Semen Hippocastani

Definition

Semen Hippocastani consists of the dried ripe seeds of *Aesculus hippocastanum* L. (Hippocastanaceae) (*1*, *2*).

Synonyms

Aesculus castanea Gilib., *A. procera* Salisb., *Castanea equina*, *Hippocastanum vulgare* Gaertner (*3*). Not to be confused with the common chestnut, *Castanea dentata* (Marshall) Burkh. (Fagaceae) (*4*) or related *Castanea* species (*5*).

Selected vernacular names

Abu farwat el hhussan, castagna amare, castagna cavallina, castagna di cavalle, castagno d'India, castan, castandas da India, castanheiro da India, castaño de Indias, chata, châtaignier de cheval, châtaignier de mer, common horse chestnut, conqueror tree, custul, gemeine Kastanie, gemeine Rosskastanie, hippocastani semen, horse chestnut, karu, marronier d'Inde, naru, paardekastanje, Pferdekastanie, qastanah baria, Rosskastanie, seiyo-tochinoki, seiyoutochinoki, semen castaneae equinae, shahbalout-e hendi, vadgesztenyemag, weisse Rosskastanie, wilde kastanje, wilde kest (*3*, *6*).

Geographical distribution

Indigenous to western Asia, is now widely cultivated in parks, gardens and along city streets of many countries worldwide, including those in Europe, and the United States of America (*7*).

Description

A tree, up to 30 m high and 2 m in circumference, with large sticky buds and dense, broad, usually orbicular, or occasionally pyramidal, crown. Leaves up to 20 cm long and 10 cm wide, with 15–20 cm long petioles; composed of 5–7 large sessile leaflets, median leaflet largest, outer leaflets much smaller. Blades obovate or oblong, tapering at the base, abruptly mucronate, irregularly serrate at the margin; dorsal side glabrous; ventral side with soft hairs. Flowers have 5 petals with an orbicular limb, imbricate at the margins, white, with yellow spot at base which later turns pink; arranged in erect dense panicles up to 20–

30 cm long; rachis and pedicel with reddish-brown hairs; calyx cylindrical to campanulate and pubescent; stamens hairy at the base; ovary covered with soft hairs and prickles. Capsules spiny, usually with 1 large seed (*7*).

Plant material of interest: dried ripe seeds

General appearance

Globulous or ovoid, 2–4 cm in diameter. The 2 large cotyledons fleshy, oily and starchy, often connate with a line of suture more or less visible; covered by a shiny dark-brown tegument with a large whitish spot corresponding to the hilum; tegument creamy white in the immature seed, takes on a brown tinge during maturation, becoming dark brown when mature. Curved radicle occupies a depression either on the commissure of the cotyledons or on the dorsal side of 1 of the cotyledons (*1*, *2*).

Organoleptic properties

Odour: slight; taste: bitter, acrid (*1*).

Microscopic characteristics

Seed envelope made up of polygonal cells radially oriented in a transverse section of the seed. Underneath the envelope are numerous layers of sclerenchyma cells with dense, roughly mottled, yellowish-brown thick walls; loose parenchyma, colourless, consisting of a few layers of cells, with rigid walls; sparse annulate or spiral vessels. Tissue of the cotyledons made up of cells with thin, colourless walls, full of starch and lipids. Characteristic starch grains found singly, either spherical (15–25 µm in diameter) or irregular (pear- or kidney-shaped); also numerous small (5–10 µm in diameter), spherical starch grains and a few grains clustered into groups of 2–4 (*1*, *2*).

Powdered plant material

Yellowish-grey. Characteristic starch grains found singly, either spherical (15–25 µm in diameter) or irregular (pear- or kidney-shaped); also numerous small (5–10 µm in diameter), spherical starch grains and a few grains clustered into groups of 2–4. Oil droplets of different sizes; fine fragments of colourless cell walls from the cotyledons; fragments of seed envelope brownish-yellow; and parenchyma and roughly mottled sclerenchyma cells (*1*).

General identity tests

Macroscopic and microscopic examinations, and thin-layer chromatography for the characteristic triterpene saponin, aescin (also known as escin) (*1*, *2*).

Purity tests

Microbiological

Tests for specific microorganisms and microbial contamination limits are as described in the WHO quality control methods for medicinal plants (*8*).

