulation such as plant-derived protein (zein, barley soy, gliadins), animal-derived proteins (whey, casein, gelatin), lipids (cardanol, phospholipids), and polysaccharides, which are now discussed (Table 16.2).

Table 16.2 GRAS certified food-grade excipients used for curcumin supplements.

S. No	Classification (food grade excipients)		Method	Properties	Reference
1	Protein	Plant derived protein			
		Zein	Water insoluble plant storage prolamine protein corn. Source: *Zea mays* L. (Corn)	↑Stability, loading capacity, prevent chemical degradation	52 and 53
		Soy	Source: *Glycine max* L. (Soybeans)	Facilitate absorption of gastric sensitive nutrients reaching intestine, ↑ solubility, ↑bioavailability and ↑ stability	54 and 55
		Gliadins (barley & wheat)	Gliadin component source: wheat gluten, desolvation method	Protect the encapsulated core ingredient from oxidation and non-withstandable gastric conditions, ↑ loading or encapsulating capacity, desirable size. ↑ efficiency, optimum entrapment efficiency, ↑stability, ↑biocompatibility and ↑ bioavailability	50, 56–59
		Animal derived protein			
		Beta lactoglobulin	Food based biopolymer. Source: cow or sheep milk (whey protein)	↑ Loading capacity, stability and facilitated targeted delivery, encapsulation of more than one nutraceutical	60–63
		Casein	Milk proteins	Good emulsifying agent, high nutrition count, flavoured, slightly viscous	10, 64 and 65
		Gelatin	Partial hydrolysis alkaline or acid, thermal or enzymatic degradation	↑Biodegradability, ↑ biocompatibility, ↑film forming ability, ↑water retention	10 and 53
2	Lipid	Cardanol	Bio based lipid mixture. Source: *Anacardium occidental* L. (cashew nut)	Abundant availability, low cost and antibacterial properties	66
		Phospholipid (soy lecithin Sunflower lecithin)	Source: soya, rapeseed, sunflower, chicken eggs, bovine milk, fish eggs	↑encapsulation efficiency, release, bioavailability, solubility, emulsifier, wetting agent, matrix forming agent	67
3	Polysaccha-rides	Starch, cellulose, chitosan, gums, alginate, pectin	Animal, plant, microbial, algae origin	↑solubility, ↑ bioavailability, ↓particle size	10, 68–71

Proteins of two categories, plant derived (zein, soy wheat gliadins, barley) and animal derived (whey protein, gelatin, casein, collagen and fibroin), are potent encapsulating agents. Although animal-derived proteins possess good quality, digestibility and water solubility, their consumption could disturb the global food system and create adverse environmental effects. Therefore, plant-derived proteins are considered as 'environmentally economical' and mostly the preferred choice as carriers for encapsulation because they are hydrophobic in nature, non-toxic, less allergic and less expensive.[10,51]

Plant-derived proteins that are commonly employed for encapsulating curcumin are zein, soy and barley proteins. Zein, approbated by the FDA, plays a magnanimous role as core, shell or carrier in nano-encapsulated formulations to increase the bioavailability of bioactive nutrients.[52] Curcumin has been successfully incorporated in zein nanoparticles by liquid–liquid dispersion or the electro spraying method. The zein nanoparticles prepared by the electro-hydrodynamic method incorporated curcumin in zein nanoparticles in the range from 1:10 to 1:500 (curcumin : zein). No disruption of either curcumin or these nanoparticles were observed from the positive results obtained from X-ray diffractometry, fluorescence microscopy images; and three months extended stability studies performed in the dark at 23 °C and 43% relative humidity further promoted curcumin application as a virtuous dietary supplement.[53] Good chemical stability and high loading capacity was obtained when curcumin was encapsulated in zein nanoparticles, which was found to be due to interparticle repulsion and hydration. Also, zein-encapsulated curcumin provided great stability in stimulated gastrointestinal conditions. It was also found that the bioaccessibility of curcumin was elevated when zein nanoparticles were combined with lipid nanoparticles to form mixed micelles by successfully forfending chemical degradation. A comparative experimentation with hollow and solid zein nanoparticles provided promising revelations that hollow nanoparticles, because of their greater surface area and smaller size, unclog curcumin release.[53]

Soy protein is another GRAS certified product that facilitates the absorption of gastric sensitive nutrients reaching the intestine. This bioactive molecule is utilized as a carrier for both hydrophobic and hydrophilic compounds to enrich the solubility, bioavailability, protein digestibility and stability of curcumin.[54] The soy protein nanoparticles formed in conjugation with folic acid by an ethanol desolvation technique used for encapsulated curcumin rendered positive outcomes in terms of loading efficiency of about 97.2%, a desired average size of about 150 nm and zeta potential of approximately −36 mV. Furthermore, the release of curcumin preceded with a biphasic pattern in phosphate buffer pH 8. In addition, there are also other methods used to encapsulate curcumin with soy protein such as the ligand binding method.[54] The treatment of soy protein with glutaminase formed SPI(E-SPI), which, when complexed with curcumin, improved the stability. This complex improved the foaming capacity, loading capacity and antioxidant activity giving an insight to the potential benefit of using curcumin–soy protein complexes.[55]

Barley proteins help protect the core-encapsulated ingredient from oxidation and non-withstandable gastric conditions because of the desirable size and high loading or encapsulating capacity.[56] Similarly, gliadin nanoparticles promote controlled release[50] and perform even better in collaboration with glutaraldehyde in terms of stability, oral bioavailability and biocompatibility.[56] It was also observed that gliadin and lecithin utilized for the preparation of curcumin nanoparticles by antisolvent coprecipitation technology improved the encapsulation efficiency, drug delivery efficiency and imparted improved ultraviolet (UV) protection.[57] A Pickering emulsion was designed in the context of delivering bioactive compounds by chitosan–gliadin nanoparticles. This emulsion system was fabricated using corn oil that was homogenized with a chitosan–gliadin mixture in different ratios and stabilized at different levels namely primary complexes, coacervates and soluble complexes. These complexes were studied for their stability and antioxidant properties. In this study, coacervates stabilized more oil through droplet bridging, forming a percolating network rendering high stability and preventing curcumin from lipid oxidation as determined by hexanal levels and low lipid hydroperoxide levels at a thermostatically accelerated condition.[58] Curcumin can be successfully impregnated in a gliadin–chitosan system (GCNPs) through pH-induced interaction. Due to the GCNPs system that was formed by electrostatic associative interaction, facilitated reduction in particle size was observed by scanning electron microscopy (SEM). Moreover, the encapsulation efficiency was tremendously improved with controlled release of curcumin through *in vitro* pepsin and trypsin digestion;[59] thus providing a powerful scope of delivering bioactive compounds *via* natural food-grade carrier agents.

