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Comprehensive review of the nutritional composition and health benefits of horse chestnut (*Aesculus hippocastanum*) seeds and nuts: A review

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Abstract
This review examines the nutritional composition and health benefits of horse chestnut seeds/nuts, emphasizing their diverse properties such as antioxidative, anti-inflammatory, antiviral, immune-modulating, spasmolytic, anticarcinogenic, and neurodepressive effects. Bioactive compounds, including saponins, flavonoids, tannins, alkaloids, glycosides, triterpenoids, steroids, and phenolic substances, contribute to these therapeutic qualities. Noteworthy constituents aesculin and aescin play key roles. Horse chestnut seeds/nuts find applications in food, animal feed, cosmetics, and toiletries. Indian Horse Chestnut Seed Extract (HCSE), initially pharmaceutical, is gaining recognition for its nutritional value, displaying varying profiles in raw and processed forms, especially in sugar and starch content. Mineral analysis reveals essential elements such as calcium, phosphorus, potassium, copper, manganese, iron, and zinc. Quantification of phytochemicals, including total phenols, tannins, and flavonoids, underscores their bioactive nature. In Serbia, urban horse chestnut trees are explored for pharmaceutical potential, examining fatty acids, flavonoids, aescin, and heavy metals across 11 genotypes. Dominance of unsaturated fatty acids, particularly oleic acid, and the presence of flavonoids like rutin, quercetin, and kaempferol, with rutin being prominent, are noted. Aescin content displays variability among genotypes, highlighting the diversity in this botanical resource.

Keywords: Escin content, spasmolytic effects, horse chestnut seed extract (HCSE), flavonoids

1. Introduction
The horse chestnut tree, scientifically known as *Aesculus indica*, is a remarkable species belonging to the *Sapindaceous* family, with its roots deeply embedded in the temperate regions of Asia, America, and Europe. It thrives at elevations ranging from 900 to 3600 meters, (Santapau and Henry 1973) [131] showcasing its resilience and adaptability to diverse climates. Known by various regional names like "Bankhor" in the state of Himachal Pradesh and "Handun" in the Kashmir, this tree is especially prevalent in India, Pakistan, Afghanistan, and Nepal, where it graces damp and shaded places, notably in the states of Uttar Pradesh, Himachal Pradesh, and Jammu and Kashmir. (Zhang *et al*. 2010; Singh 2006) [176, 137]. Despite its widespread use in afforestation efforts in the Kashmir region, the Indian horse chestnut remains relatively unexplored as an agroforestry species, primarily due to a lack of comprehensive information concerning its propagation, utility, and cultural practices (Majeed *et al*. 2009) [86]. However, it boasts immense significance in traditional and medicinal contexts, where different parts of the tree, including its leaves and seeds, find utility as fodder, charcoal material, and timber. Wildlife, including bears, monkeys, and various forest-dwelling animals, depend on its seeds as a food source. Hill tribes have traditionally turned to these seeds as sustenance during times of scarcity, following a meticulous process to remove their bitter components. Moreover, the versatile horse chestnut serves as a valuable resource in various industrial applications. Its powdered seeds are blended with wheat flour to create culinary delights, (Thakur *et al*. 2015) [155] while the timber derived from this tree finds its way into the production of furniture, cellulose, and high-quality pencils. (Bellini and Nin 2005) [12]. It is also a preferred choice in building construction, packing cases, cooperage, and a wide array of woodcraft products (Anonymous 1985) [4].

Additionally, the bark of the horse chestnut tree is harnessed for creating pastes used in the treatment of painful and dislocated joints. The tree further contributes to various sectors through the extraction of valuable compounds from its different parts. For instance, stem bark extracts exhibit fungicidal properties, while flower and leaf extracts demonstrate pesticidal potential in the cultivation of crops like sugarcane and rice. (Anwar and Jabbar 1987) [7]. The roots of the Indian horse chestnut have a historical legacy in the treatment of leucorrhoea, showcasing its medicinal prowess (Anonymous 1985) [4]. Furthermore, leaf extracts boast antioxidant properties and have shown effectiveness against various pathogenic bacteria (Chakraborty 2009; Bibi *et al*. 2011) [24, 17]. The tree's traditional uses extend to the treatment of ailments such as rheumatism, skin disorders, colic issues, leucorrhoea, and vein complaints, thanks to the presence of bioactive compounds like saponins, fatty oils, glycosides, and flavonoids (Zhang *et al.* 2010; Rana and Datt 1997; Shah and Joshi 1971) [176, 125, 135]. The horse chestnut's significance is not limited to traditional or medicinal realms; its nutritional composition is also a subject of interest. Rich in diverse chemical compounds, including polysaccharides like starch, proteins, lipids, and essential mineral elements such as nitrogen, phosphorus, potassium, calcium, sulphur, copper, iron, zinc, and manganese, this tree presents a unique nutritional profile. However, it faces a challenge posed by anti-nutritional factors, particularly saponins, which can hinder its utilization as a food ingredient. The presence of these naturally occurring compounds can lead to adverse effects on nutrient utilization and overall food quality. Furthermore, the issue of food preservation is a critical concern in the modern era. The presence of oxygen in packaged foods can trigger oxidative reactions, resulting in food deterioration, off-odours, off-flavours, adverse colour changes, and diminished nutritional quality. To combat these effects, oxygen scavengers are employed to remove oxygen from packaging, thereby retarding oxidative reactions. Similarly, moisture control agents play a pivotal role in food preservation, regulating water activity, reducing microbial growth, and preventing undesirable condensation in various food products. In light of these considerations, this investigation aims to explore the physio-chemical changes in pretreated horse chestnut flour during storage. By delving into the nutritional aspects, anti-nutritional factors, and the impact of storage conditions, we seek to enhance our understanding of the Indian horse chestnut's potential as a valuable food and agroforestry resource.