Foreign organic matter

Not more than 2% (*1*).

Total ash

Not more than 4% (*1*, *2*).

Loss on drying

Not more than 10% (*2*).

Pesticide residues

The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg (*9*). For other pesticides, see the *European pharmacopoeia* (*9*), and the WHO guidelines on quality control methods for medicinal plants (*8*) and pesticide residues (*10*).

Heavy metals

For maximum limits and analysis of heavy metals, consult the WHO guidelines on quality control methods for medicinal plants (*8*).

Radioactive residues

Where applicable, consult the WHO guidelines on quality control methods for medicinal plants (*8*) for the analysis of radioactive isotopes.

Other purity tests

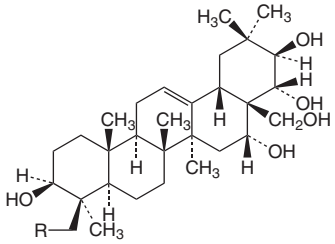
Chemical, acid-insoluble ash, sulfated ash, water-soluble extractive and alcohol-soluble extractive tests to be established in accordance with national requirements.

Chemical assays

Contains not less than 3.0% triterpene saponins, calculated as aescin (escin), determined by spectrophotometry at 540 nm (*1*, *2*). High-performance liquid chromatography (*11*) and thin-layer chromatography–densitometry (*11*, *12*) procedures for the quantitative analysis of triterpene saponins have also been developed.

Major chemical constituents

The major constituents are triterpene saponins (up to 10%), collectively referred to as aescin (also known as escin), and are considered the active therapeutic principles. Aescin exists in three forms, α-aescin, β-aescin and cryptoaescin, which are differentiated by their physical properties. β-aescin is a mixture of more than 30 different glycosides derived from the triterpene aglycones protoaescigenin (also known as protoescigenin) and barringtogenol C. Other constituents include flavonoids (e.g. quercetin, kaempferol and their glycosyl derivatives) (*3*, *5*, *7*). The structures of barringtogenol C and protoaescigenin are presented below.



barringtogenol C R = H

protoaescigenin R = OH

Medicinal uses

Uses supported by clinical data

Internally, for treatment of symptoms of chronic venous insufficiency, including pain, feeling of heaviness in the legs, nocturnal calf-muscle spasms, itching and oedema (*13–21*). Externally, for the symptomatic treatment of chronic venous insufficiency, sprains and bruises (*22–24*).

Uses described in pharmacopoeias and in traditional systems of medicine

Treatment of coronary heart disease (*25*).

Uses described in folk medicine, not supported by experimental or clinical data

Treatment of bacillary dysentery and fevers. Also as a haemostat for excessive menstrual or other gynaecological bleeding, and as a tonic (*6*).

Pharmacology

Experimental pharmacology

Anti-inflammatory activity

Intravenous administration of a 95% ethanol extract of Semen Hippocastani (0.2–0.4 ml/kg body weight) decreased histamine-induced erythema in guinea-pigs (*26*). Intragastric administration of a 30% ethanol extract of the seeds sup-

pressed carrageenan-induced footpad oedema and adjuvant-induced arthritis in rats (at doses of 0.6 and 1.5 ml/kg body weight, respectively) (*27*). Intraperitoneal administration of a saponin fraction isolated from a seed extract exhibited analgesic, anti-inflammatory and antipyretic activities in vivo; the saponin fraction also inhibited prostaglandin synthetase activity in vitro (*28*). Intragastric administration of a hydroalcoholic extract of the seeds to rats (200–400 mg/kg body weight) suppressed footpad oedema induced by peroxide and carrageenan (*29*). Intravenous or oral administration of aescin to rats (0.5–120 mg/kg body weight) inhibited footpad oedema induced by dextran, and granuloma induced by cotton pellet and formalin paper (*30–32*). Intravenous administration of aescin to rats reduced footpad oedema induced by ovalbumin, formalin and dextran (*33*, *34*).

Vasoactive effects

A hydroalcoholic extract of the seeds induced contractions in canine saphenous veins in vitro, and an intravenous bolus (25–50 mg) increased venous pressure in perfused canine saphenous veins in vivo (*29*).

