Horse Chestnut: Cultivation for Ornamental Purposes and Non-Food Crop Production

Article *in* Journal of Herbs Spices & Medicinal Plants · April 2005

DOI: 10.1300/J044v11n01_04

2 authors:

Elvio Bellini
Centro di Studio e Documentazione sul Castagno (CSDC)
133 PUBLICATIONS **1,472** CITATIONS

SEE PROFILE

Stefania Nin
Council for Agricultural Research and Economics
52 PUBLICATIONS **552** CITATIONS

SEE PROFILE

Horse Chestnut: Cultivation for Ornamental Purposes and Non-Food Crop Production

E. Bellini
S. Nin

ABSTRACT. The horse chestnut (*Aesculus hippocastanum* L.) is a common ornamental tree that possesses numerous useful derivatives (escin, cholesterol-escin complex, glycolic, soft and dry extracts, esculin) in different parts of the plant, especially the seeds and trunk bark. These derivatives are widely used in dermatology and pharmacology, mainly for the treatment of peripheral chronic venous insufficiency, but also in the cosmetic field for the treatment of unattractive features, such as couperose, cellulites and hair loss. More recently proanthocyanidin A_2, present in the fruit pericarp, bark, leaves, and buds, has shown a protective effect against UV damage. Very little, however, is known about methods for cultivation, chemical composition of seeds of various genotypes, optimum harvest-time of plant tissues, or storage conditions for seeds. This review has been done within the context of the EU research project on the "European *Aesculus* Cultivation System" in order to better understand the growth and pharmacological properties of secondary metabolites from horse chestnut and may represent the first step towards use of the plant in non-food, income-generating arboriculture of European countries.

E. Bellini and S. Nin are affiliated with the Horticulture Department, 50019 Sesto Fiorentino (FI), Italy.

Received July 10, 2002.

Journal of Herbs, Spices & Medicinal Plants, Vol. 11(1/2) 2004
http://www.haworthpress.com/web/JHSMP

Digital Object Identifier: 10.1300/J044v11n01_04

KEYWORDS. *Aesculus hippocastanum*, seed derivatives, escin, properties and uses

INTRODUCTION

Among the species belonging to the genus *Aesculus*, the horse chestnut (*A. hippocastanum* L.) is conspicuously important, used widely as an ornamental and landscape plant in parks and gardens all over the world because of the beauty and hardiness of the plant. Horse chestnut is a majestic tree having a great ornamental presence as a consequence of the growth habit, the thick and fine foliage that develops early in spring, the high shading capacity, the abundant, showy, and long-lasting flowering period, and the longevity of the plant. Sometimes this tree is used to consolidate erosion-prone slopes and for environmental forestry plantings.

The horse chestnut is also the source of numerous chemical compounds that find wide application in world pharmacopoeias. Derivatives of the seeds (for example, escin, cholesterol-escin complex, glycolic, and soft and dry extracts) have been widely investigated and are used in dermatology and pharmacology. Because of the numerous ornamental and environmental qualities and the medicinal properties, the horse chestnut, similarly to several other trees, is attracting increasing attention as an ornamental plant and as a source of organic compounds (6).

The European supplies of horse chestnut fruit for industrial use at this time are obtained almost exclusively from trees planted in gardens or along the roads (Table 1). The medicinal industry, however, demands a

TABLE 1. Common names of horse chestnut.

German:	Gemeine Rosskastanie, Weisses Rosskastanie, Judkest, Pferdekastanie, Rosskastanie, Wilde Kastanie, Wildi Kest[1]
English:	Common horse-chestnut, Conqueror tree, Horse-chestnut, Bongay
French:	Châtaignier de cheval, Châtaignier de mer, Marronier d'Inde
Italian:	Castagno amaro, Castagno cavallino, Castagño d'India, Marron d'India, Ippocastano
Portuguese:	Castanhas da India, Castanheiro da India
Spanish:	Castaño de India, Hipocastaño

[1] Data from Hänsel et al. (33), Merendi (58), and Rossi and Lotti (84).

reliable year-round supply of high quality plant materials. To meet these requirements without risking the raw vegetable material being replaced by synthetic products, appropriate cultivation techniques, rational collection methodologies, clonal selection systems (both genetically and sanitary), and methodologies for adequate processing of the product must be developed.

Today, the European demand for *Aesculus* seeds (estimated to be more than 2000 tons of dried material per year) is supplied almost exclusively by Central and East European countries (Hungary and Poland). Seeds are currently collected in urban areas from trees severely damaged by the fungus *Guignardia aesculi* and the insect *Cameraria ohridella*. To ensure an adequate and constant supply of quality plant material for the European pharmaceutical industry, a more reliable source of plant material is necessary.

For this purpose, a research project, entitled "European *Aesculus* Cultivation System" was initiated in 1993, and supported by the European Community under the coordination of Madaus AG (Köln, Germany) and with the collaboration of the Department of Horticulture of the University of Florence (Italy), the Institute of Tree Growing of the University of Sassari (Italy) and the Forestry Commission Northern Research Station (Roslin, Scotland) (6). The main objectives of this project were the collection and evaluation of germplasm with the identification of the best ecotypes, the development of efficient techniques for vegetative propagation, the development of appropriate cultivation techniques, the determination of soil and climatic effects on the growth and development, the creation of a horse chestnut clone bank, the development of analytical methodology for the rapid estimation of escin content in a large number of samples by TLC, and studies on the environmental impact and the of the horse chestnut with particular regard to prevention of soil erosion in the more erosion-prone zones. To better understand the growth and physiology of horse chestnut as well as the medicinal properties of its secondary metabolites the existing literature on this species was reviewed.

ORIGIN OF HORSE CHESTNUT

In the last century, botanists believed that the horse chestnut came originally from Asia, whence was derived the Italian name for the fruit "Castagna d'India." In reality, this species is native to the mountains of Albania, Bulgaria, and the north of Greece, where the horse chestnut

grows wild, forming mixed woods with *Alnus glutinosa*, *Juglans regia*, *Fraxinus excelsior*, and *Acer platanoides*. Fossil seeds of horse chestnut have been found in the Upper Pliocene at Frankfurt am Main, and seeds and leaves of other *Aesculus* species have been found in various parts of Central Europe (2,15,83).

Although horse chestnut was known to the Greeks, the species was named and depicted for the first time in the 16th century in the Commentaries of Mattioli (50). The tree, which was brought to northern and western Europe from Constantinople, was introduced into cultivation by Charles de l'Escluse, who sowed fruit from the tree in Vienna about 1576, and, later, by a certain Bachelier who planted the tree in his garden in Paris in 1615 (50). In Italy, the first fruit of horse chestnut arrived in 1657, and two centuries later the tree was distributed throughout Europe, so that today no city or village is without a horse chestnut tree (8,30,36,49,83).

The name *Aesculus* (from *esca*, food) was applied originally to a species of oak that, according to Pliny, was highly appreciated for its acorns, although how this name was subsequently transferred to the horse chestnut is still uncertain (2). As Loudon (52) suggested, the name *Aesculus* was probably attributed to horse chestnut because the seeds of the tree very much resemble those of the chestnut, but are not usable as food. Some writers think that the prefix *horse* is a corruption of the Welsh *gwres*, which means hot, fierce, or pungent (whence horse chestnut = bitter chestnut) in contrast to the mild, sweet chestnut. The species name *hippocastanum* is a translation of the common name given to broken-winded horses that can be cured with the seeds of the horse chestnut (30).

BOTANY OF HORSE CHESTNUT

Horse chestnut, *Aesculus hippocastanum*, is in the small Hippocastanaceae family, belonging to the genus *Aesculus* which includes horse chestnut (common, red, Japanese, Indian and dwarf horse chestnut) and buckeye trees. The genus consists of about 25 species originating from North America, South-East Europe, and East Asia to India. The systematic classification of these species is not easy because of the considerable polymorphism and frequent hybridism. This hybridization, which involves *A. hippocastanum* and four other species, assumes primary importance in studies of the genus because of the great confusion relating to the concept of species and of the problems of identifying

buckeyes in north-east America among the considerable number of mongrel forms encountered in natural populations and gardens (3,16,39).

The literature related to this group of species is conspicuous because of the economic importance attributed to various species in the genus *Aesculus*. A large part of this literature is summarized in a publication by Rehder (82) and in a guide published by the United States Department of Agriculture (13). The list of synonyms for each of species is very extensive with approximately 175 names have been attributed to 15 species and hybrids known in the genus. Most of these names have been summarized by Rehder (82) and to a minor extent in other publications (87). The following synonyms have been attributed to horse chestnut (*Aesculus hippocastanum* L.): *A. castanea* Gilib., *A. procera* Salisb., *Castanea equina*, *Hippocastanum vulgare* Gaertner (36).