Certain animal-based proteins successfully employed for encapsulating curcumin are whey proteins, caseins and gelatin. Whey proteins are extensively used as carriers in encapsulation techniques for bioactive compounds and promise to improve their biological activity. A study compared the influence of using surfactant-based emulsion and protein-based emulsion (whey protein or caseinate) and observed that the latter rendered more protection from chemical degradation than surfactant-based emulsion (Tween 80). Hence, protein-based emulsion proved promising to ameliorate stability, bioaccessibility and the oral bioavailability of curcumin.[60] Mixed hydrogels or microgels composed of aggregates of whey proteins and k-carrageenan are excellent protectors of curcumin to facilitate colon delivery.[61] Beta lactoglobulin (BLG) is another GRAS certified component that is a major component of whey protein that facilitates encapsulation and delivery of various nutraceutical compounds and is helpful in overcoming the problems of bioavailability. Recent studies explained that BLG used as an encapsulant amplified the loading capacity, stability and facilitated targeted delivery of phenolic compounds like curcumin.[62] Moreover, a study emphasized the detailed mechanism which facilitated BLG to interact with curcumin and fatty acid, thereby enhancing the binding constant of curcumin and its loading efficiency. Also, this study provided a course which promises to deliver more than one nutraceutical simultaneously.[63]

Caseins are excellent carriers for bioactive compounds of hydrophobic nature. These milk proteins are good emulsifying agents, have a high nutrition count, are lightly flavored and slightly viscous in solution.[10] The most researched bioactive compound, curcumin, was encapsulated in a casein nanoparticle by the spray dry technique resulting in improvement in its biological activity when evaluated by cell proliferation assays and antioxidant studies. The bioactive was excellently encapsulated with higher loading capacity as assessed by Fourier transform electron spectroscopy (FTIR) and fluorescence spectroscopy.[64] Encapsulating the combination of curcumin and quercetin in reassembled casein micelles improved the shelf-life solubility and particle size that in turn improved the anticancer activity especially on breast cancer cells.[65] Hence, casein is a good encapsulating candidate to improve the solubility and bioavailability of natural hydrophobic compounds.

Gelatin has excellent characteristics related to biodegradability, biocompatibility, film forming and water retention ability to make it a suitable natural carrier for bioactive compounds.[10] The application of curcumin, a bioactive polyphenol, is limited due to low solubility, therefore encapsulation of curcumin in gelatin by electrohydrodynamic atomization revealed enhanced solubility of curcumin-loaded gelatin microparticles. This increase in solubility was 38.6 times more than curcumin, which enriched the biological activities especially the anti-microbial and antioxidant properties.[53]

Other food-grade ingredients considered for encapsulation are lipids, namely cardanol and phospholipids as they are less toxic.[10] They are found to be efficient for encapsulating curcumin because of great loading capacity, gelation property, molecular recognition and targeted delivery.[50] Incorporation of cardanol in halloysite, as thermo-responsive nanocarriers, have given encouraging results for curcumin delivery.[66] Similarly, curcumin-loaded lecithin liposomes, which enhanced the bioavailability five times more than normal curcumin suspension, are crucial especially for enhancing memory, improving immunity and preventing cardiovascular disease.[67]

Lastly, curcumin formulations involve certain polysaccharides for encapsulation as they are capable of increasing the solubility, digestibility, water retention capacity, are non-toxic, easily biodegradable, and have great hydrophilicity and emulsification capacity.[10,68] Polysaccharides are vast and numerous research studies have been conducted successfully on encapsulating bioactive compounds with low solubility and bioavailability into polysaccharide-based drug delivery systems. Curcumin, which possesses countless biological activities, is circumscribed by limited solubility and bioavailability.[69] Polysaccharides such as starches, chitosan, cyclodextrin, pectin and alginate are efficient carriers proven to reduce the particle size and increase the solubility and bioavailability of curcumin.[70] The bioavailability of curcumin was enhanced by encapsulating it by a polyelectrolyte complexation method using positively charged chitosan and a negatively charged protein that is acylated cruciferin (ACRU), a rapeseed globulin nanoparticle.[71] Hence, the biological activities of curcumin are greatly improved by these GRAS certified food-grade ingredients.

16.3.2 Advanced Techniques

It is very important to select a suitable encapsulating technique to encapsulate bioactive compounds to acquire desirable results. The continuous efforts to develop advanced techniques which are cost effective, reliable and less time consuming are in progress. In this series, certain successful encapsulating techniques that have emerged recently are antisolvent technology, electrospinning or electrospraying, microfluidic technology, coacervation, extrusion, layer by layer, freeze drying, nanofiber weaving technology and PNS, which have acquired remarkable acclamation in achieving promising drug delivery systems. Technologies that are utilized for curcumin encapsulation are discussed further.