Cutaneous capillary hyperpermeability induced by chloroform, serotonin and histamine also decreased in rats and rabbits after intragastric administration of a hydroalcoholic extract of the seeds (50–400 mg/kg body weight) (*29*). Aescin (5–10 µg/ml) increased the tension of isolated human saphenous veins and rabbit portal veins in vitro. The effect was due to preferential formation of prostaglandin $F_{2\alpha}$ and was reversed by treatment with indometacin (*35*). The vasoactive effects of aescin were investigated in isolated peripheral blood vessels, isolated arteries and veins (constant-flow perfused cat rear paw, isolated perfused carotid artery of the guinea-pig and iliac veins of the pig). Aescin had a biphasic effect on blood vessels: initial transient dilation was followed by increased tone, which was long lasting in isolated arteries and veins, but was transient in isolated peripheral blood vessels (*36*). Aescin has also been shown to inhibit hyaluronidase activity in vitro (IC_{50} 149.9 µmol/l) (*37*). A hydroalcoholic extract of the seeds (250 µg/ml) reduced lipid peroxidation and had radical-scavenging properties (IC_{50} 0.24 µg/ml for superoxide dismutase radicals) (*38*).

Clinical pharmacology

Chronic venous insufficiency and related conditions

Nine placebo-controlled clinical trials (eight double-blind, one single-blind, seven with crossover design) assessed the efficacy of oral administration of a standardized Semen Hippocastani extract (250–600 mg, equivalent to 100–150 mg aescin daily) in a sustained-release form for the symptomatic treatment of patients with chronic venous insufficiency (CVI) (*13–21*). In one study, 96 patients with CVI received the extract over two treatment periods of 20 days each. Symptomatic improvement in skin colour, venous prominence, oedema, dermatosis, and pain, itching and feeling of heaviness in the legs were observed

in the treated patients (*13*). However, the methodology of this study was poor, and no statistical analysis was performed. Two later studies assessed the efficacy of the extract in 212 patients (*19*) and 95 patients (*17*) with CVI, using a numerical scale to rate the severity of symptoms. A significant symptomatic improvement ($P < 0.01$–0.05) in oedema, calf-muscle spasms, pain and feeling of heaviness in the legs was observed in patients treated with the extract (during two treatment periods of 20 days each) (*17*, *19*). The efficacy of the extract was assessed in a double-blind study of 20 female patients (13 with pregnancy-related varicose veins and seven with CVI) during two treatment periods of 14 days each. A significant reduction in leg volume (114 ml in patients with varicose veins and 126 ml in patients with CVI, $P < 0.01$) was demonstrated by water plethysmography in patients treated with the extract (*21*). Another double-blind study assessed the efficacy of the extract in the treatment of 74 patients with CVI and lower-leg oedema. In patients treated with the extract, the leg volume following induction of oedema was reduced from 32 ml to 27 ml (determined by water plethysmography); in the placebo group the leg volume increased from 27 ml to 31 ml (*18*).

Two further studies investigated the effects of the extract on the intravascular volume of the lower-extremity veins and on interstitial filtration (measured indirectly by venous-occlusion or water plethysmography) in patients with CVI (*14*, *20*). In one of the studies, after a single dose of 600 mg extract (equivalent to 100 mg aescin), the transcapillary filtration coefficient decreased by 22%, as compared with a slight increase in the coefficient of the placebo group. This study demonstrated that the extract exerted its action primarily by reducing capillary permeability (*14*). In the other study, patients treated daily with 600 mg extract (equivalent to 100 mg aescin) for 28 days showed a significant reduction in extravascular volume of the foot and ankle ($P < 0.01$), as well as a significant improvement in oedema, and feelings of tension, pain, fatigue and itching of the legs ($P < 0.05$). However, no changes in venous capacity or calf-muscle spasms were observed (*20*).

The efficacy of the extract was assessed in a randomized, parallel, double-blind study of 40 patients with venous oedema due to chronic deep-vein incompetence stage II. Patients received 369–412 mg extract (equivalent to 75 mg aescin) twice daily for 6 weeks. A significant reduction was observed in leg volume (measured by water plethysmography after oedema induction) and leg circumference in the treated group ($P < 0.01$) (*15*). A randomized, single-blind, parallel study compared the efficacy and safety of class II compression stockings with the extract or placebo in 240 patients with CVI. Patients in the treatment group received 300 mg extract (equivalent to 50 mg aescin) twice daily for 12 weeks. The lower-leg volume of the affected limbs decreased by an average of 43.8 ml in patients treated with the extract and by 46.7 ml in patients wearing compression stockings. In the placebo group, the lower-leg volume increased by 9.8 ml. Thus, treatment with the extract or wearing class II compression stockings resulted in similar decreases in lower-leg volume (*16*).