A general organizational scheme for members of the genus *Aesculus* has been proposed by Rehder (82) by means of a sub-division into four sections, based on floral, leaf and fruit morphological characteristics and types of winter buds (22). According to this botanical classification horse chestnut belongs to the section *Hippocastanum* K. Koch (synonym *Euesculus Pax*). Several varieties and botanical forms of *A. hippocastanum* are known (Table 2).

The horse chestnut, a deciduous tree that lives for more than 200 years, can grow to a height of 25 to 30 m and has ovoid, pointed buds, three to five cm long, that are sticky and dark reddish-brown in color. The leaves are palmate with long petioles and five to seven leaflets. Leaflets are sessile and can reach 20 cm in length. The flowers, which generally appear only on trees of over 14 to 15 years old, are hermaphrodite or male, approximately 2 cm long and combined in large, erect, terminal, compound racemes of pyramidal form. Not all the flowers are fertile or functionally complete.

The flower corolla is white with yellow and red spots at the base, although two less common varieties native to the southern United States and rare in Europe exist, one characterized by yellow flowers and the other by pale red flowers. The fruit is dry, dehiscent, trilocular (generally through failure 1-2 bilocular), capsule type that is a brilliant green in color that becomes a brownish color on the exterior and white internally. At maturity, the fruit, which can reach a diameter of 6 cm and has a thick, sub-globose coriaceous with a spiny pericarp and a fine pubescence, opens in three valves. Each fruit contains from one to four brown-colored, globose or faceted, exalbuminate seeds with two irregular, large, fleshy, oily, and amylaceous cotyledons that are often joined (5,18,55,82,97).

TABLE 2. Varieties and botanical forms of *A. hippocastanum*.

Varieties and forms[1]	Characteristics
baumanii Schneid. var. (f.) syn. *flore pleno* Lemaire	Double white flowers, without fruits, the best of the ornamental forms
incisa Dipp. var. (f.)	Short and broad leaflets, deeply and doubly serrate to incisely lobed
henkelii Henkel var. (f.)	Similar to the incisa, but with narrower leaves
lanciniata Leroy var. *lanciniata* Jacques var., and *lanciniata* Dipp. f. syn. *asplenifolia* Hort., *dissecta* Hort., and *heterophylla* Hort.	Narrow, deeply and irregularly incised leaflets
memmingeri Bean var. *memmingeri* (K. Koch) Rehd. var. *memmingeri* Sim.-Louis var. *memmingeri* Rehd. F.	White spotted leaflets
pumila Dipp. var.(f.) syn. *digitata* Hort.	Dwarf form
pyramidalis Nichols. var. *pyramidalis* Henry var. *pyramidalis* S. Louis f.	Compact narrow-pyramidal habit
schirnhoferi Rosenth. var. *schirnhoferi* Voss. F.	Double yellowish-red flowers
tortuosa Bth. f.	Twisted branches
umbraculifera Rehd. var. *umbraculifera* Jeag. var. *umbraculifera* Rehd. f.	Compact roundish head
pendula Puvilland *pendula* Pinelle var.	Pendulous branches
variegata Loud. Var. (f.)	Yellow-colored leaflets
luteo-variegata West. var.	Yellow variegated leaflets
albo-variegata West. var.	White variegated leaflets

[1] Data from Bailey (5), Dirr (18), Hänsel et al. (33), Hegi (36), Rehder (82), and Traverso (97); var. = variety, f. = form.

DRUGS FROM HORSE CHESTNUT

Horse chestnut is listed as a medicinal plant in pharmacopoeias published by the Ministries of Health in many countries, both officially and for popular tradition (Table 3). Among these are: Austria, Belgium, Brazil, Chile, Denmark, Germany, France, Greece, India, Japan, Mexico, Paraguay, Portugal, Romania, Spain, ex-USSR countries, Sweden, Switzerland and Venezuela (65). In Italy horse chestnut is absent both from the Official Pharmacopoeia of the Italian Republic (VIII Ed.) of the Ministry of Health in 1972 and from the list of plants declared officinal in Decree 772 of 1932.

CHEMICAL COMPOSITION AND ACTIVE INGREDIENTS OF HORSE CHESTNUT

Horse chestnut seeds have a sour, bitter taste and are toxic as a consequence of a high level of pentacyclic triterpene saponins. An important result from investigations on the constituents of horse chestnut during

TABLE 3. Drug forms of horse chestnut.

Drug term[1]	Plant tissue
Hippocastani folium	Dried horse chestnut leaves
Hippocastani oleum	Horse chestnut oil extracted from peeled seeds
Hippocastani semen	Dried horse chestnut seeds, DAB10 and dried mature horse chestnut seeds, AB-DDR
Aesculus hippocastanum hom. HAB1	Fresh peeled seeds
Aesculus hippocastanum & cortice, äthanol. decoctum hom. HAB1	Horse chestnut cortex
Aesculus hippocastanum & floribus hom. HAB34	Fresh white and red flowers
Aesculus hippocastanum hom. HPUS78	Mature and fresh seeds without coats

[1] Information from Hänsel et al. (33).

the first twenty years of this century was the extraction of the saponin escin (a complex mixture of more than 30 triterpene glycosides derived from protoaescigenin and barringtogenol-C) from the seeds (1). Several organic acids are bound to the terpenic moiety, giving rise to a large number of compounds very similar to each other in terms of polarity and biological activity. Thus, escin consists of a mixture of diacyl derivatives of tetra- and penta-hydroxy-β-amyrin types that have a glucuronic acid in position 3 with two sugar residue substituents. The esterifying organic acids are acetic, butyric, isobutyric, angelic, and tiglic, while the sugar moieties after acid hydrolysis are glucuronic acid, glucose, xylose, and galactose. The isomeric forms of escin, β-escin, and cryptoescin, carry an acetyl group in different positions, the 22-α-hydroxyl and 28-hydroxyl, respectively. The α-escin, which has both of the acetyl groups, possesses unusual stability in aqueous solutions (9,32). Escin may exist in crystalline or amorphous form, although the amorphous form is the more bioavailable (64). More recently, five triterpene oligoglycosides, named escin-Ia, Ib, IIa, IIb, and IIIa (saponin fractions) have been isolated from seeds of horse chestnut by Yoshikawa et al. (105).

The seeds also contain coumarin glycosides (among which is esculoside), flavonoid derivatives (including glucosides of quercetin and campherol), starch (23-48%), tannins, oil (4-6%), cellulose (3%), protein (8-12%) (adenine, adenosine, guanine, uric acid), sucrose (8-17%), glucose (5-6%), albumin (7-11%), proanthocyanidins (including leucoanthocyanins and catechin), and dyes. Also present are salts of calcium, magnesium, copper, and manganese, methionine, B group vitamins, vitamin C, vitamin [illegible], and provitamin D. In the seeds, as in the seed coat, however, coumarin, esculin, and fraxin are lacking (24,33,61,89,103). Various proanthocyanidins are observed in the pericarp and seed coat, including condensed tannins, leucoanthocyanins, and epicatechin (59).

The oil extracted from the peeled seeds consists of the following acids: oleic (65%), linoleic (21%), palmitic (4.5%), stearic (3.7%), and linolenic (2.3%) (33). The constituents of the unsaponifiable portion of horse chestnut oil remained unidentified for a long time. Although the unsaponifiable portions constitute only a small part of the oil (2-3%), these portions consist of important compounds such as sterols, triterpenes, aliphatic alcohols, vitamins, hydrocarbons, pigments, and other compounds. According to analyses by Stankovic et al. (90,91), campesterol, cholesterol, stigmasterol, sitosterol, α-spinasterol, stigmastenol, and

triterpenes can be found in the unsaponifiable substance (0.01% of the dry weight of the seeds).

The reserves of the seeds consist of fructose, galactose, and amylose (33), starch, oil and albumin (7-11%), plus phytosterols and triterpenes in the unsaponifiable portion of the oil (91). Escin also constitutes a reserve substance. The seed pericarp contains hippocastanoside, a compound that differs from escin essentially in the aglycone portion (99).