2. Botanical Description

The horse chestnut, scientifically known as *Aesculus hippocastanum*, is a deciduous tree belonging to the small family *Hippocastanaceae* within the genus *Aesculus*. This genus encompasses approximately 25 species originating from North America, South-East Europe, East Asia, and India. However, classifying these species is challenging due to their considerable variability and frequent hybridization, particularly involving *A. hippocastanum* and four other species. This hybridization complicates the identification of buckeye trees in northeastern America, leading to confusion in both natural populations and gardens. The scientific literature on the Aesculus genus is substantial primarily due to the economic value given to various species within it (Rehder, A. 1940) [127]. Numerous synonyms have been assigned to the 15 known species and hybrids, with about 175 names documented. Aesculus is divided into four parts according to a botanical categorization scheme (Rehder, A. 1940) [127], based on the morphology of the flowers, leaves, and fruits, as well as types of winter buds (Engler, A. 1988) [39]. The horse chestnut falls into the section known as *hippocastanum* K. Koch) (Synonym *Aesculus Pax*).There are several botanical forms and varieties of *A. hippocastanum*.

The horse chestnut tree is a long-lived species, frequently exceeding 200 years in age, and can reach heights of 25 to 30 metres. It is characterized by ovoid, sticky, dark reddish-brown buds measuring three to five centimetres in length. Its leaves are palmate, featuring long petioles and five to seven leaflets, which can grow up to 20 centimetres long. The tree produces hermaphrodite or male flowers, typically appearing on trees older than 14 to 15 years, arranged in large, erect, pyramidal racemes. Not all of these flowers are fertile or complete in their reproductive function. Although there are rarer variations with yellow or pale red flowers, the corolla of the horse chestnut flower has red, yellow, and white spots at the base. The fruit is a trilocular (Sometimes bilocular), dry, dehiscent capsule that is bright green at first but eventually turns brown on the exterior and white inside as it matures. When completely ripe, the fruit has a thick, sub-globose, spiky pericarp with fine pubescence, and it can reach a diameter of 6 cm. It opens into three valves, with each containing one to four brown, faceted, or round seeds lack of albumen. These seeds have two irregular, large, fleshy, oily, and starchy cotyledons that may be fused. (Bailey, L.H. 1947. Maugini, E. 1988. Traverso, O. 1926) [10, 93, 160]. The horse chestnut tree may reach a certain height of 100 feet and is medium to large in size. It features a short, straight, cylindrical trunk, a spreading crown, with smooth light brown bark. The dark five or seven lance-shaped leaflets are united at one point on palmate green leaves. The tree bears 4-6-inch, multi-coloured flowers that bloom in May and June. The majority of the blooms are white or creamy white. After fertilisation, these flowers produce a significant number of seeds every October and November. (Wani *et al*. 2014) [169]. The seeds are dark and lustrous brown, enclosed in light brown fruit capsules with green patches. October to early December, the tree sheds its leaves; new leaves appear in April. It is particularly attractive when in bloom during May and June. Additionally, the leaves and shoots are used as fodder, while the fruits are consumed by cattle.

Table 1: Varieties and botanical forms of *A. hippocastanum*

Varieties and forms	Characteristics
Baumannii 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
Laciniata Leroy var. *laciniata* Jacques var., and *laciniata* 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
Schirnhofer Rosenth. var. *Schirnhofer* Voss. F.	Double yellowish-red flowers
Tortuosa Bth. f.	Twisted branches
Umbraculifera Rehd. var. *umbraculifera* Jeag. var. *umbraculifera* Rehd. f.	Compact roundish head
pendula Puvill and *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

Source: (Bailey, L.H. 1947. Maugini, E. 1988, Traverso, O.1926) [10, 93, 160]

3. Cultivation

Aesculus hippocastanum (Horse chestnut) is a highly versatile plant with a global presence in temperate and cold-temperate regions. Its successful growth hinges on specific soil conditions, shelter from harsh winds, and considerations for its sensitivity to various environmental factors.

3.1. Preferred growth conditions

Horse chestnut thrives in siliceous, deep, and fertile soils. These soils should ideally be well-draining to prevent waterlogging, as the plant does not tolerate drought conditions. While it can adapt to full sun, it prefers partial shade, similar to the hornbeam. This adaptable tree has an impressive altitudinal range, growing at elevations up to 1300 meters and even thriving in northern regions like Sweden and Norway, where the climate can be quite severe. However, it's crucial to note that the horse chestnut tree is sensitive to salinity, air pollution, and aggressive pruning techniques. (Allegri, E. 1935. De Martino, A. 1982, Merendi, A. 1969) [2, 30, 96].

3.2. Resilience and susceptibility

After its juvenile growth phase, horse chestnut demonstrates resilience, enduring low temperatures and frost. However, it can be vulnerable to nonparasitic leaf scorch, particularly during dry seasons, but wet seasons can also lead to significant damage. While several cryptogams and insects have been indicated as probable causes of horse chestnut disorders, the tree generally remains resistant to pests and diseases (Hepting, G.H. 1971, Pirone, P.P. 1978) [58, 109]. The most significant fungal threat is Guigtiardia aesculi, causing leaf blotch (Stewart, V.B. 1916) [150], which has been problematic in North America, Italy, and beyond (Goidanich, G. 1983.) [48]. In severe instances, particularly within nursery environments, this disease has the potential to lead to early and total leaf loss (Scaramuzzi, G. 1954) [133]. It is suspected that the disease can be transmitted through infected seeds and subsequently spread via sexual reproduction (Orton, C.R. 1931) [103]. The observation that certain genotypes exhibit reduced vulnerability implies that there is potential for the identification and cultivation of more resilient clones (Hepting, G.H. 1971) [58].

3.3. Cultivation techniques

Historically, horse chestnut cultivation has predominantly served ornamental purposes in urban areas, parks, and large gardens. Information regarding large-scale fruit production remains limited.

3.4. Propagation by seeds

Horse chestnut propagation primarily relies on seeds. However, the vitality of these seeds diminishes rapidly after dehydration, necessitating their timely collection when capsules turn yellowish or when they fall to the ground (McMillan-Browse, P.D.A. 1971.) [94] Seeds should be either sown without delay or subjected to stratification or prechilling, wherein they are stored in airtight polyethylene bags at temperatures ranging from 2 to 4°C, while maintaining moderate humidity levels. This is done in preparation for spring planting (McMillan-Browse, P.D.A. 1971. Suszka, B. 1966, Suszka, B. 1982) [94, 152, 153]. If stratification is not employed, an alternative method involves soaking the seeds in water for approximately 48 hours. Following that, remove approximately one-third of the seed at the scar end without removing the seed coat. Germination, which is typically hypogeal, typically occurs within three to four weeks, as indicated by sources. (Allegri, E. 1935., Rudolf, P.O. 1974) [2, 130].