A randomized, double-blind trial compared the efficacy of a standardized extract (360–412 mg, equivalent to 75 mg aescin, twice daily) and oxerutins (1000 mg *O*-(β-hydroxyethyl)-rutinosides twice daily) in 40 patients with CVI and peripheral venous oedema. A reduction in oedema (based on measurement of leg circumference) was observed in both treatment groups (*39*). Another randomized, double-blind study compared the efficacy of a standardized seed extract with oxerutins in the treatment of 137 postmenopausal women with chronic deep-vein incompetence stage II. Following a 1-week placebo run-in, patients were treated daily with either 600 mg extract (equivalent to 100 mg aescin) or 1000 mg oxerutins for 12 weeks, or 100 mg oxerutins for 4 weeks followed by 500 mg oxerutins for 12 weeks. Patients were observed for 6 weeks after treatment; the group treated with 1000 mg oxerutins had the greatest decrease in leg volume (*40*).

A placebo-controlled, double-blind crossover study assessed the effect of a standardized seed extract in the symptomatic treatment of 52 pregnant women with venous insufficiency. Patients were treated with either one capsule containing 300 mg extract (equivalent to 50 mg aescin) or a placebo twice daily for 2 weeks. The extract was superior to the placebo in reducing oedema and symptoms such as leg pain, fatigue and itching. Patients treated with the extract also showed a greater resistance to oedema induction (*41*). The ability of a standardized seed extract to reduce oedema was investigated in a randomized, double-blind, placebo-controlled trial of 30 patients with CVI. A significant reduction in leg circumference was found in the treatment group ($P < 0.05$) as compared to the placebo group ($P < 0.05$) (*42*).

A double-blind placebo-controlled study investigated the effect of a standardized seed extract (one dose of 600 mg, equivalent to 100 mg aescin) on vascular capacity and filtration in the arms and legs of 12 healthy volunteers. Using vein plethysmography, the study showed a decrease in both vascular capacity and filtration coefficient in subjects treated with the extract (*43*). The effect of a standardized seed extract (one dose of 1800 mg) on the flow velocity of venous blood between the instep and the groin was quantitatively determined in 30 patients with varicose veins by the xenon-133 appearance method. Blood flow increased by >30%, with a lasting effect observed after 12 days of treatment. Blood viscosity was also reduced and there was a 73% improvement in subjective complaints (*44*). A randomized double-blind study assessed the effect of a standardized seed extract on lower-leg oedema in 10 healthy volunteers during a 15-hour airflight. A single dose of the extract (600 mg, equivalent to 100 mg aescin) completely prevented or significantly reduced the increase in ankle and foot oedema ($P < 0.05$), determined by measuring the circumference of the ankle and heel before and after flying (*45*). A post-marketing surveillance study of over 5000 patients suffering from CVI demonstrated that treatment with a standardized seed extract (equivalent to 75 mg aescin) twice daily for 4–10 weeks reduced the symptoms of leg pain, fatigue, oedema and itching (*46*). In a multicentre study without controls, 71 patients with CVI were treated daily with a topical gel containing 2% aescin. After

6 weeks of treatment, a significant reduction in ankle oedema (reduction of 0.7 cm in the ankle circumference, $P < 0.001$) and a significant reduction in the symptom score (60%, $P < 0.001$) was reported (*24*). In a postmarketing surveillance study involving over 4000 patients with CVI, treatment with a standardized extract of the crude drug (equivalent to 50 mg aescin) twice daily improved typical symptoms in more than 85% of patients (*47*).

A criteria-based systematic review assessed the randomized, double-blind, placebo-controlled trials of standardized seed extracts for symptomatic treatment of CVI. The data were extracted from the trials in a standardized manner, and the methodological quality and outcome of each trial were assessed by two independent reviewers. In all trials, the extract was shown to be superior to the placebo. Use of the extract was associated with a decrease in lower-leg oedema, and a reduction in the circumference of the calf and ankle. Other symptoms such as leg pain, itching and fatigue were reduced. Results from five comparative trials demonstrated that the extract was as effective as oxerutins, and one of the five trials showed that the extract was as effective as compression therapy (*48*).