Antisolvent technology is an upcoming technique that immensely improves the bioavailability and solubility of curcumin. This technology utilizes polyvinylpyrrolidone and methyl-β-cyclodextrin with curcumin by ARISE, which were co-precipitated utilizing CO_2 at pressure 95 bar and temperature 30 °C from organic solutions (Figure 16.2a).[72] Likewise, another formulation utilized Pluronic® 127 and Eudragit® L100 with Tween 20 by SAS for increasing the solubility and stability of curcumin extract (Figure 16.2b).[73] Electrospinning is another emerging technology that has proved successful for encapsulating bioactive components such as curcumin. Besides, other technologies that produce nanofibers namely drawing, self-assembly, template synthesis, and phase separation were enormously time consuming, involved complex procedures, uncontrolled sizes and suffered specific polymer usage. Electrospinning was thus a breakthrough that manages to overcome these limitations and proved to be cost effective, flexible and reliable to be used at industrial scale (Figure 16.2c).[74] This technique utilized the electric field to melt the polymer solution into fine fibers. Various successful formulations were fabricated using electrospinning technology for encapsulating curcumin *via* chitosan/phospholipid, amaranth protein isolate (API) and zein nanofibers that improved the release kinetics and increased the antimicrobial and antioxidant activity of curcumin.[50] Microfluidic or microreactor technology is an upcoming technique that channels the control and miniature flow of fluids, which affects the viscosity and facilitates rapid mass and heat transfer by high surface area to volume ratio. This technology is economical, promotes high output and encapsulation of bioactive compounds utilizing a microfluidic technique providing the desired size and greater bioavailability. Encapsulation of curcumin in nanoparticles by utilizing a microfluidic reactor (gas–liquid) resulted in a reduction of particle size (50 nm).[75] Utilization of a microfluidizer technique in the preparation of palm oil based nanoemulsions for encapsulating curcumin followed by response surface methodology (RSM) resulted in improved droplet size (275.5 nm), polydispersity index (0.257), viscosity (446 cp) and zeta potential (−36.2). Thus, this technique facilitates improved particle size and bioavailability in order to achieve promising biological activities.[76] Besides, to preserve the bioactive contents, layer by layer technology was introduced

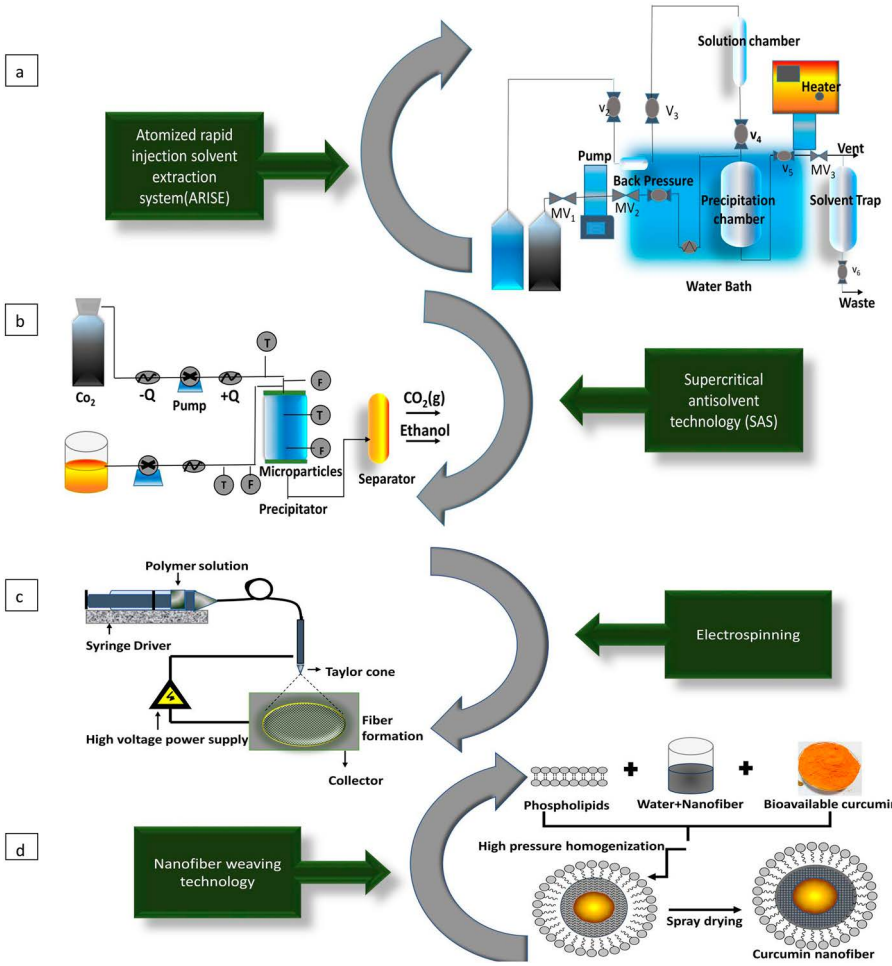


Figure 16.2 Advanced techniques: (a) atomized rapid injection solvent extraction system (ARISE); (b) supercritical antisolvent technology (SAS); (c) electrospinning; (d) nanofiber weaving technology.

which uses polyelectrolytes of opposite charges gathered in layers on the desired surface to form ultra-thin multicomposite films. This technology has gathered immense recognition for encapsulating drugs to attain sustained release drug delivery systems.[77] Numerous studies were conducted to improve the encapsulation efficacy, and drug release of curcumin using layer by layer technology which improved its biological activities.[78] Similarly, complex coacervation technology is the formation of a complex in between oppositely charged, previously soluble polyelectrolytes forming a colloidal dispersion in a dispersed phase. Complex coacervation is governed by strong electrostatic forces, hydrogen bonds, hydrophobic interactions and van der

Waals interaction. This technique utilizes two or more biopolymers to microencapsulate bioactive compounds like curcumin to produce the desired particle size, increase encapsulation efficiency and stability.[79] To produce solid dispersions of uniform shape and densities, a hot-melt extrusion method was utilized. This technology is less labor-intensive, produces better reproducibility, no organic solvent and is automatically well sound. Hot-melt technology formed stable solid dispersions of curcumin that increased solubility and produced the desired sustained release of this bioactive molecule.[80] Other technologies that improved the characteristics of curcumin were spray drying and freeze drying. Spray drying is the preferred method in the food and nutraceutical industries because it facilitates usage of different biopolymers as carriers and process ease; for example, manufacturing of curcumin microcapsules by the spray drying technique using different carrier agents such as alginate, gum arabic and chitosan showcased better physicochemical properties including shape and size and demonstrated different release profiles related to the biopolymer used.[81] A dry powder inhaler of curcumin was successfully developed by using freeze drying technology as it resulted in excellent aerosol formation with enhanced solubility. This formulation resulted in ameliorating the antimicrobial activity of curcumin.[82]