3.5. Propagation by grafting

Horse chestnut trees can also be easily grafted into immature one or two-year-old seedlings. Various grafting techniques such as triangle, saddle, horseback, wedge, apical side veneer, and simple cleft grafts are commonly employed during the period from February to March. Bark grafting, on the other hand, is typically performed in March-April. This process involves using dormant apical scions, which are obtained in January or February. Alternatively, one can select medium-sized buds from the centre area of the branch in late summer to achieve good budding. (McMillan-Browse, P.D.A. 1971., McMillan-Browse, P.D.A. 1982., Merendi, A. 1969) [94, 95, 96].

3.6. Propagation by cuttings

The technique of scion grafting, including methods like the double English wedge, simple wedge, and lateral wedge, can be attempted in the field, but it's worth noting that it often yields unsatisfactory results (Leiss, J. 1967.) [78]. Interestingly, the Aesculus genus, to which horse chestnut belongs, exhibits a high success rate when subjected to bench grafting. While not commonly practiced, an alternative method for propagating horse chestnut involves the use of cuttings. These cuttings consist of terminal shoots, approximately 8 cm in length, collected during the summer. To enhance root formation, about 75 percent of the leaves are removed from these cuttings. Rooting is facilitated under misty conditions using a substrate composed of peat, bark, and perlite in a 1:1:1 ratio. The most favourable outcomes are achieved when using semi hardwood cuttings collected between May and June (Chapman, D.J., and S. Hoover. 1982) [26].

3.7. *In vitro* culture

In terms of agricultural innovation, horse chestnut offers a compelling alternative to traditional farming strategies for producing secondary metabolites and bioactive chemicals. The scientific literature has particularly focused on the *In vitro* cultivation of *Aesculus hippocastanum*, with an emphasis on two critical aspects: callogenesis and somatic embryogenesis. Researchers have successfully generated somatic embryos from various plant tissues, including microspores (Radojevic, L. Radojevic, L. 1980, Chalupa, V. 1991, Radojevic, L. 1988) [122, 25 123]. anther filaments (Jorgensen, J. 1959, Kiss, J., L. Heszky, E. Kiss, and G. Gyulai. 1992) [65, 74]. Numerous studies have demonstrated high efficiency adventive development on somatic embryos of anther, filament, and immature proembryos origin in horse chestnut. (Dameri *et al*. 1986. Callus Profumo *et al*. 1986) [28, 117]. (P. Gastaldo. 1992, P, P. Gastaldo *et al.* 199l) [113, 116]. These findings notably demonstrate that the synthesis of escin, a valuable compound, can occur *In vitro* without the need for plant growth regulators. Furthermore, it has been established that the quantity of escin produced is independent of the specific tissue (Kamenicka *et al.* 1988, P. Gastaldo *et al.* 1991) [68, 116]. Aescin concentration in embryogenic callus and in embryoids derived from *Aesculus hippocastanum* leaf explants. (Dameri, and P. Modenesi. 1980) [18]. Aescin content in calluses from *Aesculus hippocastanum* cotyledons grown *In vitro.* Interestingly, escin synthesis naturally occurs in various parts of the plant *in vivo*, with embryos serving as the primary site for accumulating this bioactive component. These outcomes hold significant promise, as *In vitro* cultures present an avenue for augmenting the synthesis of these bioactive substances. This can be achieved by subjecting the cultures to various treatments, including modifications to culture conditions and the composition of the growth medium, ultimately enhancing their capacity for biosynthesis.

3.8. Field trials and training systems

Regarding the cultivation of horse chestnuts, initial field experiments were conducted at Florence University as part of the EU's "European Aesculus Cultivation System" project. The trials revealed that horse chestnut trees often respond well to a variety of training and pruning methods. The most appropriate trees for training were those with a well-developed central axis. Having a leading limb enables the tree to achieve a suitable balance in nutrient distribution. Training techniques that adhered to the tree's natural development pattern were effective. The study results suggested that harnessing the horse chestnut's strong desire to grow upright and its branching structure by shaping trees into a modified central leader shape proved beneficial. Furthermore, trees were simple to train into the palmette system; however, there were concerns because this form may prevent horse chestnut trees from growing a large enough crown for major yields when they reached maturity. In the open-centre tree method, the primary advantage was the ability to utilize the natural phyllotaxis of the horse chestnut and establish three to four symmetrical side scaffold branches (at 120° or 90° angles). However, challenges included the need for disbudding after topping and pollarding the main axes and the requirement for divaricators (Such as sticks, canes, weights, and bindings) to encourage the growth of neo-formed shoots, which typically grew upward with tightly closed angle crotches, sometimes leading to limb breakage during bending and binding.

3.9. Seed collection and preservation

The method used to grow horse chestnuts is to collect seeds in the autumn and sowing them in the early spring. It is imperative that seeds be preserved properly, such as by storing them in sand to avoid mould and rot throughout the winter. (Khan *et al*. 1993) [72]. The seeds exhibit faster germination when they are soaked in water ((Bhagat *et al.* 1993) [16]. These seeds can thrive in various weather circumstances and soil varities, but they particularly excel in sandy loam soil, requiring minimal care during their growth ((Maithani *et al*. 1990) [83] Collecting fully mature seeds is essential for maintaining viability, especially since these seeds fall into the temperate recalcitrant category with limited viability becauseof the high moisture content (Uniyal and Nautiyal 1996; Harrington 1970) [163, 55].

4. Biochemical composition

The seeds of the horse chestnut display considerable diversity in their biochemical makeup, and it is essential to create suitable conditions for preserving these seeds for germination without significantly reducing their moisture content (Bonner *et al*. 1994) [21]. Carbohydrates, specifically starch, serve as the primary component responsible for seed germination by providing the necessary energy for growth. Generally, the various biochemical substances present have an impact on the germination process and the production of high-quality seedlings. The seed's biochemical composition influences its germination potential. (Stein *et al*. 1974) [149]. Adequate levels of water and starch promote germination, whereas fat content acts as an energy source. Additionally, proteins are essential for preserving the structure of seeds and preventing excessive drying.