Bruises

The efficacy of a topically applied gel containing 2% aescin in reducing the tenderness to pressure haematoma (experimentally induced by injection) was tested in a randomized, placebo-controlled, single-dose study involving 70 healthy volunteers. Based on tonometric sensitivity measurements, the aescin gel significantly reduced the tenderness to pressure haematoma ($P < 0.001$). This effect was seen 1 hour after treatment and lasted for 9 hours (*49*).

Other trials have assessed the efficacy and safety of a topically applied gel containing 2% aescin for the treatment of bruises and sprains (*22*, *23*).

Contraindications

Semen Hippocastani is contraindicated in cases of known allergy to plants of the Hippocastanaceae family.

Warnings

No information available.

Precautions

Drug interactions

Two suspected cases of toxic nephropathy probably due to very high doses of aescin were reported (*50*). Therefore, Semen Hippocastani should not be administered with other drugs known to be nephrotoxic, such as gentamicin.

Carcinogenesis, mutagenesis, impairment of fertility

A 30% ethanol extract of Semen Hippocastani was not mutagenic in the *Salmonella*/microsome assay using *S. typhimurium* strains TA98 and TA100

(200 µl/ml) (*51*). Sodium aescinate had no effect on the fertility of male rats (*52*).

Pregnancy: teratogenic effects

A 40% ethanol extract of Semen Hippocastani was not teratogenic or embryotoxic in rats or rabbits following intragastric administration of 1.6 ml/kg body weight (*53*). Intragastric administration of a 40% ethanol extract of the seeds to rats (100–300 mg/kg body weight) or rabbits (100 mg/kg body weight) was not teratogenic. However, when pregnant rabbits were given 300 mg/kg body weight, a reduction in birth weight was observed (*54*).

Pregnancy: non-teratogenic effects

Semen Hippocastani has been used in clinical trials involving pregnant women with no ill effects (*21*, *41*). However, the drug should not be administered during pregnancy without medical supervision.

Paediatric use

There is no therapeutic rationale for the use of Semen Hippocastani in children.

Other precautions

No information available on general precautions or precautions concerning drug and laboratory test interactions; or nursing mothers. Therefore, Semen Hippocastani should not be administered during lactation without medical supervision.

Adverse reactions

Case reports have indicated gastrointestinal side-effects such as nausea and stomach discomfort (*47*, *55*). Allergic reactions have also been reported (*56*).

Dosage forms

Crude drug and extracts (*7*). Store away from light and humidity (*1*).

Posology

(Unless otherwise indicated)
Daily dosage: 250.0–312.5 mg twice daily of a standardized powdered extract of the crude drug (equivalent to 100 mg aescin) containing 16–20% triterpene glycosides, calculated as aescin (*55*); topical gels containing 2% aescin (*22–24*, *49*).