Another achievement is 'nanofiber weaving technology' to surmount the restraints of low bioavailability and solubility. This technology pledges to conserve the functional properties, meliorate the stability and favor controlled release of the active moiety curcumin. Positive results were obtained from the physicochemical characteristics that were studied including the morphology, particle size distribution, rheological properties and zeta potential.[83] Recently, turmeric nanofiber weaving technology was utilized for incorporating curcumin complex and asafoetida, a formulation named 'gut health product', that provided promising results on rat models exhibiting clear anti-colitis activity.[84] This technology promotes health, paving the way for an improvised version of curcumin in the market (Figure 16.2d). Apart from this, the Aurea Biolabs team brought a novel technology to the research rostrum, 'Cureit', developed through incessant efforts of our research team to revive a complete natural turmeric matrix (CNTM) to form improved bioavailable curcuminoids by polar-nonpolar sandwich technology (PNS).[85]

16.3.3 Novel Curcumin Encapsulates

There are various formulations that have cropped up in recent years to enrich curcumin delivery to the target site as well as to enhance the bioavailability and solubility of the same. Liposomes, especially theranostic liposomes, nutriosomes, phytosome complex, nanotubes, nanomesh and marinosomes, are various recent nanotechnological developments rendering therapeutic potential, site specific delivery of bioactive compounds with the least cytotoxicity (Figure 16.3) (Table 16.3). Nutriosomes are an upcoming novel discovery, which not only increase the bioavailability and biodistribution of curcumin but also facilitate its oral delivery. These nanosized phospholipid

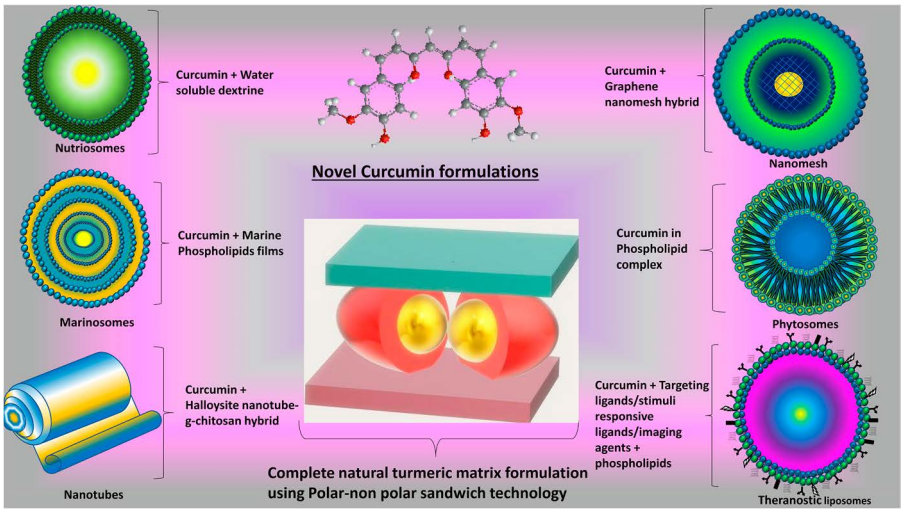


Figure 16.3 Novel curcumin formulations.

vesicles are about 168 nm and fabricated successful intestinal protection.[86] In addition, liposomes of krill lipids known as marinosomes have recently fueled delivery of curcumin enhancing their bioavailability and providing cost-effective nutraceuticals, displaying an increase in encapsulation efficiency, Herculean antioxidant property and oxidative stability inhibiting the proliferation of lung cancer cells (A549) in a dose-related manner thereby exhibiting improved anticancer properties of curcumin; hence, tailoring a promising pathway for the targeted delivery of curcumin.[87] Moreover, there are recently developed halloysite nanotubes which utilize aluminum silicate clay for the fabrication of a hollow tubular structure of a range of sub-micrometers, having the capability to remove macrophages from living cells. The inner lumen contains aluminol groups and the outer surface contains a siloxane group and the capability of modification of their surfaces by supramolecular interactions or chemically, broadens their scope in various applications. These halloysites are considered safe and have the capability to cross the cell barrier to penetrate the nuclei. Hence, halloysite nanotubes are a promising drug carrier for oral or topical delivery of bioactive compounds.[88] Beside this, a report suggested that the antibacterial activity of curcumin is refined when it is functionalized with graphene nanomesh produced by an electrochemical exfoliation procedure. The pyrolytic graphite in ammonium persulfate solution of 1M is the basis of forming graphene nanomesh. Both curcumin as a control and a hybrid of curcumin/nanomesh were compared which demonstrated that the latter possesses greater antibacterial activity against *Staphylococcus aureus*, gram positive bacteria.[89] In this series, phytosomes have emerged as a promising delivery system for bioactive molecules, which were fabricated by applying specific conditions to mix different molar ratios of active constituents with phospholipids.

Table 16.3 Recently developed formulations for curcumin supplements.

S. No	Formulation	Method	Physicochemical parameters	Improvements	Reference
1	Nutriosomes	Soluble dextrin (Nutriose FM06)	Mean dia.:127–183 nm Zeta pot. (mV): −12 to −38	Bioavailability, biodistribution	86
2	Marinosomes	Reflux, thin drug lipid film method	Mean dia.: −127–183 nm Zeta pot. (mV): −13 to −14	Bioavailability	87
3	Nanotubes	—	Length: 0.2–1.5 μm Inner dia.: 10–30 nm Outer dia.: 40–70 nm Zeta pot. (mV): −50–60	Removal of macrophages, easily cross cell barrier	88
4	Nanomesh	Electrochemical exfoliation	Mean dia.: −15 nm Zeta pot. (mV): −30 to −41	Antibacterial activity	89
5	Phytosomes	Solvent evaporation/freeze drying/antisolvent precipitation	Mean size: −50 nm–100 μm Zeta pot.: 153 nm to 10.09 mV	↑ plasma absorption and bioavailability	90
6	Theranostic liposomes	Double solvent displacement/ solvent injection/thin film hydration & extrusion/micro-fluidization/super critical carbon-dioxide	Dia.: −178–182 nm Zeta pot. (mV): 0.95 to 1.10	Improved site-specific targeting/imaging/ biomarker/response to external &internal stimuli/controlled drug release profile	11 91

To exemplify, a promising phytosomal formulation used as a chemopreventive drug, reported improved plasma absorption and increased the oral bioavailability of curcumin.[90] Recently, a promising formulation known as 'theranostic liposomes' has emerged, which utilizes both diagnosis and therapy to deliver efficient site-specific controlled targeting with the caliber of imaging, biomarker and internal or external stimuli. These liposomes comprise phospholipid, stimuli-responsive lipids or targeting ligands, therapeutic components and imaging agents.[11] The stimuli responsive properties of theranostic liposomes are designed by incorporating reactive oxygen species (ROS) responsive functions that have been studied through a laboratory built nano-flow cytometer. These studies revealed proper functionalism of liposomes with controlled release and promising ROS sensing ability.[91] Thus, these liposomes could decrease side effects with increased therapeutic outcomes which facilitate their usage in efficient tumor diagnosis and reduction, brain targeting and so on.