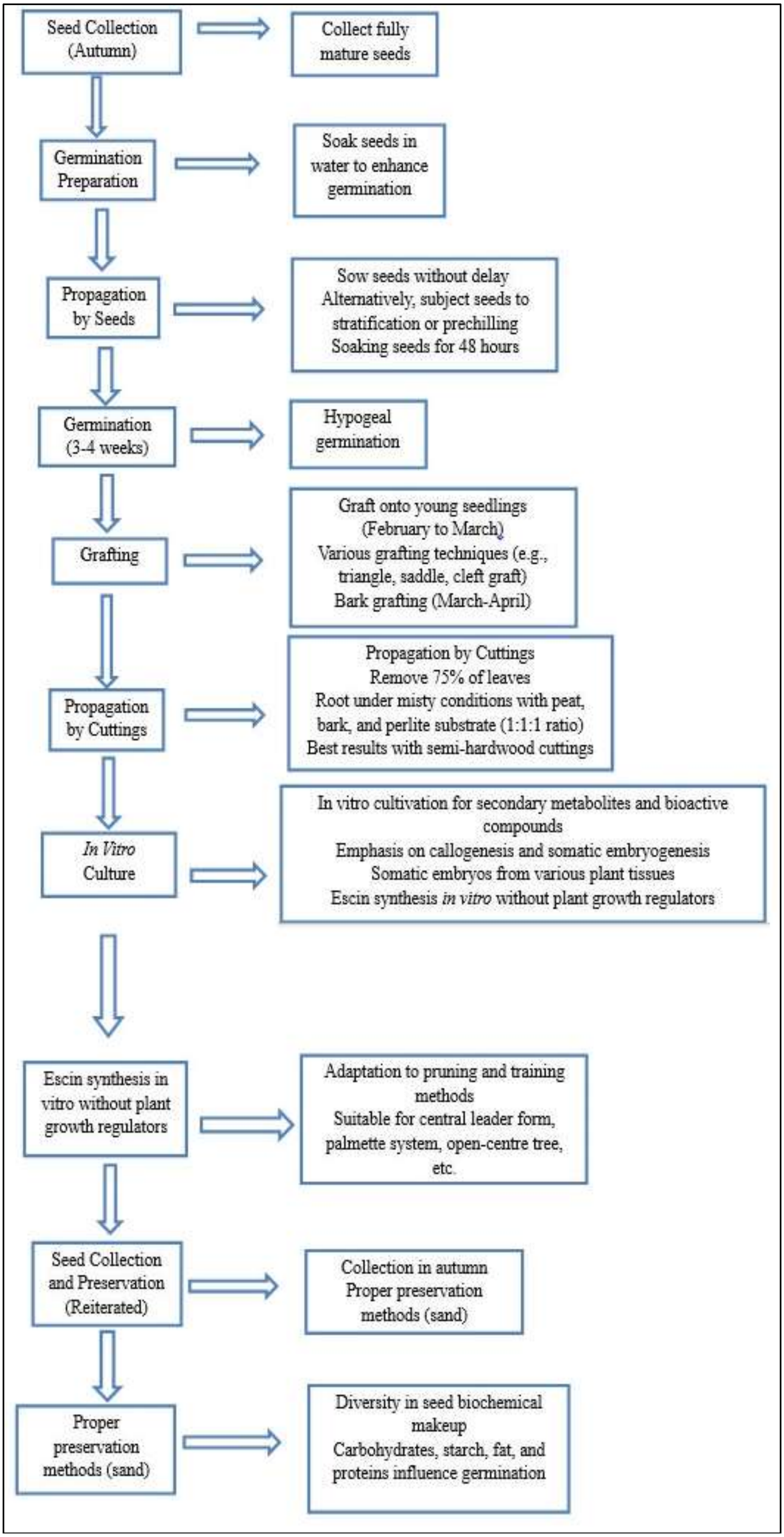

Fig 1: Process of Cultivation

5. Drugs from horse chestnut

Horse chestnut holds a recognized place in the pharmacopoeias of numerous countries, both through official inclusion and within traditional medicinal practices. These countries include Austria, Belgium, Brazil, Chile, Denmark, Germany, France, Greece, India, Japan, Mexico, Paraguay, Portugal, Romania, Spain, former USSR nations, Sweden, Switzerland, and Venezuela. (Penso, G.1983) [107].

Table 2: Drug forms of horse chestnut

Drug name	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 horn. HAB1	Fresh peeled seeds
Aesculus hippocastanum & cortice, ethanol. decoctumhom. HAB1	Horse chestnut cortex
Aesculus hippocastanum & floribus horn. HAB34	Fresh white and red flowers
Aesculus hippocastanum horn. HPUS78	Mature and fresh seeds without coats

Source: (Hansel, R., K. Keller, H. Rimpler, and G. Schneider. 1992) [52].

6. Chemical composition of horse chestnut

Horse chestnut seeds are recognized for their sour and bitter taste, attributed to their high pentacyclic triterpene saponin content, making them toxic. Extensive research conducted in the early 21st century yielded valuable insights into the chemical constituents of horse chestnut.

6.1. Escin: a complex saponin

Escin, an important discovery, is a complex mixture of over 30 triterpene glycosides produced from protoaescigenin and barringtogenol-C (Acar, A.M., and S. Paksoy. 1993) [1]. Since different organic acids are bonded to the terpenic moiety, it displays a wide range of characteristics. Escin's composition includes diacyl derivatives of tetra- and penta-hydroxy-β-amyrin types, with a glucuronic acid at position 3 and two sugar residue substituents. The esterifying organic acids include acetic, butyric, isobutyric, angelic, and tiglic acids, and the sugar moieties after acid hydrolysis include glucuronic acid, glucose, xylose, and galactose.β-escin and cryptoescin are the isomeric forms of escin that are identified by the presence of an acetyl group at the 22-α-hydroxyl and 28-hydroxyl positions, respectively. Remarkably, α-escin, bearing both acetyl groups, exhibits exceptional stability in aqueous solutions. (Bombardelli, E., and P. Morazzoni. 1996) [19]. Escin can occur in crystalline or amorphous forms, and the latter is more bioavailable. (Pedretti, M. 1990) [106]. Recent studies have shown the five triterpene oligoglycosides, namely escin-Ia, Ib, IIa, IIb, and IIIa, isolated from horse chestnut seeds. (Yoshikawa *et al*. 1994) [175]. EscinsIa, Ib, IIa, and IIIa are bioactive triterpene oligoglycosides produced from the seeds of *Aesculus hippocastanum* L. They reduce ethanol absorption and have hypoglycemic efficacy in glucose tolerance tests.