The horse chestnut seeds are collected at maturity and dried at 60°C. The content of escin is variable and may fluctuate from 3 (43) to 13 (31) to 28 percent (44), according to the genotype, the time of year, and the maturity of the seeds. According to Profumo et al. (75), the highest concentrations of escin are observed in the cotyledons in January. By contrast, Karuza-Stojakovic et al. (44) reported that the content of escin, which is absent during the first phase of seed development, increased rapidly until the concentration reached a maximum in August (when the diameter of the seeds was about 30 mm) and then decreased subsequently in relation to the increase in biomass (when the mean diameter of the seeds was about 38 mm). Escin is absent from the seeds and the roots (about 5 months after germination); moreover the content of escin decreases significantly after germination demonstrating that the escin takes part in metabolism associated with plant development.

The bark contains numerous substances, among which are esculitannic acid, coumarin glycosides (2-3%) (including esculoside, fraxoside and scopoloside), coumarins (including esculin) (7.2%), fraxin (2.8%), scopolin (0.15%), esculetin (0.2%), scopoletin (trace), and argirin plus allantoin, resin, catechic tannins (leucocyanidin, leucodelphinidin, and epicatechins of the proanthocyanin group), dyes, citric acid, and a fatty oil. In contrast to these chemical constituents, the bark of horse chestnut is devoid of saponins (15,33,59). The content of coumarins is lower in the bark of young branches: esculin (0.7%), esculetin (0.2%), fraxin (0.1%), fraxetin (0.05%), and scopolin and scopoletin (traces), however, the bark does contain large amounts of quebrachitol and allantoin.

The content of coumarins in the leaves is lower than that of the bark (esculin at 0.02% and fraxin in traces), while the stalks and veins contain saponins. Hydrolysed extracts contain flavonols, such as quercetin, isoquercetin and campherol, in addition to leucocyanidin and p-coumaric acid (33). Furthermore, adenine, adenosine, guanine, uric acid and other free amino acids such as alanine, valine, cysteine, phenylalanine, tyrosine, α-aminobutyric acid, leucine, isoleucine, arginine, glutamic acid (asparagine and glutamine) have been identified. Campesterol,

stigmasterol, and β-sitosterol are present in the petroleum ether extract. Acids, including: lauric, myristic, palmitic, palmitoleic, stearic, linoleic and linolenic, are in leaves. In addition, the leaves contain α-carotene, vitamin C, tannins, resin (88). Young leaves in particular contain many tannins and large amounts of quebrachitol.

Harborne (35) identified five flavonoid glycosides (campherol-3-arabinoside, campherol-3-glucoside, campherol-3-rhamnoside, campherol-3-rutinoside and quercetin-3-rutinoside) in the flowers and the leaves of the horse chestnut. According to Hsiao and Li (40), quercetin-3-rhamnoside, quercetin-3-glucoside, and quercetin-3-arabinoside are also present in the leaves.

The main compounds in the flowers are campherol-3-arabinoside, campherol-3-glucoside, campherol-3-rhamnoglucoside, isoquercetin and rutin. In addition, adenine, adenosine, guanine and uric acid have been identified (33).

MEDICINAL PROPERTIES AND THERAPEUTICAL USES OF HORSE CHESTNUT

In folk medicine, horse chestnut, which has been used for centuries, is still used as a remedy for treatment of gastralgia of atony, diarrhoea, chronic catarrh, uterine hemorrhage, mammary indurations, and cancer. Mattioli, in the 1565 edition of his Commentaries, wrote of the astringent quality of horse chestnuts, while Tablet in 1708-1709, discovered the stimulatory effects of the powder and use in disturbances of the circulation (50). Bon of Montpellier in 1720, used the bark of the branches as an febrifuge to substitute for cinchona (50). Studies by Artault of Vevey (7,50), between 1896 and 1909, established horse chestnut as an anti-inflammatory and anesthetic agent in the phytotherapy of venous circulation and hemorrhoids. Numerous pharmacological and clinical studies over subsequent years have defined the mechanism of action and the value of this plant.

Seeds. The drug used for medicinal purposes in pharmacology consists essentially of the seeds and the bark. The drug is odorless and initially sweet, later becomes bitter to the taste. Accôrding to pharmacological research, horse chestnut extract (HCE) has been demonstrated to possess antioxidant antiedemigenous, antiexudative, vasoprotective, and decongestive actions, properties that seem to be exclusively due to the triterpenic saponin escin (20,51). Escin is essentially a vasoconstrictor,

but also acts to reduce pain, perhaps due to the hemolytic action of the saponins that leads to a decrease in the viscosity index of venous blood. Certain coumarins (such as esculoside and esculetol), like some flavone derivatives (such as rutoside, xanthorhammoside, quercetoside, luteolol, quercetol, and rhamnetol), are capable of increasing capillary resistance, relieving pain, and reducing the fragility of blood vessels (47). Clinically, HCE is widely used in the treatment of venous stasis, varicosis, and venous insufficiency (19,20,21,25,31,44,98).

A large number of pharmacological and clinical investigations during the 1960s and 1970s evaluated the anti-edema and anti-inflammatory activities of escin. The biochemical mechanisms proposed for the anti-edema effect of escin included a tensio-active effect, an increase in the capacity of the erythrocytesa to bind water (leading to plasmatic border flow developing a passive sodium pump independent from metabolism), a normalization of the exchange of extra- and intra-cellular fluids, and a direct site of action on connective tissue and cell membrane. In contrast, escin was reported to be effective only on models representing the initial stage of inflammation and characterized by an alteration in vascular permeability (impermeabilising the capillary walls through which water exchange occurs) (4,60,68,69,102).

Several years experimentation have ensured the safety of escin in doses normally used in human therapy. Accumulation of escin in tissue has not, except for the excretory organs, been observed, side effects have been absent, and toxicity has been noted only at very high doses. Escin has proven to be well tolerated throughout treatments lasting 50 consecutive days (28,51,53,100).

Because of the pharmacological characteristics and the high degree of tolerability of escin, the drug is widely used in medical practices. For example, because escin action is displayed at the cellular level the drug has been used successfully in treatment of localized edema. At first escin was used only by intravenous or oral administration, but today gels or ointments containing escin are used externally for treatment of varices, contusions, and hematomas and irrigation with escin is used for treating hemorrhoids. Escin is particularly effective in patients subjected to thrombophlebits with cerebral edemas following cranial fractures and traumas (with or without retrograde amnesia), suffering from a recent apoplectic attack and hemiparesis, from cerebral tumors of different types, and from cerebral sclerosis meningitis, encephalitis, and cerebral abscesses. Following treatment with escin, a complete, long-lasting remission of symptoms or a reduction in frequency and severity of the disorders is mostly rapid and spectacular (27,37,41,62,101).

Escin can be also used in the field of obstetrics and gynecology for the resolution of edematous states and in otorhinolaryngology and odontoiatria for treatment of tonsillar abscesses, acute raucedo, epiglottic edema, and gingival hemorrhages. A therapeutic effect from the anti-inflammatory and anti-edema action of escin on multiple sclerosis has been reported (23,41,95). Due to the vasoprotective action of escin, the drug is also used in dermatology, although a complex of escin with β-sitosterol and phosphatidylcholine has been shown to be more active than escin alone (9).

A degree of anti-viral activity has been reported for escin by Rao et al. (81) and may be attributable to triterpene saponins containing acylated aglycones of the β-amyrin type. Significant anti-microbial activity against pathogenic bacteria has been documented by Margineanu et al. (54). More recently, Yoshikawa et al. (105) have assigned a powerful hypoglycemic activity to the compounds escin-Ia, Ib, Iia, Iib, and IIIa, while Konoshima and Lee (46) have isolated two components (hippoesculin and barringtogenol-C21-angelate) from the fruit that have cytotoxic activity, thus placing the horse chestnut among the medicinal plants of major interest as a source of new potential anti-tumor agents of natural origin.

Another important, clinically-tested molecule that stimulates healing and exerts a venotonic activity, normalizing impaired capillary permeability and fragility due to vitamin P deficiency, is proanthocyanidin A_2. This compound is endowed with potent antioxidant and anti-enzymatic activity, therefore playing a significant role in humans in preventing/reducing photobiological damage of the skin (26).

Bark. The bark of the horse chestnut is collected in spring (or autumn) from two to three-year-old branches and dried in a dry and airy place. The bark in commerce is in pieces 10 to 12 cm long, about 2.5 to 4 cm wide and 2.5 cm thick, grayish-brown in color externally with elongated and corky scales and internally brown in color with fine longitudinal striations (50). Hose chestnut bark lacks an aroma, but possesses a bitter and astringent taste.