16.4 Imminent Curcumin Encapsulated Supplements

There are numerous marketed bioavailable products developed from varied encapsulated formulations such as phytosomes, liquid micelles, micronized, colloidal nanoparticles, solid lipid nanoparticles, cyclodextrin formulations and complete natural turmeric matrix formulations using PNS technology. The research associated with improvement in curcumin supplementation also utilizes food-grade excipients because of the difficulty related to storage and alteration in physical chemical properties experienced with non-food grade excipients. Recent research described a water-soluble formulation known as curcumagalactomannosides (CGM), which promised enhanced oral bioavailability and safety. This formulation incorporated galactomannans, a food-grade hydrocolloid derived from fenugreek. Furthermore, preclinical studies on Wistar rats exemplified their safety profile by studying their sub-chronic, acute toxicity and genotoxicity. The efficacy of the formulation was reported by measuring post-blood plasma curcumin levels at regular time intervals by an electrospray ionization tandem mass spectrometer (ESI-MS/MS) and high-performance liquid chromatography with a photodiode array detector (HPLC-PDA). The results showed 10 times higher bioavailability and safe intake up to 2.0 g kg^{-1} per day compared to curcumin that was unformulated.[5]

Our research team, with their continuous efforts, developed a breakthrough formulation which gave the medicinal market a plenary of a more bioavailable form of curcumin benevolent to relieve human suffering. This formulation is devoid of the usual liposomes, micelles, or phospholipid matrix and rather involves PNS incorporating water soluble protein portions of turmeric and dietary fibers. Initially, our team tested the authenticity of 'Cureit' by elastase inhibition activity through a cell culture study. Elastase,

a degrading enzyme, is localized in the epithelial cells and their inhibition is responsible for the anti-inflammatory activity. For this study, porcine elastase was used to study different concentrations of 'Cureit' and was analyzed spectrophotometrically. The outcome revealed that a higher concentration (100 mg L^{-1}) of Cureit was beneficial and bio-efficient. Also, the co-culture study of keratinocytes and melanocytes were conducted for Cureit, which showed that they are promising to keep the functionality, integrity and morphology of the cell consistent.[92] In the same way, Gopi *et al.* (2014), through a cell culture study, provided ample information regarding the hyaluronidase inhibition property of Cureit, which was up to 42% promoting it as an anti-aging agent. They even explored the improved antioxidant property of Cureit through a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay which demonstrated promising results that were equivalent to ascorbic acid.[93] Even the anticancer potential of Cureit was studied by conducting a 3-(4, 5-Dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) assay on adenocarcinoma (LnCAP), human embryonic kidney (HEK 293 T), breast cancer (MCF-7) cells to observe their cell proliferation capability. The outcome of the results demonstrated that Cureit is a potent anticancer agent. This novel technology was thoroughly tested through pilot cross-over clinical studies on 12 human volunteers divided into groups of two (six each) who were given a 500 mg capsule of Cureit and curcumin 95%, respectively. The chromatographic analysis of the plasma proved that patients who were given Cureit capsules showed better tolerance and were safer than those who were given curcumin 95%.[94] In this series, another clinical trial on double-blind, randomized, placebo-controlled, parallel-group research was conducted to reveal the dose effective benefits of Cureit on patients suffering from rheumatoid arthritis (RA). In this study, two groups, each with 12 members, were subjected to 250 and 500 mg oral dose twice daily for 90 days, respectively, and the responses from the subjects were analyzed through visual analog scale (VAS), rheumatoid factor values (RF), c-reactive protein (CRP), American college of rheumatology response (ACR), disease activity score 28 (DAS28) and erythrocyte sedimentation rate (ESR). The remarkable changes in these values suggested that this natural turmeric matrix is beneficial at low (250 mg) as well as high (500 mg) doses as they are reasonably tolerated with no side effects, displaying their anti-inflammatory and analgesic properties to cure RA.[95]

These studies led the team to delve into the ostentatious applications of Cureit by analyzing it through scanning electron microscopy (SEM), Fourier transform infrared (IR), quadrupole time-of flight mass spectrometry (Q-TOF), thermogravimetric analysis (TGA), X-ray diffraction (XRD) and nuclear magnetic resonance spectroscopy (NMR). In which, the clarity of structure of Cureit was attained by SEM images denoting well dispersed spherical morphology with distinct layers of a polar and non-polar matrix. XRD, IR, TGA, DSC all entrenched the presence of curcuminoids in the PNS matrix that involved GRAS certified carrier agents. Through Q-TOF, the presence of different components was confirmed such as curcuminoids, sesquiterpenes, lactones in the polar layer and the non-polar layer comprised

turmeronol, dihydroturmerone, aromatic turmerone, bisacurone and curdione. Also, the metabolic profile of Cureit was studied through a mass spectrometer. The interlinking of the polar and non-polar layers by hydrogen bonding in the PNS matrix was affixed by NMR spectral data. Moreover, the study promised a potent delivery profile of a more bioavailable form of curcumin possessing various biological activities without any alteration in structure and physical properties, definitely proving economical and possessing minimal adverse effects. Furthermore, the potency of Cureit was established by Gopi *et al.* (2017) by comparing it with other two bioavailable formulations of curcumin, one containing a volatile oil composition and the other a phospholipid-cellulose formulation through clinical trials on 15 adults (male). These subjects were given an oral supplement of 500 mg of each capsule and blood aliquots were studied at distinct intervals through liquid chromatography mass spectrometry (LCMS) for a period of 24 h. The superiority of Cureit rooted in blood plasma evaluation revealed that Cureit exhibited a greater bioavailability than the other two formulations.[96] Hence, Cureit is definitely an upcoming advancement promoting the green drug curcumin, reducing its flaws by improving the bioavailability to be utilized in various applications. However, more clinical trials must be conducted to affix its position to be recognized in the medical market.