6.2. Additional seed components

Horse chestnut seeds contain various other compounds, such as coumarin glycosides, including esculoside, flavonoid derivatives (e.g., glucosides of quercetin and campherol), starch (Comprising 23-48% of the seed's composition), tannins, oil (4-6%), cellulose (3%), proteins (Including adenine, adenosine, guanine, uric acid, accounting for 8-12% of the composition), sucrose (8-17%), glucose (5-6%), albumin (7-11%), proanthocyanidins (Including leucoanthocyanins and catechin), dyes, and salts of calcium, magnesium, copper, and manganese. Essential nutrients like methionine, B group vitamins, vitamin C, vitamin K, and provitamin D are also present. Notably, coumarin, esculin, and fraxin are notably absent from both the seeds and their seed coat (pericarp). (G. Hildebrand. 1955, G. Schneider. 1992. A.M. 1994, V.N. 1966, W. 1968) [43, 52].

6.3. Proanthocyanidins in pericarp and seed coat

The pericarp and seed coat contain proanthocyanidins, which include epicatechin, leucoanthocyanins, and condensed tannins. (G.-L. Nonaka *et al*. 1987) [98].

6.4. Oil composition from seeds

The oil extracted from peeled horse chestnut seeds predominantly contains oleic acid (65%), linoleic acid (21%), palmitic acid (4.5%), stearic acid (3.7%), and linolenic acid (2.3%). The unsaponifiable portion of horse chestnut oil, though constituting only a small part (2-3%) of the oil, contains essential compounds such as sterols, triterpenes, aliphatic alcohols, vitamins, hydrocarbons, pigments, and others. Analytical studies have identified compounds like campesterol, cholesterol, stigmasterol, sitosterol, α-spinasterol, stigmasterol, and triterpenes in this fraction (Stankovic *et al.* 1984) [147].

6.5. Reserve substances in seeds

Reserve substances in horse chestnut seeds include fructose, galactose, and amylose (Hänsel, R., K. Keller, H. Rimpler, and G. Schneider. 1992) [52] along with starch, oil, albumin (7-11%), and phytosterols. The unsaponifiable portion of the oil contains phytosterols and triterpenes (Stankovic *et al.* 1985) [147]. Escin functions as a reserve material as well. Hippocastanoside, which is present in the seed pericarp, is a chemical that mostly varies from escin in its aglycone part. (B. Proksa *et al.* 1989) [164]. The structure of hippocastanoside, a novel saponin derived from the horse chestnut seed pericarp.

6.6. Escin content variation

The content of escin in horse chestnut seeds can vary significantly, ranging from 3% (Kamenicka, A., and M. Rypak. 1988) [68] to 13% (Handa, S.S., A.S. Chawla, and A.K. Sharma. 1992) [50] to 28% (Karuza-Stojakovic, L., J. Petricic, and Z. Smit. 1991 [69]. The total triterpene glycoside content expressed as aescin in horse chestnut seeds during vegetation influenced by factors such as genotype, time of year, and seed maturity. Escin concentrations are highest in the cotyledons in January. (P. Gastaldo, *et al.* 1987) [115], though others reported that escin content increases rapidly until reaching a maximum in August. Crucially, five months after germination, escin is not present in the roots or seeds, and its concentration drastically drops post germination, indicating that it plays a role in metabolism related to plant development.

6.7. Bark composition

Horse chestnut bark contains an array of substances, including esculitannic acid, coumarin glycosides (2-3%), coumarins (7.2%), fraxin (2.8%), scopolin (0.15%), esculetin (0.2%), scopoletin (trace amounts), and argirin. It also includes allantoin, resin, catechic tannins (e.g., leucocyanidin, leucodelphinidin, and epicatechins of the proanthocyanin group), dyes, citric acid, and fatty oil. Notably, the bark lacks saponins, but it contains substantial quantities of quebrachitol and allantoin.

6.8. Leaf composition

Horse chestnut leaves have a lower coumarin content than the bark, with esculin at 0.02% and fraxin found in trace amounts. However, the stalks and veins of the leaves contain saponins. Hydrolysed extracts from leaves contain flavonols such as quercetin, isoquercetin, and campherol, along with leucocyanidin and p-coumaric acid (Hänsel, R., K. Keller, H. Rimpler, and G. Schneider). Furthermore, free amino acids like adenine, adenosine, guanine, uric acid, and others (e.g., alanine, valine, cysteine, phenylalanine, tyrosine, α-aminobutyric acid, leucine, isoleucine, arginine, glutamic acid, asparagine, and glutamine) have been identified. The petroleum ether extract of leaves contains campesterol, stigmasterol, and β-sitosterol, along with various fatty acids (e.g., lauric, myristic, palmitic, palmitoleic, stearic, linoleic, and linolenic acids). Leaves also contain α-carotene, vitamin C, tannins, and resin (Souleles, Chr. and K. Vayas. 1986) [142] with young leaves in particular having high tannin content and significant amounts of quebrachitol.

6.9. Flower composition

Flowers of the horse chestnut contain compounds such as campherol-3-arabinoside, campherol-3-glucoside, campherol-3-rhamnoglucoside, campherol-3-rutinoside and quercetin-3-rutinoside. (Harborne, J.B. 1967) [54]. In addition to other compounds, the leaves of the horse chestnut tree also contain quercetin-3-rhamnoside, quercetin-3-glucoside, and quercetin-3-arabinoside (J.-Y *et al*. 1973) [60]. The primary constituents found in the flowers consist of campherol-3-arabinoside, campherol-3-glucoside, campherol-3-rhamnoglucoside, isoquercetin, and rutin. Adenine, adenosine, guanine, uric acid, and other compounds have also been identified. (Hänsel *et al*. 1992) [52].