The bark possesses tonic, astringent, detergent, antiseptic, narcotic, febrifuge, anti-hemorrhagic and anti-hemorrhoidal properties (2,33,88). The astringency is conferred by the tannins, while the febrifuge properties commended by numerous physicians are attributable to esculin. The coumarin esculin finds a well established clinical application, especially for the treatment of hemorrhoids. The main pharmacological activities of esculin concern the improvement of capillary permeability

full development of the trees. In Central Europe, horse chestnut is also planted in hunting reserves and in wooded avenues to nourish the game that eat the fruit (15). Sometimes the tree is used to consolidate unstable slopes or to form arboreal belts as defense against fires (58).

Domestic and Food Use. The flour obtained from the seeds has been known for a long time as a means for washing linen and wool. During the war, the flour was mixed together with clay (kaolin) to form a soap, known as "Bolous" soap, that was especially effective against spots of fat, but was not suitable for washing white garments because the iron in the clay discolored the cloth. The flour, which has been added to water used in watering flowers with the aim of removing earthworms, is also constituent of "Schneeberger" snuff. The leaves are sometimes used to adulterate tobacco and, when young, leaves may serve as a substitute for hops. Necklaces may be prepared with horse chestnuts to put in wardrobes to combat moths and it is said that always keeping a horse chestnut in the pocket during winter protects against colds.

Following lixiviation and processing of the seeds at 40°C a very good raw flour is obtained that resembles corn flour; while a pasty flour is obtained after processing of the seeds at 100°C: both are used for soup, cakes and confectionery in general. In the past, the bitter flour was mixed six to eight times with soda water and allowed to stand for 12 h each time, washed with water, filtered through a cloth and then dried in the sun or in an oven, and then used to make bread in a 1:1 mixture with corn flour (15,36).

In many countries, and in particular in ex-Czechoslovakia, *A. hippocastanum* is a useful source of nectar for bees and a moderate source of pollen in spring, with a nectar production of 1.4 to 1.6 mg, in 24 h and a sugar content varying between 40 and 69 percent. The yield of honey is a minimum of 383 kg/ha (34,66).

Cosmetics. Escin, like other extracts of the seeds, is widely used in the cosmetic sector, thanks to the astringent and decongestive activities of the extracts, and is frequently a constituent of bath foams, shampoos, and foaming footbaths, providing the products tonic, refreshing, calming, and astringent actions. The extract is also a constituent of refreshing and anti-reddening lotions for the scalp, toothpastes, pastes used for hygiene of the oral cavity for gingivitis and periodontitis, and other personal hygiene products. Above all, however, the extract is used in a vast range of products for the body (in the form of a cholesterol-escin complex), including such products as lotions, milks, and creams or gels for the treatment of delicate skin subject to rosacea; sunscreens in general; after-bath and creams for anti-cellulite coadjuvant massages; breast

consolidation and soothing and tonic applications; creams, gels, lotions, and compresses for the legs and feet to prevent and alleviate swellings, venous dilatation, heaviness, and tiredness. By contrast, in cosmetics esculin improved skin trophism and was effective in cellulitis. Topically applied esculin increases the "capillary density" (number of capillaries open to flow per surface unit) and the morphology of the smallest blood vessels. In man, it is particularly indicated for a series of conditions characterized by a chronic deficiency of districtual skin microcirculation and has been successfully employed for the treatment of aging skin and cellulitis and the reduction of hair loss, seborrhea and alopecia. The extract is principally used for the production of creams or sun gels (61,76,77).

Industrial and Technological. The dextrins contained in the seeds are used for the production of liquors and for the preparation of glues used by bookbinders, by flax weavers and by box manufacturers (36). The raw flour, after boiling in water, yields a rot-resistant and solid glue recommended for labels and for the adhesive board of herbaria. The powder from the seeds also serves as a foaming agent for extinguishers and for the preparation of concrete. By contrast, the oil is an emulsifying agent for whale oil, paraffin oil, drilling oil, detergent cream and for pastes used in cleaning metals in the galvanizing industry. The seeds are also used for the preparation of anti-parasitic and insecticidal agents, and in the textile dyeing industry, while the leaves have been used in the past to dye wool a pale chestnut color. The oil extracted from the seeds is used in the manufacture of soap, as a lubricating oil for machinery, for animal feed and also for human nutrition. The extract of the bark is used for tanning or for dyeing wool yellow (15,36).

The wood from the horse chestnut, yellowish-white or sometimes also reddish, is soft and light, waxy, confusable by the naked eye with conifer wood, with a fine, compact and uniform grain and sometimes with a shiny surface. Horse chestnut, considered by many to be one of the worst timbers both for industry and for heating, is, according to others (50), not appreciated only because the wood is little-known. The timber is easily worked, but cannot be cut cleanly, because the lumber twists and warps easily and decays rapidly when exposed to atmospheric agents. The wood, which is particularly dry, burns vigorously, but only for a short time. Lumber from horse chestnut, which can be adapted to numerous uses including the interior of furniture, trelliswork, cooking implements, and pianos, is actually seldom used except for production of boxes for vegetables and fruit and for pyrography. The wood does take a good polish and readily absorbs a black stain, indicat-

ing why the wood is used to imitate ebony. In the past, horse chestnut was used for sculpture because the white paint used as a cover before gilding, concealed any defects (2,15,50).

Animal Nutrition. The seeds are used as fodder for fattening cattle and game animals and especially for pigs, goats, sheep, and fish. In Eastern countries, seeds are used for feeding horses and cattle. The cattle become habituated to the fodder, a little at a time, and, thanks to the high nutritional value, appears to have an effect on the increase in milk production. Pigs decisively reject the seeds. Chicken are not poisoned by the seed, but hens grow thin and no longer lay eggs; ducks die. Deer do not like horse chestnut seeds (50).

In preparation for use as feed, seeds are soaked in lime-water to remove the bitter flavor, ground into meal, and then mixed with ordinary provender (2,33). Nevertheless, a more satisfactory way to prepare feed from horse chestnut seeds consists of soaking partially crushed seed in cold water for one night, boiling the mixture for about one and one-half hours and then removing the water. The residue can be subsequently dried, partially peeled, and reduced to a flour which, although slightly bitter, has a pleasant taste and appearance (30).

CULTIVATION OF HORSE CHESTNUT

Horse chestnut is a very adaptable plant and is spread through all the temperate and cold-temperate regions of the globe. For optimum growth, the plant prefers siliceous, deep, light, and rather fertile soils in a locality sheltered from strong winds that could damage the wide and dense foliage that characterize the plant. Horse chestnut does not grow well in droughty conditions. After the juvenile growth phase, the plant is relatively hardy and tolerates low temperatures and even frost well. The plant also tolerates full sun, although sciaphilous, similar to the hornbeam. The range of the horse chestnut extends to 1300 m in altitude and to north of Sweden and Norway to the 65° parallel where the tree still succeeds in bringing fruit to maturity, despite the harshness of the climate. Horse chestnut is sensitive to salinity, atmospheric pollution and energetic pruning which is practiced to contain the foliage (2,14,58). In the juvenile phase, horse chestnut are sensitive to water stress and, as a consequence of a water imbalance, the leaves will turn yellow and fall to the ground (nonparasitic leaf scorch). Scorch is more prevalent in dry seasons, but serious damage also has been observed in wet seasons.

Different cryptogams and insects have been reported as responsible of horse chestnut disorders (38, 67), however, the tree is seldom affected by pests and diseases. The ascomycete *Guignardia aesculi* (agent of the leaf blotch) represents the most important fungal disease (92). This disease has caused serious damage in North America and has become very widespread and frequent in the world, even in Italy (29). In the most serious cases, especially in nurseries, the disease may cause premature and complete defoliation of the plants (86). According to Orton (63), the disease may be carried by infected seeds and then transmitted by gamic propagation. The lower susceptibility shown by some genotypes suggests the possibility of selecting and propagating the more resistant clones (38).