16.5 Conclusion and Future Perspectives

The present study deals with the potency of curcumin in dietary supplements. Curcumin supplements and their benevolent effects in curing various disease conditions associated with changes in lifestyle and diet such as cardiovascular, gastrointestinal, hormonal, bone, bacterial conditions, as wells as viral infections and diabetes, along with evidence-based studies prove promising in this advanced technological era. Certain curcumin supplements that employ food-grade carrier agents (GRAS certified) and recent encapsulating technologies and formulations are discussed in this review, so that they can be utilized for more formulations of curcumin and other bioactive compounds to oppugn the malignant disease state.

Although there are numerous curcumin formulations available in the market such as curcumin extract (95%), curcumin phytosomes, colloidal dispersion of curcumin, optimized curcumin extract and so on, which improve the bioavailability and help to increase the therapeutic activities of curcumin, this review particularly focuses on Cureit, a bioavailable formulation of curcumin with proven clinical benefits. Moreover, this review tries to establish an inquisitiveness among the scientific society as there is still a lacuna of knowledge about curcumin supplements. In addition, a need for more rigorous studies exploring the encyclopedic properties and mechanisms involved that facilitate these food-derived carrier agents (lipids, proteins and phospholipids), advanced encapsulation techniques (ARISE, SAS, electrospinning, nanofiber weaving technology, PNS) and formulations (nutriosomes, marinosomes, nanotubes, nanomesh and complete natural turmeric matrix)

to serve versatile and efficient delivery systems for bioactive drug complexes is warranted. Finally, more evaluations *in vivo* or excessive clinical studies have yet to be conducted involving these upcoming revelations. Incorporation of more GRAS certified plant-grade carrier agents along with comparison with other non-food grade carrier agents have yet to be explored. Also, the lack of large-scale insight needs to be addressed to generate a safe and efficient profile for human survival. These prospects can be worked upon by comprehending and elucidating these delivery systems. Incorporating curcumin supplements with the utilization of food-grade excipients can help establish their status in the medical market.

Conflict of Interest

The authors profess no conflict of interest in this chapter.

Acknowledgements

The authors take this chance as an esteemed privilege to show their gratitude to the management of Aurea Biolabs (P) Ltd., Cochin, India, who were an invariable source of support to put pen to paper and encouraged us during the entire course of this chapter. We also grab this golden opportunity to express deep appreciation to our colleagues for their valuable support that accelerated the successful completion of this task.

References

1. I. Magrath and J. Litvak, *J. Natl. Cancer Inst.*, 1993, **85**, 862.
2. Z. Teng, R. Xu and Q. Wang, *RSC Adv.*, 2015, **5**, 35138.
3. S. Wanninger, V. Lorenz, A. Subhan and F. T. Edelmann, *Chem. Soc. Rev.*, 2015, **44**, 4986.
4. H. Vogel and J. Pelletier, *J. Pharm.*, 1815, **2**, 50.
5. V. B. Liju, K. Jeena, D. Kumar, B. Maliakel, R. Kuttan and I. M. Krishnakumar, *J. Food Funct.*, 2014, **6**, 275.
6. S. Prasad and B. Aggarwal, *Biotechnol. Adv.*, 2014, **32**, 1053.
7. A. Amalraj, A. Pius, S. Gopi and S. Gopi, *J. Tradit. Complementary Med.*, 2017, **7**, 205.
8. R. Jamwal, *J. Integr. Med.*, 2018, **16**, 367.
9. E. Pagano, B. Romano, A. A. Izzo and F. Borrelli, *Pharmacol. Res.*, 2018, **134**, 79.
10. L. Zou, B. Zheng, R. Zhang, Z. Zhang, W. Liu and C. Liu, *RSC Adv.*, 2016, **6**, 3126.
11. M. R. I. Shishir, N. Karim, V. Gowd, X. Zheng and W. Chen, *Trends Food Sci. Technol.*, 2019, **85**, 177.
12. H. Yu and Q. Huang, *Agric. Food Chem.*, 2012, **60**, 5373.
13. R. Ravichandran, *J. Biomater. Nanobiotechnol. Sci. Res.*, 2013, **4**, 291.