References

1. *Pharmacopée française*. Paris, Adrapharm, 1996.
2. *Deutsches Arzneibuch*. Stuttgart, Deutscher Apotheker Verlag, 1998.
3. Blaschek W et al., eds. *Hagers Handbuch der pharmazeutischen Praxis*. *Folgeband 2: Drogen A–K*, 5th ed. Berlin, Springer-Verlag, 1998.
4. Youngken HW. *Textbook of pharmacognosy*, 6th ed. Philadelphia, PA, Blakiston, 1950.
5. Bruneton J. *Pharmacognosy, phytochemistry, medicinal plants*. Paris, Lavoisier, 1995.
6. Farnsworth NR, ed. *NAPRALERT database.* Chicago, University of Illinois at Chicago, IL, February 9, 1998 production (an online database available directly through the University of Illinois at Chicago or through the Scientific and Technical Network [STN] of Chemical Abstracts Services).
7. Bombardelli E, Morazzoni P. *Aesculus hippocastanum* L. *Fitoterapia*, 1996, 67:483–510.
8. *Quality control methods for medicinal plant materials.* Geneva, World Health Organization, 1998.
9. *European pharmacopoeia*, 3rd ed. Strasbourg, Council of Europe, 1996.
10. *Guidelines for predicting dietary intake of pesticide residues*, 2nd rev. ed. Geneva, World Health Organization, 1997 (document WHO/FSF/FOS/97.7).
11. Kockar OM et al. Quantitative determination of escin. A comparative study of HPLC and TLC-densitometry. *Fitoterapia*, 1994, 65:439–443.
12. Vanhaelen M, Vanhaelen-Fastre R. Quantitative determination of biologically active constituents in medicinal plant crude extracts by thin-layer chromatography–densitometry. *Journal of Chromatography*, 1983, 281:263–271.
13. Alter H. Zur medikamentösen Therapie der Varikosis. *Zeitschrift für Allgemeine Medizin*, 1973, 49:1301–1304.
14. Bisler H et al. Wirkung von Rosskastaniensamenextrakt auf die transkapilläre Filtration bei chronischer venöser Insuffizienz. *Deutsche Medizinische Wochenschrift*, 1986, 111:1321–1328.
15. Diehm C et al. Medical edema protection—clinical benefit in patients with chronic deep vein incompetence. A placebo-controlled double-blind study. *Vasa*, 1992, 21: 188–192.
16. Diehm C et al. Comparison of leg compression stocking and oral horse-chestnut seed extract therapy in patients with chronic venous insufficiency. *Lancet*, 1996, 347: 292–294.
17. Friederich HC et al. Ein Beitrag zur Bewertung von intern wirksamen Venenpharmaka. *Zeitschrift für Hautkrankheiten*, 1978, 53:369–374.
18. Lohr E et al. Ödemprotektive Therapie bei chronischer Veneninsuffizienz mit Ödemneigung. *Münchener Medizinische Wochenschrift*, 1986, 128:579–581.
19. Neiss A, Böhm C. Zum Wirksamkeitsnachweis von Rosskastaniensamenextrakt beim varikösen Symptomenkomplex. *Münchener Medizinische Wochenschrift*, 1976: 213–216.
20. Rudofsky G et al. Ödemprotektive Wirkung und klinische Wirksamkeit von Rosskastaniensamenextrakt im Doppelblindversuch. *Phlebologie und Proktologie*, 1986, 15: 47–54.
21. Steiner M, Hillemanns HG. Untersuchung zur ödemprotektiven Wirkung eines Venentherapeutikums. *Münchener Medizinische Wochenschrift*, 1986, 31:551–552.
22. Götz AK, Giannetti BM. Naturstoffe in der Therapie stumpfer Sportverletzungen—heute noch zeitgemäss? *Erfahrungsheilkunde*, 1990, 6:362–371.
23. Calabrese C, Preston P. Äscin bei der Behandlung von Hämatomen—eine randomisierte doppelblind-Studie. *Zeitschrift für Phytotherapie*, 1994, 60:112.
24. Geissbühler S, Degenring FH. Behandlung von chronisch venöser Insuffizienz mit Aesculaforce Venengel. *Schweizerische Zeitschrift für Ganzheits Medizin*, 1999, 11: 82–87.