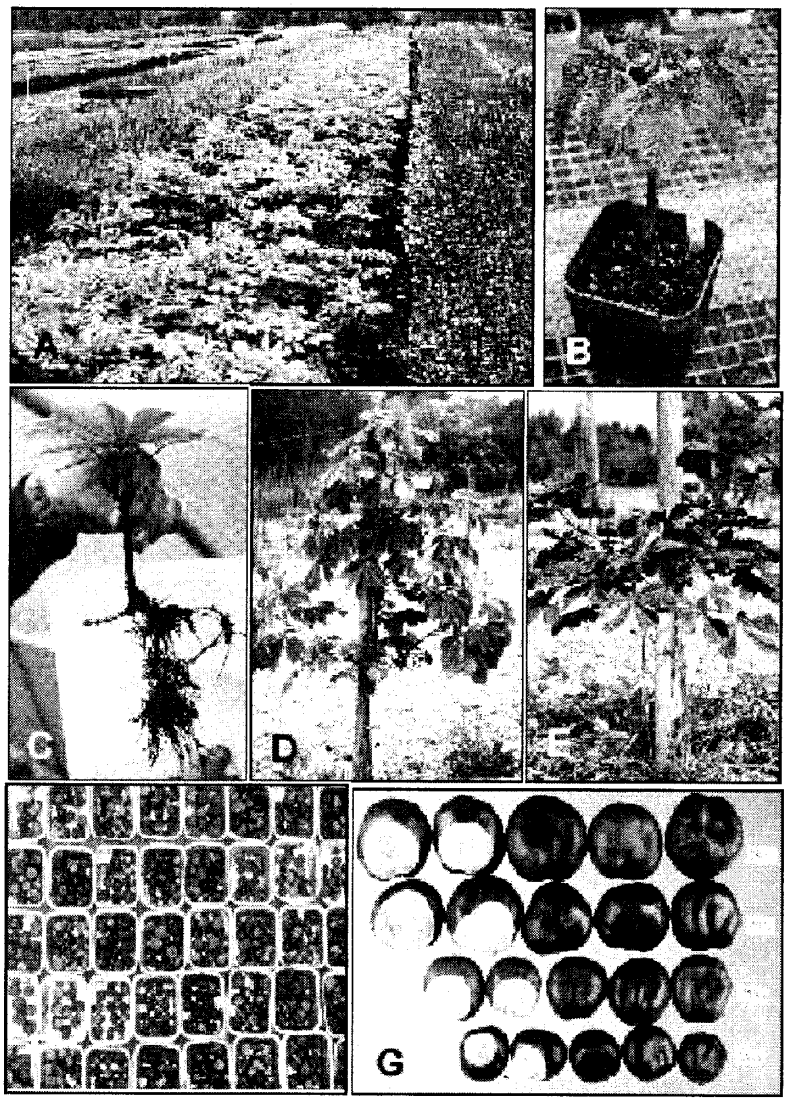
The known techniques for cultivation of horse chestnut concern exclusively the ornamental sector for raising plants in urban areas, in parks and in large gardens, and not in nurseries. No information is available about raising this plant for industrial purposes in large plantings for fruit production.

Propagation by seed is the method used with great success both for the horse chestnut and for the other species belonging to the genus *Aesculus*. Nevertheless, the vitality of the seeds decreases rapidly following dehydration. Consequently the fruits should be collected from the tree as soon as the capsule turns yellowish and starts to split open or from the ground immediately after they have fallen (56). Sowing should take place as soon as possible or the seeds should be stratified or prechilled (stored in hermetically sealed polyethylene bags at a temperature of 2 to 4°C and in the presence of moderate humidity) promptly for spring sowing (56,93,94,96). Without stratification, seeds should be soaked in water for about 48 h and one-third of the seed should be removed by cutting at the scar end without removing the seed coat. The germination is hypogeal and usually is complete after three to four weeks (2,85).

The horse chestnut may be also grafted without difficulty onto 1 or 2-year-old seedlings (Figures 1A and 1B). Triangle, saddle, (horseback), wedge, apical side veneer and simple cleft grafts are practiced in February-March, while bark graft in March-April. Dormant apical scions are used, taken in January or February. Budding may also be carried out with success in late summer using buds of medium size chosen from the central portion of the branch (56,57,58).

The scion graft (double English wedge, simple and lateral wedge) may also be performed in the field, but does not give good results (48). The genus *Aesculus*, however, responds particularly well to bench

FIGURE 1. Horse chestnut: propagation, training systems and seed evaluation.



A = Seedlings of horse chestnut in a Scottish nursery, B = Grafted plant from a Florentine Clone, C = Rooted cutting (photo supplied by FCNRC, Scotland). D = Young tree trained to free palmette, E = An open vase at the Monna Giovanella Agricultural Experimental Centre of the University of Florence, Antella (Florence), F = Seed sample preparation for escin extraction (partly dried nuts collected in Florence), G = Dry nuts of different sizes collected in Florence.

grafting. Although not commonly practiced, horse chestnut may be propagated by cuttings (Figure 1C) using terminal shoots (about 8 cm long) taken in summer and stripped of 75 percent of the leaves. Rooting under mist conditions in a substrate consisting of peat, bark and perlite in the proportions 1:1:1 provides satisfactory rooting rates (60%). According to Chapman and Hoover (11), the best results are obtained using semihardwood cuttings taken from May to June.

As a valid alternative to traditional agriculture for the production of secondary metabolites and active principles, horse chestnut can be *in vitro* cultured. The literature on the *in vitro* culture of *A. hippocastanum* especially regards callogenesis and somatic embryogenesis. Somatic embryos have been obtained by numerous authors starting from microspores (78,79), immature zygotic embryos (10,80), anther filaments (42,45), leaves (12,74) and cotyledons (70,73). The results show clearly that the synthesis of escin takes place *in vitro* from different types of explant, even in the absence of phytoregulators and, moreover, that the amount of escin does not depend on the source of the explant used (43,71,72). In fact, the escin synthesis takes place *in vivo* in many parts of the plant, although the embryos appear to be the preeminent place for accumulation of the active principle. These results are of great interest since *in vitro* cultures offer the possibility of increasing the yield of active principles by submitting the cultures to treatments (different culture conditions and medium compositions) which improve their biosynthetic capacity.

As for horse chestnut plantation, first field experimental trials have been done at the Florence University within the EU "European Aesculus Cultivation System" project, showing that generally horse chestnut trees adapt well to different pruning and training systems. Trees with a complete central axis were the most suitable for training operations. The presence of a leader limb allowed the tree to reach a proper trophic-hormonal balance. Free training systems better fitted with the natural growth pattern of tree. Study results indicated that advantage could be made of the strong apical dominance of the horse chestnut by shaping trees to a central leader; the modified central leader seemed the best form for fitting horse chestnut strong tendency to grow upright and branch anatomy. Moreover, trees were easy trained to the palmette system (Figure 1D), although some perplexity arise from the consideration that this form will not allow horse chestnut trees to develop a large enough volume of crown and achieve heavy production at maturity. In the open-center tree, the main advantages was the possibility to exploit the natural horse chestnut phyllotaxis and constitute three to four sym-

metrical side scaffold branches (at 120° or 90°, respectively) (Figure 1E). Problems, however, were encountered: first, the necessity of disbudding after topping and pollard of main axes and second, the need of divaricators (sticks, canes, weights, bindings) to open the neo-formed shoots, which usually grew upwards with very closed angle crotches; this operation often caused limb breaking off during bending and binding.

QUALITY OF HORSE CHESTNUT

The world production of horse chestnut today is based on naturally growing trees and on trees planted for ornamental purposes. Seed yield from the trees varies greatly in quantity, from 2 to 3 kg to 25 kg of fresh seeds/tree, and in quality, from 0.1 to 15 percent escin content/seed dry weight. Under the present conditions no possibility of preventing and controlling pests and diseases on the trees exists. Moreover, the acquisition of plant-derived chemicals in the future could be difficult as a consequence of the decrease of wild plant availability and the increase of urban pollution. For this reason the development of highly productive plants of good quality by clonal selection (both genetic and sanitary) of existing plant material, combined with appropriate cultivation techniques is necessary.

A first attempt towards the selection of superior clones from the existing materials for commercial aims has been provided by the EU "European *Aesculus* Cultivation System" project. Horse chestnut populations evaluated over four seasons demonstrated considerable variability in all of the examined characters (Figures 1F and 1G). In particular, this variability was large for all productive characters, such as mean seed dry weight, size and yield of nuts, and seed escin content, suggesting that the selection of superior individuals for productivity may give quite good results and economical advantages.

The major problems, however, were encountered in escin evaluation. Indeed, contrary to the behavior of other characters, escin content was subjected to great fluctuations from one year to another within a genotype (Table 4). This caused great difficulties in the selection of high yielding individuals. Probably, environmental and bio-physiological factors have a strong influence on seed escin accumulation as evidenced by negative effect of very dry weather conditions (Table 4) and by the positive effect of cold storage on seed escin content (Table 5). How and to what extent these environmental and bio-physiological factors may

TABLE 4. Escin content of horse chestnut seeds in the Bologna area.