14. S. Prasad, A. K. Tyagi and B. B. Aggarwal, *Cancer Res. Treat.*, 2014, **46**, 2.
15. H. Li, A. Sureda, H. P. Devkotad, V. Pittalàe, D. Barreca, A. S. Silvag, D. Tewari, S. Xuj and S. M. Nabavik, *Biotechnol. Adv.*, 2020, **38**, 107343.
16. J. Zou, S. Zhang, P. Li, X. Zheng and D. Feng, *Nutr. Res.*, 2018, **56**, 32.
17. B. B. Aggarwal and K. B. Harikumar, *Int. J. Biochem. Cell Biol.*, 2009, **41**, 40.
18. C. Vernieria, F. Nichettia, A. Raimondia, S. Pusceddua, M. Plataniaa and F. Berrinoc, *Crit. Rev. Oncol. Hematol.*, 2018, **123**, 57.
19. G. Derosa, G. Catena, R. Raddino, G. Gaudio, A. Maggi and A. D. Angelo, *Phytomed*, 2018, **42**, 75.
20. H. Kaur, A. Bal and R. Sandhir, *Pharmacol., Biochem. Behav.*, 2014, **125**, 55.
21. S. Sharma, Y. Zhuang, Z. Ying, A. Wu and F. Gomez-Pinilla, *Neuroscience*, 2009, **161**, 1037.
22. A. Wu, E. E. Noble, E. Tyagi, Z. Ying, Y. Zhuang and F. Gomez-Pinilla, *Biochim. Biophys. Acta, Mol. Basis Dis.*, 2014, **1852**, 951.
23. H. Khandaker, A. Aubry, R. Ravenelle, D. Gordian, C. Ubri, G. Schafe and N. Burghardt, *Biol. Psychiatry*, 2017, **81**, S62.
24. R. Shegokar, *Emerging Nanotechnol. Immunol.*, 2018, 203.
25. R. Bhandari and A. Kuhad, *Life Sci.*, 2015, **141**, 156.
26. A. Amalraj, N. P. Sukumaran, A. B. Kunnumakkara and S. Gopi, in *Curcumin for Neurological and Psychiatric Disorders, Neurochemical and Pharmacological Properties*, ed. T. Farooqui and A. A. Farooqui, Academic press, USA, 2019, vol. 6, pp. 105–127.
27. J. Jiang, X. Y. Wu, X. Q. Zhou, L. Feng, Y. Liu and W. D. Jiang, *Aquaculture*, 2016, **463**, 174.
28. H. K. Mahmoud, A. A. Al-Sagheer, F. M. Reda, S. A. Mahgoub and M. S. Ayyat, *Aquaculture*, 2017, **475**, 16.
29. T. Arablou and R. Kolahdouz-Mohammadi, *Biomed. Pharmacother.*, 2018, **97**, 91.
30. L. Ge, Y. Xu, X. Li, L. Yuan, H. Tan and D. Li, *ACS Sustainable Chem. Eng.*, 2018, **6**, 9153.
31. W. Xu, W. Jin, L. Lin, C. Zhang, Z. Li and Y. Li, *Carbohydr. Polym.*, 2014, **101**, 961.
32. Y. Lyu, M. Yu, Q. Liu, Q. Zhang, Z. Liu, Y. Tian, D. Li and C. Mu, *Carbohydr. Polym.*, 2020, **230**, 115573.
33. M. D. Baldissera, C. F. Souza, C. C. Zeppenfeld, S. D. Descovi, V. S. Machado and C. V. Roberto, *Microb. Pathog.*, 2018, **116**, 237.
34. D. Mathew and W. L. Hsu, *J. Funct. Foods*, 2017, **40**, 692.
35. B. C. Mounce, T. Cesaro, L. Carraua, T. Vallet and M. Vignuzzi, *Antiviral Res.*, 2017, **142**, 148.
36. J. Ming, J. Ye, Y. Zhang, Q. Xu, X. Yang, X. Shao, J. Qiang and P. Xu, *Fish Shellfish Immunol.*, 2020, **97**, 540.
37. N. Parsamanesh, M. Moossavi, A. Bahrami, A. E. Butler and A. Sahebkar, *Pharmacol. Res.*, 2018, **136**, 181.
38. S. Rivera-Mancía, M. C. Lozada-García and J. Pedraza-Chaverri, *Eur. J. Pharmacol.*, 2015, **75**, 30.

39. I. S. V. D. Melo, A. F. D. Santos and N. B. Bueno, *Pharmacol. Res.*, 2018, **128**, 137.
40. M. Hie, M. Yamazaki and I. Tsukamoto, *Eur. J. Pharmacol.*, 2009, **621**, 1.
41. Y. Henrotin, F. Priem and A. Mobasheri, *SpringerPlus*, 2013, **2**, 56.
42. J. Jacob, A. Amalraj, K. K. J. Raj, C. Divya, A. B. Kunnumakkara and S. Gopi, *J. Tradit. Complementary Med.*, 2019, **9**, 346.
43. N. Yang, K. Sampathkumar and S. C. J. Loo, *Clin. Nutr.*, 2017, **36**, 968.
44. J. M. Cooney, M. P. G. Barnett, Y. E. M. Dommels, D. Brewster, C. A. Butts and W. C. McNabb, *J. Nutr. Biochem.*, 2016, **27**, 181.
45. G. Padmanaban and P. N. Rangarajan, *Trends Pharmacol. Sci.*, 2016, **37**, 1.
46. A. S. Strimpakos and R. A. Sharma, *Antioxid. Redox Signal.*, 2008, **10**, 511.
47. B. J. Douglass and D. L. Clouatre, *J. Am. Coll. Nutr.*, 2015, **34**, 347.
48. L. Zou, W. Liu, C. Liu, H. Xiao and D. J. McClements, *Food Funct.*, 2015, **6**, 2475.
49. P. Wen, M. H. Zong, R. J. Linhardt, K. Feng and H. Wu, *Trends Food Sci. Technol.*, 2017, **70**, 56.
50. Z. L. Wan, J. Guo and X. Q. Yang, *Food Funct.*, 2015, **6**, 2876.
51. R. R. Balandran-Quintana, A. M. Mendoza-Wilson, G. R. C. Montfort and J. A. Huerta-Ocampo, in *Proteins: Sustainable Source, Processing and Applications*, ed. C. M. Galanakis, 2019, vol. 4, pp. 97–130.
52. M. R. Kasaai, *Trends Food Sci. Technol.*, 2018, **79**, 184.
53. J. Gomez-Estaca, M. P. Balaguer, R. Gavara and P. Hernandez-Munoz, *Food Hydrocolloids*, 2012, **28**, 82.
54. F. P. Chen, S. Y. Ou, Z. Chen and C. H. Tang, *J. Agric. Food Chem.*, 2017, **65**, 1707.
55. H. Xiang, D. Sun-waterhouse, C. Cui, W. Wang and K. Dong, *J. Food Chem.*, 2018, **268**, 504.
56. R. Mohammadinejad, S. Karimi, S. Iravani and R. S. Varma, *Green Chem.*, 2015, **18**, 20.
57. S. Yang, L. Dai, C. Sun and Y. Gao, *Food Hydrocolloids*, 2018, **85**, 185.
58. M. F. Li, Z. Y. He, G. Y. Li, Q. Z. Zeng, D. X. Su and J. L. Zhang, *J. Food Hydrocolloids*, 2019, **90**, 482.
59. Q. Z. Zeng, M. F. Li, Z. Z. Li, J. L. Zhang, Q. Wang and S. L. Feng, *Food Sci. Technol.*, 2019, **105**, 79.
60. L. Zou, W. Liu, C. Liu, H. Xiao and D. J. McClements, *Food Funct.*, 2015, **6**, 2475.
61. F. Alavi, Z. Emam-Djomeh, M. S. Yarmand, S. Momen and A. A. Moosavi-Movahedi, *Food Hydrocolloids*, 2018, **85**, 267.
62. Z. Teng, Y. Li and Q. Wang, *J. Agric. Food Chem.*, 2014, **62**, 8837.
63. J. Liu, L. Lei, F. Ye, Y. Zhou and H. G. R. Younis, *Food Funct.*, 2017, **9**, 491.
64. K. Pan, Q. Zhong and S. J. J. Baek, *J. Agric. Food Chem.*, 2013, **61**, 6036.
65. N. Ghayoura, S. M. H. Hosseini, M. H. Eskandari, S. Esteghlal, A. R. Nekoei, H. N. Gahruie, M. Tatar and F. Naghibalhossaini, *Food Hydrocolloids*, 2019, **87**, 394.
66. M. Massaro, R. Amorati, G. Cavallaro, S. Guernelli, G. Lazzara, S. Milioto, R. Noto, P. Pomad and S. Riela, *Colloids Surf., B*, 2016, **140**, 505.