7. Production of horse chestnut

Currently, horse chestnut production worldwide depends upon naturally growing trees cultivated for ornamental purposes. However, there are significant challenges associated with this production. The yield of seeds from horse chestnut trees varies widely in terms of both quantity and quality. Some trees may produce as little as 2 to 3 kg of fresh seeds, while others can yield as much as 25 kg per tree. Additionally, the quality of the seeds, as measured by the escin content (A key bioactive compound), can range from 0.1 to 15 percent of the seed's dry weight. One major concern in horse chestnut production is the inability to effectively prevent and control pests and diseases in these trees. Furthermore, the future acquisition of plant-derived chemicals, such as escin, may become challenging due to decreasing availability of wild horse chestnut plants and increasing urban pollution. To address these challenges, it is necessary to cultivate highly productive horse chestnut plants of superior quality. This can be accomplished by clonal selection, which involves both the genetic and hygienic aspects of existing plant materials, combination with appropriate cultivation methods.

Efforts towards the selection of superior horse chestnut clones for commercial purposes have been initiated, notably through projects like the EU's "European Aesculus Cultivation System." These projects have evaluated horse chestnut populations over multiple seasons and have identified significant variability in various traits. Notably, there is substantial variation in productive characteristics, including seed weight, size, yield, and escin content. This suggests that selecting individuals with high productivity can be advantageous both in terms of quantity and economic benefits. However, one of the challenges faced during clonal selection has been the evaluation of escin content. Escin content in horse chestnut seeds can fluctuate significantly from one year to another within the same genotype. This makes it difficult to select consistently high-yielding individuals. Environmental and bio-physiological factors appear to play a substantial role in escin accumulation, with dry weather conditions negatively impacting escin content and cold storage having a positive effect. Additionally, the optimal time for fruit collection and storage procedures can vary among different genotypes. In the existing literature, there is considerable variability in reported escin content, ranging from 3 to 15 to 28 percent. This variability is attributed to factors such as genotype, time of year, seed maturity, and seed status. Furthermore, the quantitative determination of escin can be challenging, and analytical methods, including TLC (Thin-layer chromatography), may not always provide highly accurate results. Additionally, the identification and quantification of different escin isomers or derivatives, which may possess varying therapeutic properties, present analytical challenges.

7.1. Medicinal attributes and clinical applications of horse chestnut

7.2. Seeds

For centuries, horse chestnut has been used in folk medicine. It has been employed as a remedy for various ailments, including gastralgia of atony, diarrhoea, chronic catarrh, uterine haemorrhage, mammary indurations, and even cancer. In the early 18th century, it was discovered that horse chestnut powder had stimulatory effects and could be used to address circulatory disturbances (Lieutaghi, P. 1975) [80]. In 1720, Bon of Montpellier began using the bark from horse chestnut branches as a substitute for cinchona (Lieutaghi, P. 1975) [80], employing it as a febrifuge. Subsequently, between 1896 and 1909, Artault of Vevey conducted studies that identified horse chestnut as an effective remedy for anti-inflammatory and anesthetic purposes in the treatment of venous circulation issues and haemorrhoids (Benigni, R., C. Capra, and P.E. Cattorini. 1964, Lieutaghi, P. 1975) [14, 80]. Over the years that followed, numerous pharmacological and clinical investigations have further elucidated the mechanism of action and the therapeutic benefits associated with this plant.

Pharmacological research has shed light on the therapeutic potential of horse chestnut seeds. The primary bioactive compound in horse chestnut seeds is escin, a triterpenicsaponin (Enghofer, E., R. Eisenburger, and K. Seibel. 1984, Lorenz, D., and M.L. Marek. 1960) [37, 81]. Escin has been found to possess several beneficial properties, including antioxidant, antioedematous (Reducing edema), antiexudative, vasoprotective, and decongestive actions. These effects are mainly attributed to escin. Escin, while primarily acting as a vasoconstrictor, also helps reduce pain, possibly due to the haemolytic action of saponins, which leads to a decrease in the viscosity index of venous blood. Other compounds found in horse chestnut seeds, such as certain coumarins and flavone derivatives, can increase capillary resistance, relieve pain, and reduce blood vessel fragility (Lavollay, J. 1945) [76]. Clinically, horse chestnut extract (HCE) is widely used in the treatment of conditions related to venous stasis, varicosis, and venous insufficiency (Dustmann, *et al*. 1984, Enghofer, *et al.* 1984, A.K. Sharma. 1992, L., J. Petricic, and Z. Smit. 1991) [36, 37, 50, 69]. Horse chestnut (*Aesculus hippocastanum*) seeds' total triterpene glycoside content during vegetation is expressed as aescin.

Several pharmacological and clinical studies undertaken in the 1960s and 1970s focused on examining the anti-inflammatory and anti-edema properties of escin. Researchers proposed various biochemical mechanisms to explain escin's anti-edema effects, including its ability to act as a surfactant, enhance erythrocyte water binding capacity (Resulting in the development of a passive sodium pump independent of metabolism), normalize the exchange of fluids between cells, and directly affect connective tissue and cell membranes. Notably, escin was discovered to be beneficial exclusively in models depicting the early phases of inflammation characterised by increased vascular permeability, specifically in rendering capillary walls impermeable to water exchange. (Ascher, P.W., and F. Paltauf. 1970, Preziosi, P., and P. Manca. 1964, Preziosi, P., and P. Manca. 1965) [8, 112, 111]. Many years of extensive testing verified the safety of escin at dosages commonly used in human medicine. Except for excretory organs, escin accumulation in tissues was not seen. Side effects were infrequent, and toxicity occurred only at very high doses. Escin was well tolerated, even in treatments lasting up to 50 consecutive days (Girerd *et al*. 1961, Lorenz, D*et al*, M.L. Marek *et al*. 1960) [147, 81].

Due to its pharmacological characteristics and high tolerability, escin is widely employed in medical practices. Initially administered intravenously or orally, escin is now available in gels or ointments for external use in treating conditions such as varicose veins, contusions, hematomas, and hemorrhoids. Escin irrigation is also utilized for hemorrhoid treatment. The effectiveness of escin is particularly notable in patients with conditions like thrombophlebitis with cerebral edema following head injuries, recent stroke with hemiparesis, various types of cerebral tumors, cerebral sclerosis, meningitis, encephalitis, and cerebral abscesses. Treatment with escin frequently results in rapid and impressive remission of symptoms or a reduction in their frequency and its severity. (Gemaehlich *et al*. 1965. Heppner *et al.* 1967, Hutzel, M. 1963, Niedling, H. 1967. Vogel, G., *et al*. 1970) [46, 57, 63, 102, 167]. Escin is used in obstetrics, gynaecology for edema resolution, otorhinolaryngology, and dentistry to treat tonsillar abscesses, acute hoarseness, epiglottic edoema, and gingival haemorrhage. Some reports suggest that escin's anti-inflammatory and anti-edema properties may be beneficial in managing multiple sclerosis. (Ferrero *et al*. 1968, Tillmanns, H. *et al*. 1969) [42, 158].