Year	Escin content				Weather conditions
	Mean value[1]	Maximum	Minimum	Variability[2]	
	(%)				
1993	4.1 ± 0.5	1.46	7.66	43.6	Average
1994	2.5 ± 0.1	0.35	3.90	35.8	Very dry
1995	3.8 ± 0.2	2.30	7.30	31.2	Average
1996	3.8 ± 0.2	2.04	5.25	18.8	Warm, late winter

[1] Mean ± S.E.
[2] Coefficient of variability.

TABLE 5. Effect of harvest time and cold storage on escin content of seeds collected in Florence.

Harvest time, 1996	Seed escin content
	(%)
September 26	4.3 ± 0.1ab
October 3	4.2 ± 0.2ab
October 10	4.0 ± 0.2b
October 17	4.2 ± 0.2ab
Cold stored for 6 months	5.3 ± 0.3a

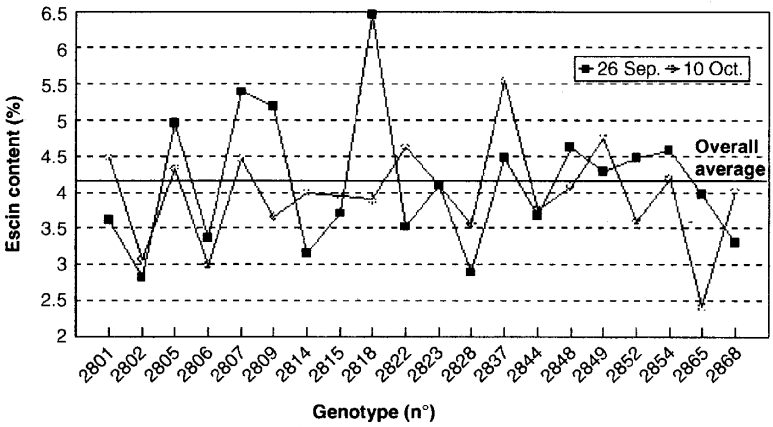
[1] Means followed by the same letter are not significantly different according Duncan's Multiple range test for P = 0.01.

have had and effect on escin content during the years of trial. Also the best fruit collection time and storage procedure appeared to vary with genotype (Figure 2).

According to various authors, the content of escin in the seed is very variable and may fluctuate from 3 to 15 to 28 percent, probably according to the genotype, the time of the year, the maturity and the status of the seeds. Moreover, the literature concerning escin accumulation is often contradictory and the quantitative data are based on a casual sampling without any information about variability, genetics, or selection.

Part of the problems concerned also the analytical screening of samples, in particular the TLC method which allowed the record of escin content in a large number of samples, but on the other side did not assure a very realistic quantitative determination, as well as the isolation, identification and determination of escin isomers or other escin derivatives, which may possess different phytoterapic activity.

FIGURE 2. Effect of seed collection time on escin content of seeds.



CONCLUSIONS AND FUTURE PROSPECTS FOR HORSE CHESTNUT

Because of numerous desirable properties, horse chestnut occupies a position of primary importance among medicinal plants and in the future this plant might assume a significant role as a plant for ornamental and for pharmaceutical use. Currently, horse chestnut extracts and several escin-based compounds are used in pharmaceutical products of therapeutic importance in treatment of edemas and peripheral chronic venous insufficiency and in cosmetics for skin protection and treatment of cellulitis and hair loss.

As for seed derivatives, essentially escin and other active principles have been widely described and investigated for extensive use in dermatology and pharmacology. A number of reports on both horse chestnut *in vivo* and *in vitro* propagation have been published, but information on cultural practices as well as those on genetic and variability of the species are completely lacking. Further studies are needed to improve our fundamental understanding of the variables controlling escin accumulation, to define the best fruit collection time (which appeared to vary depending on single trees), and the best storage procedures to maximize escin yield. Systematic studies will be needed to relate escin accumulation to fruit and seed growth stages. Special emphasis should be given to the accumulation depending on both genotype and environmental factors (such as temperature and water stress). Additional exper-

iments should be done in different environments with replicated clones to distinguish between the genetic and the non-genetic parts of the global variance of individuals and to compute heritability because the knowledge of characters to be improved and the genetic systems that control the inheritance of these factors is fundamental in improving the efficiency of breeding programs.

Research should also be directed to escin quantitative determination and optimization of horse chestnut seed extract in order to develop an analytical system for escin evaluation which can be used for an efficient and more rapid characterization of samples as well as for a more accurate and realistic quantitative determination using HPLC and HPTLC approach coupled with a spectrophotodensitometer. In particular, the supercritical fluid extraction system may offer considerable advantages, being characterized by a high selectivity and a very low consumption of solvents.

Finally, recommendations of cultural practices for efficient horse chestnut seed production should be developed. The introduction of environmentally friendly cultivation systems efficient from a productive point of view will contribute to the development and diversification of the rural economy, giving a solution compatible with the environment and providing an additional income to farmers.

REFERENCES

1. Acar, A.M., and S. Paksoy. 1993. Derivative spectrophotometric determination of aescin in *Aesculus hippocastanum* L. seeds. *Pharmazie* 1:65-66.

2. Allegri, E. 1935. Gli ippocastani. *L'Alpe Riv. Forestale Ital.* 14:454-457.

3. Anderson, E. 1953. Introgressive hybridization. *Biol. Rev.* 28:280-307.

4. Ascher, P.W., and F. Paltauf. 1970. Klinische Untersuchungen ueber die Pharmakodynamik von tritiummarkiertem Aescin. *Aerztl. Forsch.* 24: 294-297.

5. Bailey, L.H. 1947. *The Standard Cyclopedia of Horticulture.* Vol. I. The McMillan Company, New York. pp. 226-228.

6. Bellini, E., S. Nin, and R. Liersch. 1996. L'ippocastano, coltura ornamentale e per usi non alimentari. *Inf. Agr.* 48:39-55.

7. Benigni, R., C. Capra, and P.E. Cattorini. 1964. L'ippocastano. *In:* Inverni e Della Beffa, eds. *Piante Medicinali, Chimica, Farmàcologia e Terapia.* Milano. Vol. II. pp. 763-772.

8. Benson, L. 1957. *Plant Classification.* D.C. Heath and Company, Boston. pp. 177-179.

9. Bombardelli, E., and P. Morazzoni. 1996. *Aesculus hippocastanum* L. *Fitoterapia* 6:483-511.

10. Chalupa, V. 1991. Somatic embryogenesis and plant regeneration in *Picea, Quercus, Betula, Tilia, Robinia, Fagus* and *Aesculus. Communicationes Instituti Forestalis Cechosloveniae* 15:133-148.

11. Chapman, D.J., and S. Hoover. 1982. Propagation of shade trees by softwood cuttings. *Comb. Proc. Int. Plant Prop. Soc.* 31:507-511.

12. Dameri, R.M., L. Caffaro, P. Gastaldo, and P. Profumo. 1986. Callus formation and embryogenesis with leaf explants of *Aesculus hippocastanum* L. *J. Plant Physiol.* 1:93-96.

13. Dayton, W.A. 1952. *United States Department of Agriculture Bibliographical Bulletin.* Washington, DC.

14. De Martino, A. 1982. Le malattie dell'ippocastano. *Inf. Fitopatol.* 1:15-18.

15. De Philippis, A. 1941. L'ippocastano. *Riv. della R. Soc. Toscana Orticoltura* 26:83-90.

16. DePamphilis, C.W., and R. Wyatt. 1990. Electrophoretic confirmation of interspecific hybridization in *Aesculus* (Hippocastanaceae) and the genetic structure of a broad hybrid zone. *Evolution* 5:1295-1317.

17. Digonnet, L., J. Cahn, M. Dubrasquet, and R. Pierre. 1952. Etude sur certaines substances freinatrices de la hyaluronidase. *Thérapie* 7:254-264.

18. Dirr, A.M. 1990. *Manual of Woody Landscape Plants–Their Identification, Ornamental Characteristics, Culture, Propagation and Uses.* Stipes Publishing Company, Champaign, IL. pp. 82-91.

19. Dustmann, H.O, G. Godolias, and K. Seibel. 1984. Verminderung des Fussvolumens bei der chronischen venoesen Insufficienz im Stehversuch durch eine neue Wirkstoffkombination. *Therapiewoche* 34/36:5077-5086.