67. K. Oehlke, M. Adamiuk, D. Behsnilian, V. Graf, E. Mayer-Miebach and E. Walz, *Food Funct.*, 2014, **5**, 1341.
68. X. Lu, J. Chen, Z. Guo, Y. Zheng, M. C. Rea, H. Su, X. Zheng, B. Zheng and S. Miao, *Trends Food Sci. Technol.*, 2019, **86**, 311.
69. R. Richa and A. R. Choudhury, *Int. J. Biol. Macromol.*, 2020, **156**, 1287–1296.
70. L. Z. Cheong, Z. Guo, B. M. Lue, R. Miklos, S. Song, W. Panpipat and X. Xu, in *Lipids in Nanotechnology*, ed. M. U. Ahmad, Academic Press and AOCS press, USA, 2012, vol. 2, pp. 15–51.
71. F. Wang, Y. Yang, X. Ju, C. C. Udenigwe and R. He, *J. Agric. Food Chem.*, 2018, **66**, 2685.
72. F. Kurniawansyah, L. Quachie, R. Mammucari and N. R. Foster, *Int. J. Pharm.*, 2017, **521**, 239.
73. A. Arango-Ruiz, A. Martin, M. J. Cosero, C. Jiménez and J. Londono, *Food Chem.*, 2018, **258**, 156.
74. R. Leidy and Q. C. M. Ximena, *Trends Food Sci. Technol.*, 2019, **85**, 92.
75. L. Zhang, Q. Chen, Y. Ma and J. Sun, *ACS Appl. Bio Mater.*, 2020, **3**, 107.
76. R. Raviadaran, D. Chandran, L. H. Shin and S. Manickam, *LWT–Food Sci. Technol.*, 2018, **96**, 58.
77. O. S. Sakr and G. Borchard, *Biomacromolecules*, 2013, **14**, 2117.
78. P. Priya, R. M. Raj, V. Vasanthakumar and V. Raj, *Arabian J. Chem.*, 2020, **13**, 694.
79. N. Shahgholian and G. Rajabzadeh, *Food Hydrocolloids*, 2016, **59**, 17.
80. W. Fan, W. Zhu, X. Zhang and L. Di, *J. Pharm. Sci.*, 2020, **109**, 1242.
81. J. Lucas, M. Ralaivao, B. N. Estevinho and F. Rocha, *Powder Technol.*, 2020, **362**, 428.
82. H. Yu, T. T. Tran, J. Teo and K. Hadinoto, *Colloids Surf.*, 2016, **504**, 34.
83. S. Gopi, A. Amalraj, J. Jacob, N. Kalarikkal, S. Thomas and Q. Guo, *New J. Chem.*, 2018, **42**, 5117.
84. S. Gopi, A. Amalraj, S. Jude, K. Varma, T. R. Sreeraj, J. T. Haponiuk and S. Thomas, *Mater. Sci. Eng., C*, 2017, **81**, 20.
85. A. Amalraj, S. Jude, K. Varma, J. Jacob, S. Gopi and O. S. Oluwafemi, *Mater. Sci. Eng., C*, 2017, **75**, 359.
86. A. Catalan-Latorre, M. Pleguezuelos-Villa, I. Castangia, M. L. Manca, C. Caddeo and A. Nacher, *Nanoscale*, 2017, **10**, 1957.
87. S. Ibrahima, T. Tagami, T. Kishi and T. Ozeki, *Int. J. Pharm.*, 2018, **540**, 40.
88. M. Massaro, G. Lazzara, S. Milioto, R. Notoa and S. Riela, *J. Mater. Chem. B*, 2017, **5**, 2867.
89. Z. M. Markovic, D. P. Kepic, D. M. Matijasevic, V. B. Pavlovic, S. P. Jovanovic and N. K. Stankovic, *RSC Adv.*, 2017, **7**, 36081.
90. M. Lu, Q. Qiu, X. Luo, X. Liu, J. Sun and C. Wang, *Asian J. Pharm. Sci.*, 2018, 1.
91. C. Chen, K. Gao, H. Lian, C. Chen and X. Yan, *Biosens. Bioelectron.*, 2019, **131**, 185.
92. S. Gopi, R. George and V. T. Sriraam, *Br. Biomed. Bull.*, 2014, **2**, 545.
93. S. Gopi, R. George, S. Jude and V. T. Sriraam, *J. Chem. Pharmacol. Res.*, 2014, **6**, 96.

94. S. Gopi, R. George, M. Thomas and S. Jude, *Asian J. Pharm. Technol. Innovation*, 2015, **3**, 92.
95. A. Amalraj, K. Varma, J. Jacob, C. Divya, A. B. Kunnumakkara and S. J. Stohs, *J. Med. Food*, 2017, **20**, 1022.
96. S. Gopi, J. Jacob, K. Varma, S. Jude, A. Amalraj, C. A. Arundhathy, R. George, T. R. Sreeraj, C. Divya, A. B. Kunnumakkara and S. J. Stohs, *Phytother. Res.*, 2017, **31**, 1883.

Subject Index