25. *Materia medica of Chinese herbology*. Shanghai, State Administration of Traditional Chinese Medicine, Shanghai Scientific and Technical Press, 1996.
26. Arnold M, Przerwa M. Die therapeutische Beeinflussbarkeit experimentell erzeugter Ödeme. *Arzneimittel-Forschung,* 1976, 26:402–409.
27. Leslie GB. A pharmacometric evaluation of nine Bio-Strath herbal remedies. *Medita,* 1978, 8:3–19.
28. Cebo B et al. Pharmacological properties of saponin fractions obtained from domestic crude drugs: *Saponaria officinalis*, *Primula officinalis* and *Aesculus hippocastanum. Herba Polonica,* 1976, 22:154–162.
29. Guillaume M, Padioleau F. Veinotonic effect, vascular protection, anti-inflammatory and free-radical scavenging properties of horse chestnut extract. *Arzneimittel-Forschung,* 1994, 44:25–35.
30. Aizawa Y et al. Anti-inflammatory action of aescin. Intravenous injection. *Oyo Yakuri,* 1974, 8:211–213.
31. Damas P et al. Anti-inflammatory activity of escin. *Bulletin de la Société royale des Sciences de Liège,* 1976, 45:436–440.
32. Tarayre JP et al. Pharmacological study of some capillary-acting substances. *Annales de Pharmacie française*, 1975, 33:467–469.
33. Girerd RJ et al. *Archives for International Pharmacodynamics*, 1961, 133:127–130.
34. Preziosi P, Manca P. The anti-edematous and anti-inflammatory effects of aescin and its relation to the hypophyseal–adrenal system. *Arzneimittel-Forschung,* 1965, 15:404–413.
35. Longiave D et al. The mode of action of aescin on isolated veins: relationship with $PGF_{2\alpha}$. *Pharmacological Research Communications,* 1978, 10:145–152.
36. Felix W et al. Vasoaktive Wirkungen von alpha-Aescin. *Ergebnisse der Angiologie*, 1984, 30:93–105.
37. Facino RM et al. Anti-elastase and anti-hyaluronidase activities of saponins and sapogenins from *Hedera helix*, *Aesculus hippocastanum,* and *Ruscus aculeatus*: factors contributing to their efficacy in the treatment of venous insufficiency. *Archiv der Pharmazie (Weinheim),* 1995, 328:720–724.
38. Masaki H et al. Active-oxygen scavenging activity of plant extracts. *Biological and Pharmaceutical Bulletin,* 1995, 18:162–166.
39. Erler M. Rosskastaniensamenextrakt bei der Therapie peripherer venöser Ödeme. *Die Medizinische Welt,* 1991, 42:593–596.
40. Rehn D et al. Comparative clinical efficacy and tolerability of oxerutins and horse chestnut extract in patients with chronic venous insufficiency. *Arzneimittel-Forschung,* 1996, 46:483–487.
41. Steiner M, Hillemanns HG. Venostatin retard in the management of venous problems during pregnancy. *Phlebology,* 1990, 5:41–44.
42. Pilz E. Ödeme bei Venenerkrankungen. *Die Medizinische Welt,* 1990, 41:1143–1144.
43. Pauschinger P. Neuere Untersuchungen zur Wirkung von Venostasin retard auf die kapilläre Funktion. *Ergebnisse der Angiologie*, 1984, 30:129–137.
44. Klemm J. Strömungsgeschwindigkeit von Blut in variköse Venen der unteren Extremitäten. *Münchener Medizinische Wochenschrift,* 1982, 124:579–582.
45. Marshall M, Dormandy JA. Oedema of long-distance flights. *Phlebology,* 1987, 2: 123–124.
46. Greeske K, Pohlmann BK. Rosskastaniensamenextrakt—ein wirksames Therapieprinzip in der Praxis. *Fortschritte der Medizin,* 1996, 114:196–200.
47. Masuhr T et al. Nutzen-Risiko-Bewertung von Venoplant® retard, einem auf Aescin standardisierten Präparat aus Rosskastaniensamenextrakt, bei Patienten mit chronischer Veneninsuffizienz. *Top Medizin,* 1994, 8:21–24.
48. Pittler MH, Ernst E. Horse-chestnut seed extract for chronic venous insufficiency. A criteria-based systematic review. *Archives of Dermatology,* 1998, 134:1356–1360.

49. Calabrese C, Preston P. Report on the results of a double-blind, randomized, single-dose trial of a topical 2% aescin gel versus placebo in the acute treatment of experimentally induced hematoma in volunteers. *Planta Medica,* 1993, 59:394–397.
50. Grasso A, Corvaglia E. Due casi di sospetta tubulonefrosi tossica da escina. *Gazzetta Medica Italiana,* 1976, 135:581–584.
51. Schimmer O et al. An evaluation of 55 commercial plant extracts in the Ames mutagenicity test. *Pharmazie,* 1994, 49:448–451.
52. Kreybig H, Prechtel K. Toxizitäts- und Fertilitätsstudien mit Aescin bei der Ratte. *Arzneimittel-Forschung,* 1977, 7:1465–1466.
53. Leslie GB, Salmon G. Repeated dose toxicity studies and reproductive studies on nine Bio-Strath herbal remedies. *Swiss Medicine,* 1979, 1:1–3.
54. Liehn HD et al. A toxicological study of extractum Hippocastani seed (EHS). *Panminerva Medicine,* 1972, 14:84–91.
55. Blumenthal M et al., eds. *The complete German Commission E monographs*. Austin, TX, American Botanical Council, 1998.
56. Escribano MM et al. Contact urticaria due to aescin. *Contact Dermatitis,* 1997, 37:233–253.