In dermatology, escin is used for its vasoprotective effects. However, combination ofescin with β-sitosterol and phosphatidylcholine has shown greater efficacy than escin alone. (Bombardelli, E. *et al*. 1996) [19]. Furthermore, escin has shown some antiviral action, which may be linked to triterpene saponins that include acylated aglycones of the β-amyrin type. (Rao. *et al*. 1974) [126]. Moreover, it has demonstrated strong antimicrobial activity against pathogenic bacteria. Twelve triterpene saponins that are obtained from indigenous plants have antimicrobial properties. (Yoshikawa *et al*. 1994) [175]. *Aesculus hippocastanum* L. seeds contain bioactive triterpene oligoglycosides named escinsIa, Ib, IIa, and IIIa. These oligoglycosides have the ability to block ethanol absorption and hypoglycemic activity in glucose tolerance tests. (Chem. Pharm. Bull. 6:1357-1359)It has highlighted the potent hypoglycemic activity of escin compounds such as escin-Ia, Ib, Iia, Iib, and IIIa. (Konoshima, T. *et al*. 1986 [75], Antitumor agents, 82. Cytotoxic sapogenols from *Aesculus hippocastanum*. J. Nat. Prod. 49:650-656.) Moreover, the separation of elements from horse chestnut fruit such as hippoesculin and barringtogenol-C21-angelate has created opportunities to investigate the fruit's potential as a natural source of anti-tumordrugs. Proanthocyanidin A2 is another chemical with venotonic activity and healing properties that has been clinically tested. This substance has strong antioxidant and anti-enzymatic properties and helps to restore capillary permeability and fragility caused by vitamin P deficiency.In humans, it helps to prevent and reduce photobiological damage to the skin. (Gabetta *et al*. 1990) [45].

7.3. Bark

The horse chestnut tree's bark also has medicinal applications. It is harvested in spring or autumn from branches that are two to three years old and then dried.The bark used in commerce typically comes in small pieces measuring approximately 10 to 12 centimetres in length, and they are roughly 2.5 to 4 centimetres wide and 2.5 centimetres thick. These bark pieces usually have an external appearance that is greyish-brown in colour and are characterized by elongated and corky scales. Internally, the bark is brown and displays fine longitudinal striations (Lieutaghi, P. 1975) [80]. It should be noted that while Hose chestnut bark has an astringent and bitter flavour, it does not have a distinct aroma. The bark has a variety of characteristics, including tonic, astringent, detergent, antiseptic, narcotic, febrifuge, anti-haemorrhagic, and anti-hemorrhoidal. (Allegri, E. 1935, Hänsel, R. *et al*. 1992, Chr. and K. Vayas. 1986) [2, 52, 142]. The astringency of the bark is attributed to its tannin content, while the febrifuge properties are linked to esculin, a coumarin found in the bark. Esculin has clinical applications, especially in the treatment of haemorrhoids.