20. Enghofer, E., R. Eisenburger, and K. Seibel. 1984. Venentoniserende Wirkung und therapeutischen Wirksamkeit von Rosskastaniensamenextrakt bei der orthostatischen Fehlregulation. *Therapiewoche* 34/27:4130-4143.

21. Enghofer, E., K. Seibel, and F. Hammersen. 1984a. Die antiexsudative Wirkung von Rosskastaniensamen-extrakt. *Therapiewoche* 34/27:4130-4144.

22. Engler, A. 1988. *Englers Syllabus der Pflanzenfamilien.* Bornträger-Verlag, Stuttgard.

23. Ferrero, A., M.L. Sacrini, and M. Tiengo. 1968. Impiego dell'escina (Reparil) nelle gestosi e nell'eclampsie. *Min. Anest.* 34:1414-1418.

24. Fidler, U., and G. Hildebrand. 1955. Aminopurine aus *Aesculus hippocastanum* L. *Arzneim. Forsch.* 5:447-453.

25. Friederich, H.C., H. Vogelsberg, and A. Neiss. 1978. Ein Beitrag zur Bewertung von intern wirksamen Venenpharmaka. *Z. Hautkr.* 53:369-374.

26. Gabetta, B., M.J. Magistretti, and P. Dostert. 1990. Therapeutic use of proanthocyanidine A2 as a cytoprotective agent. U.S. Patent 4, 925,870, May 15.

27. Gemaehlich, M., and H.-D. Derbacher. 1965. Vitalmikroskopische studien der Aescinwirkung auf die Gefaessreaktionen nach Histamin. *Artzliche Forsch.* 19:98-100.

28. Girerd, R.J., G. Di Pasquale, B.G. Steinetz, V.L. Beach, and W. Paerl. 1961. The anti-edema properties of aescin. *Arch. Int. Pharmacodyn.* 133:127-137.

29. Goidanich, G. 1983. Le avversità degli alberi da ornamento. *Inf. Fitopatol.* 3:11-18.

30. Grieve, F.R.H.S. 1967. *A Modern Herbal.* Vol. I. Hafner Publishing Co., New York. pp. 192-193.

31. Handa, S.S., A.S. Chawla, and A.K. Sharma. 1992. Plants with antiinflammatory activity. *Fitoterapia* 1:3-31.

32. Hänsel, R., and H. Haas. 1983. *Therapie mit Phytopharmaka.* Springer-Verlag, Berlin. pp. 78-93.

33. Hänsel, R., K. Keller, H. Rimpler, and G. Schneider. 1992. Drogen A-D. *In:* F. Von Bruchhausen et al., eds. *Hagers Handbuch der Pharmazeutischen Praxis. 5. Vollständig Neueberarbeitete Auflage.* Vol. 4. Springer-Verlag, Berlin. pp. 108-122.

34. Haragsim, O. 1977. Nectar production of horse chestnuts (*Aesculus* spp.). *Apicultural Abstracts* 31:536.

35. Harborne, J.B. 1967 *Comparative Biochemistry of the Flavonoids.* Academic Press, London. pp. 35-44.

36. Hegi, G. 1906-1931. *Illustrierte Flora von Mittel Europa.* Part I, Vol. V. J.F. Lehmanns Verlag, München. pp. 296-309.

37. Heppner, F., W.P. Ascher, and G. Argyropoulus. 1967. Neuere Beobachtungen ueber die Wirkung von aescin in der Neurochirurgie. *Wiener Med. Wschr.* 29/31:706-709.

38. Hepting, G.H. 1971. *Disease of Forest and Shade Trees of the United States.* United States Dept. Agric., Washington, DC. pp. 62-66.

39. Hoar, C.S. 1927. Chromosome studies in *Aesculus. Bot. Gaz.* 84:156-170.

40. Hsiao, J.-Y., and H.-L. Li. 1973. Chromatographic studies on the red horse chestnut (*Aesculus* × *carnea*) and its putative parent species. *Brittonia* 1:57-63.

41. Hutzel, M. 1963. Erfahrungen mit Reparil in der chirurgie und Praxis. *Med. Welt* 23:1279-1281.

42. Jorgensen, J. 1989. Somatic embriogenesis in *Aesculus hippocastanum* L. by culture of filament callus. *J. Plant Physiol.* 2:240-241.

43. Kamenicka, A., and M. Rypak. 1988. Wirkung mancher Wuchsregulatoren auf den Wuchs der kalluskultur der Rosskastanie (*Aesculus hippocastanum* L.) *in vitro. Folia Dendrologica* 15:207-224.

44. Karuza-Stojakovic, L., J. Petricic, and Z. Smit. 1991. The total triterpene glycoside content expressed as aescin in horse chestnut seeds (*Aesculus hippocastanum*) during vegetation. *Pharmazie* 4:303-303.

45. Kiss, J., L. Heszky, E. Kiss, and G. Gyulai. 1992. High efficiency adventive embryogenesis on somatic embryos of anther, filament and immature proembryos origin in horse-chestnut. (*Aesculus hippocastanum* L.) tissue culture. *Plant Cell Tiss. Org. Cult.* 30:59-64.

46. Konoshima, T., and K.-H. Lee. 1986. Antitumor agents, 82. Cytotoxic sapogenols from *Aesculus hippocastanum. J. Nat. Prod.* 49:650-656.

47. Lavollay, J. 1945. Sur la vitamine P. action de l'esculoside et de l'esculétol sur la résistance des capillaires. *Compt. Rend. Soc. Biol.* 139:270-273.

48. Leiss, J. 1967. Grafting outdoors deciduous and broadleaf. *Proc. Int. Plant Prop. Soc.* 17:303-305.

49. Li, H.-L. 1956. The story of the cultivated horse-chestnut. *Morris Arbor. Bull.* 7:35-39.

50. Lieutaghi, P. 1975. *Il libro degli alberi e degli arbusti.* Rizzoli editore, Milano. Vol. I, pp. 445-451.

51. Lorenz, D., and M.L. Marek. 1960. Das Therapeutisch wirksame Prinzip der Rosskastanie (*Aesculus hippocastanum*). *Arzeinm.-Forsch.* 10:263-290.

52. Loudon, J.C. 1869. *An Encyclopaedia of Trees and Shrubs.* Frederick Warne & Co., London. pp. 123-134.

53. Manca, P., and E. Passarelli. 1965. Aspetti farmacologici dell'escina, principio attivo dell'*Aesculus. Clin. Terap.* 32:297-328.

54. Margineanu, C., I. Hintz, V. Cucu, L. Grecu, C. Cioaca, M. Rosca, V. Hodisan, and M. Tomas. Antimicrobial action of 12 triterpene saponins obtained from indigenous plants. *Clujul Med.* 3:254-259.

55. Maugini, E. 1988. *Manuale di botanica farmaceutica.* Piccin Editore, pp. 364-365.

56. McMillan-Browse, P.D.A. 1971. Propagation of *Aesculus. Plant Prop.* 2:4-6.

57. McMillan-Browse, P.D.A. 1982. Propagation of the hardy horse chestnuts and buckeyes. *Plantsman* 3:150-164.

58. Merendi, A. 1969. Ippocastano. *Enciclopedia Agraria Italiana.* Reda, Roma. Vol. VI, pp. 401-404.

59. Morimoto, S., G.-L. Nonaka, and I. Nishioka. 1987. Tannins and related compounds. LIX. Aesculitannins, novel proanthocyanidins with doubly-bonded structures from *Aesculus hippocastanum* L. *Chem. Pharm. Bull.* 12:4717-4729.

60. Mussgnug, G. 1969. Zur biochemie der Entzuendung und Oedembildung sowie der Pharmakokinetik des Aescins. *Arzneim.-Forsch.* 19:1588-1593.

61. Nanni, A.M. 1994. Ippocastano cosmetico. *Erboristeria Domani* 12:38-41.

62. Niedling, H. 1967. Erfahrungen ueber die Anwendung eines neuen Venenmittels. *Med Welt* 36:2137-2139.

63. Orton, C.R. 1931. Seed borne parasites. A bibliography. *Agr. Exp. St. West VirginiaUniv. Bull.* 245 p.

64. Pedretti, M. 1990. *Chimica e Farmacologia Delle Piante Medicinali.* Studio Edizioni, Milano (2nd ed.). 19 p.

65. Penso, G. 1983. *Index plantarum medicinalium totius mundi eorumque sunonymorum.* Oemf, Milano. 24 p.

66. Petkov, V. 1975. Determining the nectar-bearing capacity of *Mahonia aquifolium, Lonicera tatarica, Symphoricarpos orbiculatus, Aesculus hippocastanum* and *Acer tataricum. Rasteniev dni Nauki* 10:157-163.

67. Pirone, P.P. 1978. *Diseases and Pests of Ornamental Plants.* John Wiley & Sons Inc., New York (5th ed.). pp. 119-121.

68. Preziosi, P., and P. Manca. 1964. L'azione antiedema-antiinfiammatoria dell'escina nei rapporti con l'asse ipofisi-surrene. *Follia Endocrinol.* 17:527-530.

69. Preziosi, P., and P. Manca. 1965. Antioedem-und die antiinflammatorische Wirkung von Aescin und ihre Beziehung zur Hypophysen-Nebennieren-Achse. *Arzneim.-Forsch.*15:404-406.

70. Profumo, P., A.M. Caviglia, and P. Gastaldo. 1992. Formation of aescin glucosides by calli tissue from cotyledonary explants of *Aesculus hippocastanum* L. *Plant. Sci.* 85:161-164.

71. Profumo, P., A.M. Caviglia, P. Gastaldo, and R.M. Dameri. 1991. Aescin content in embryogenic callus and in embryoids from leaf explants of *Aesculus hippocastanum. Planta Med.* 57:50-52.

72. Profumo, P., R.M. Dameri, and P. Modenesi. 1980. Aescin content in calluses from explants of *Aesculus hippocastanum* cotyledons grown *in vitro*. *Giorn. Bot. Ital.* 1/2:25-28.

73. Profumo, P., P. Gastaldo, L. Bevilacqua, and S. Carli. 1991. Plant regeneration from cotyledonary explants of *Aesculus hippocastanum* L. *Plant Sci.* 1:139-142.

74. Profumo, P., P. Gastaldo, R.M. Dameri, and L. Caffaro. 1986. Histological study of calli and embryoids from leaf explants of *Aesculus hippocastanum* L. *J. Plant Physiol.* 1:97-103.

75. Profumo, P., P. Gastaldo, and R. Martinucci. 1987. Variations in escin content in *Aesculus hippocastanum* seed during the year. *Fitoterapia* 3:184-186.

76. Proserpio, G., S. Gatti, and P. Genesi. 1980. Cosmetic use of horse-chestnut (*Aesculus hippocastanum*) extracts, of escin and of the cholesterol/escina complex. *Fitoterapia* 5:113-128.

77. Proserpio, G., A. Martinelli, and G.F. Patri. 1986. *Elementi di Fitocosmesi.* Sepem, Milano.

78. Radojevic, L. 1978. *In vitro* induction of androgenic plantlets in *Aesculus hippocastanum*. *Protoplasm* 3/4:369-374.

79. Radojevic, L. 1980. Somatic and androgenic embryogenesis in some woody species. *Bull. Soc. Bot. Fra., Actualités Botaniques* 3/4:99-107.

80. Radojevic, L. 1988. Plant regeneration of *Aesculus hippocastanum* L. (horse chestnut) through somatic embryogenesis. *J. Plant Physiol.* 3:322-326.

81. Rao, G.S., J.E. Sinsheimer, K.W. Cochran. 1974. Antiviral activity of triterpenoid saponins containing acylated-amyrin aglycones. *J. Pharmac. Sci.* 3:471-473.

82. Rehder, A. 1940. *Manual of Cultivated Trees and Shrubs.* (2nd ed.). McMillan Publishing Co., New York. pp. 586-591.

83. Rendle, A.B. 1952. *The Classification of Flowering Plants.* Vol. II. University Press, Cambridge. pp. 300-302.

84. Rossi, M. and P. Lotti. 1993. *L'interprete di Erboristeria Domani.* Dizionario multilingue delle piante officinali. Studio Edizioni, Milano. 2 p.

85. Rudolf, P.O. 1974. *Aesculus* L. Buckeye, horsechestnut. *In*: *Seeds of Woody Plants in the United States*, Forest Service, U.S. Dept. Agr. Agric. Handbook 450. Washington, DC.

86. Scaramuzzi, G. 1954. Sul seccume delle foglie di ippocastano. *Ann. Sper. Agr.* Vol. VIII, 4:1265-1281.

87. Schneider, C.K. 1912. *Illustrierte Handbuch der Laubholzkunde.* Jena. pp. 52-65.

88. Souleles, Chr. and K. Vayas. 1986. Constituant des feuilles d'*Aesculus hippocastanum*. *Fitoterapia* 3:201-202.

89. Spiridonov, V.N. 1966. Quercetin glycosides in *Aesculus hippocastanum*. *Dokl. Akad. Nauk SSSR* 169:126-127.

90. Stankovic, S.K., M.B. Bastic, and J.A. Jovanovic. 1984. Composition of the sterol fraction in horse chestnut. *Phytochem.* 11:2677-2679.

91. Stankovic, S.K., M.B. Bastic, and J.A. Jovanovic. 1985. 4-methylergasta-8,24(28)-dien-3 ol, a minor sterol in the seed of horse chestnut. *Phytochem.* 10:2466-2469.

92. Stewart, V.B. 1916. The leaf-blotch disease of horse-chestnut. *Phytopathology* 1:5-19.

93. Suszka, B. 1966. Conditions for breaking of dormancy of germination of the seeds of *Aesculus hippocastanum* L. *Arbor. Kornicke* 11:203-220.

94. Suszka, B. 1982. Storage conditions for woody plant seed with high water content. *In:* B.S.P. Wang and J.A. Pitel, eds. *Factors Affecting Seed Storage*. Proceedings of the International Symposium on Forest Tree Seed Storage, Canadian Forestry Service, Chalk River, Ontario, Canada. pp.161-177.

95. Tillmanns, H., and P. Pfoertner. 1969. Wundheilung von Episiotomien unter Nachbehandlung mit Aescin. *Muench. Med. Wschr.* 46:2410-2412.

96. Tompsett, P.B., and H.W. Pritchard. 1993. Water status changes during development in relation to the germination and desiccation tolerance of *Aesculus hippocastanum* L. seeds. *Ann. Bot.* 2:107-116.

97. Traverso, O. 1926. *Botanica orticola. Descrizione, illustrazione e cenni per la coltivazione delle piante ornamentali, fruttifere, ortensi, agrarie, utili, nocive*. Edagricole, Bologna. Rist. 1990.

98. Tschesche, R., and G. Wulff. 1973. *Chemie und Biologie der Saponine in Fortschritte der Chemie organischer Naturstoffe*. Springer-Verlag, Wien. Vol. 30, 461 p.

99. Vadkerti, A., B. Proksa, and Z. Voticky. 1989. Structure of hippocastanoside, a new saponin from the seed pericarp of horse-chestnut (*Aesculus hippocastanum* L.). 1-Structure of the aglycone. *Chemical Papers-Chemicke Zvesti* 6:783-791.

100. Vogel, G., M.L. Marek, and R. Oertner. 1968. Zur Frage der Wertbestimmung antiexsudativ wirkender Pharmaka. *Arzneim.-Forsch.* 18:426-429.

101. Vogel, G., M.L. Marek, and R. Oertner. 1970. Untersuchungen zum Mechanismus der therapeutischen und toxischen Wirkung des Rosskastanien-Saponins Aescin. *Arzneim.-Forsch.* 20:699-703.

102. Vogel, G., M.L. Marek, and I. Stoeckert. 1963. Weitere Untersuchungen zum Wirkungsmechanismus des Rosskastanien-Saponins Aescin. *Arzneim.-Forsch.*13:59-63.

103. Winkler, W. 1968. Die Aescinbestimmung in Rosskastanienextrakten und in Extract bzw. Aescin enthaltenden Zubereitungen. *Arzneim. Forsch.* 18:1031-1035.

104. Wiskemann, A. 1968. Pharmakologischer Lichtschutz. *Strahlentherapie* 135: 500-503.

105. Yoshikawa, M., E. Harada, T. Murakami, H. Matsuda, N. Wariishi, J. Yamahara, N. Murakami, and I. Kitagawa. 1994. Escins-Ia, Ib, IIa e IIIa, bioactive triterpene oligoglycosides from the seeds of *Aesculus hippocastanum* L.: Their inhibitory effects on ethanol absorption and hypoglycemic activity on glucose tolerance test. *Chem. Pharm. Bull.* 6:1357-1359.