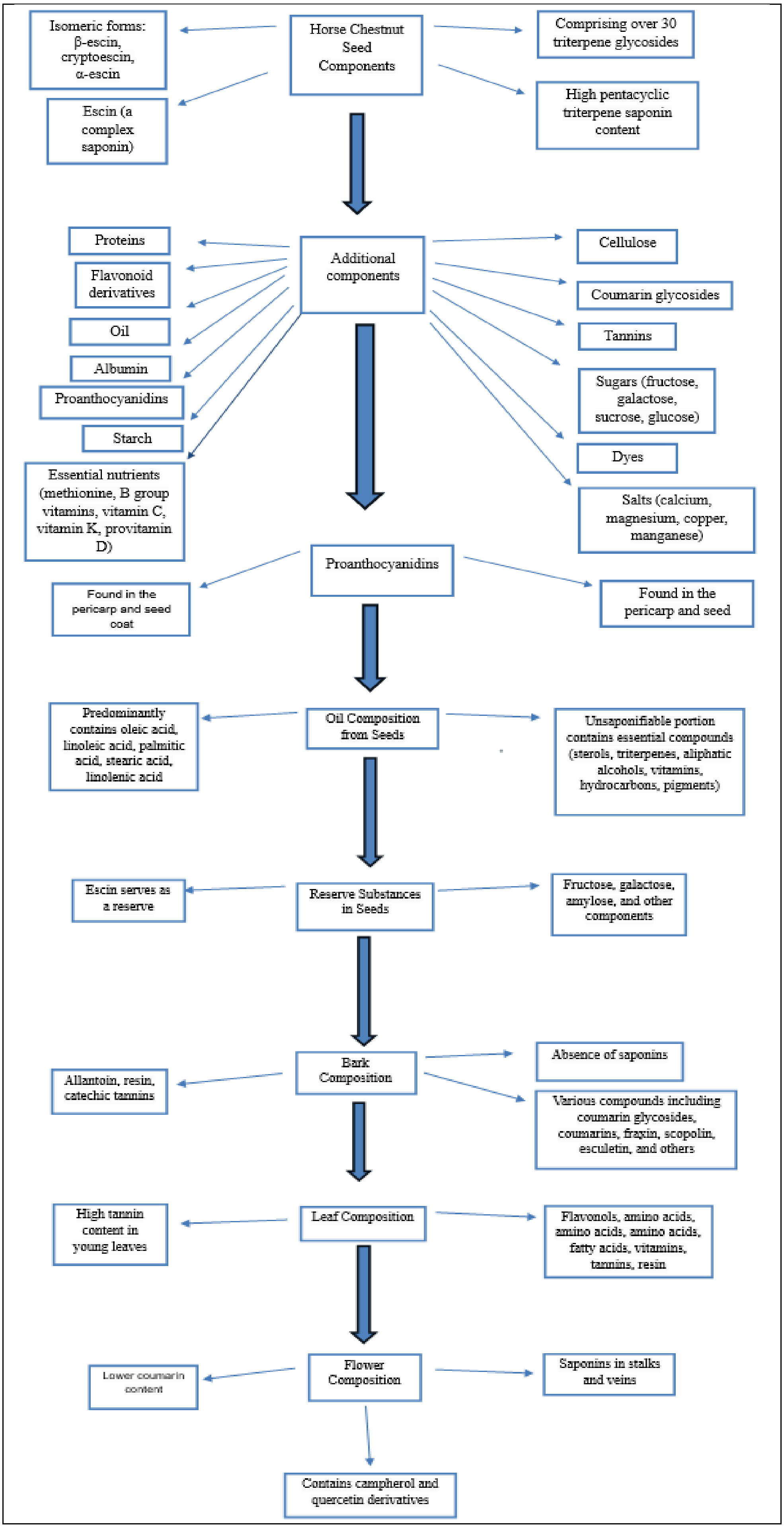

Fig 2: Chemical composition of horse chestnut

Esculin, a compound found in horse chestnut, has several significant pharmacological effects. It primarily focuses on enhancing the permeability and resilience of capillaries while also inhibiting enzymes such as hyaluronidase and collagenase. Laboratory studies have confirmed that both esculin and esculetin, derivatives of this compound, possess analgesic and antipyretic properties. Additionally, esculin has demonstrated anti-inflammatory effects, particularly when dealing with UV-induced erythema (Digonnet, *et al*. 1952, Wiskemann, A. 1968) [34, 173]. Horse chestnut bark is also noteworthy for its vitamin P properties, thanks to the presence of esculoside (Maugini, E. 1988) [93]. Esculin, when hydrolyzed by the bacterial enzyme esculinase (β-glucosidase), serves as a tool for phenotypic characterization and bacterial identification. (Hänsel, *et al*. 1992) [52]. Horse chestnut bark is used as an infusion in traditional medicine to cure intermittent fevers, although it requires extremely high doses of the whole bark to be effective. On the other hand, disorders including diarrhoea, general enteritis, and passive haemorrhages are treated using bark decoctions. (Grieve, F.R.H.S. 1967) [49]. When used externally, it has valuable antibacterial, wound-healing, and cleansing capabilities because of the presence of allantoin, a chemical recognised for its tissue-regenerating activity. As a result, it is used to heal ulcers, hemorrhoids, and chilblains (Lieutaghi, P. 1975) [80].

The leaves and flowers of the horse chestnut tree have medicinal uses. During spring, one can gather the leaves and later the flowers, which, once properly dried, offer remedies for various conditions related to superficial blood circulation, including rosacea, varices, hemorrhoids, chilblains, contusions, and rheumatism. Dried leaves are commonly used in the form of tinctures or infusions. Leaf extracts are employed to treat varices, leg pain, general arthritis, swelling, inflammation, thromboses, hemorrhoids, and cramps, and they also possess tonic and febrifuge properties (Hänsel, *et al.*1992, Chr. and K. Vayas. 1986) [52, 142]. Meanwhile, rheumatism and gout are treated with horse chestnut flowers, which are recognised for their tonic and astringent properties in traditional medicine. (Souleles, Chr. and K. Vayas. 1986) [142].

7.4. Landscape

The horse chestnut tree is largely used for aesthetic purposes and to provide shade in urban areas, private gardens, and parks. It is particularly renowned for creating picturesque avenues and becomes especially captivating during the spring when it is in full bloom. The tree's widespread popularity can be attributed to its consistent leaf coverage, early leaf production, profusion of flowers, attractive foliage, and rapid growth. In urban landscaping, it is advisable to use horse chestnut trees in locations where the avenues are spacious enough to accommodate their full growth potential. Furthermore, in Central Europe, horse chestnut trees are intentionally planted in hunting reserves and along wooded avenues to provide nutrition to animals who enjoy its fruit. On occasion, these trees are employed to stabilize precarious slopes or to establish wooded belts as a means of fire protection (Merendi, A. 1969) [96].

7.5. Domestic and food use

Historically, horse chestnut seeds have been used for household and food applications. The flour extracted from the seeds has been utilised in a variety of ways, including washing linen and wool. During the war, it was utilised to manufacture a soap known as "Bolous" soap, which is good against fat stains but not ideal for white garments due to potential discoloration. Horse chestnut flour has also been used in gardening to deter earthworms and prepare snuff. The leaves, when young, can be used as a substitute for hops and may be used to adulterate tobacco. Horse chestnut necklaces have been used as wardrobe repellents for moths, and it was once thought that carrying a horse chestnut in your pocket would shield you from colds. After undergoing a leaching and processing procedure at 40 °C, high-quality raw flour resembling corn flour is obtained from the seeds. Alternatively, processing the seeds at 100 °C yields a more viscous flour. Both types of flour find applications in soups, cakes, and various confectionery products. Historically, the bitter flour obtained from these seeds was subjected to a multi-step treatment involving mixing with soda water six to eight times, followed by 12-hour periods of standing, water washing, cloth filtration, and finally drying in the sun or an oven. After that, this treated flour mixed 1:1 with corn flour to make bread (De Philippis, A. 1941) [31]. In many countries, particularly in former Czechoslovakia, the horse chestnut tree (*A. hippocastanum*) provides a valuable source of nectar for bees as well as a moderate amount of pollen during the spring. The nectar output ranges from 1.4 to 1.6 mg in a 24-hour period, with sugar content varied between 40 and 69%. This yields at least 383 kg of honey per hectare from horse chestnut nectar production (*Aesculus spp*.). (Haragsim, O. 1977) [53].

7.6. Cosmetics

Because of its astringent and decongestive qualities, escin and other seed extracts are widely used in the cosmetics sector. It is a common ingredient in various personal care products such as bath foams, shampoos, and foaming footbaths, where it imparts toning, refreshing, calming, and astringent effects. This extract is also found in refreshing and anti-redness scalp lotions, toothpaste formulations, oral hygiene products for treating gingivitis and periodontitis, and numerous other personal hygiene items. However, its primary application lies in a wide range of body care products, often in the form of a cholesterol-escin complex. These products include lotions, milks, creams, and gels designed for the treatment of sensitive skin prone to conditions like rosacea. It is also a key component in sunscreens, after-bath creams for cellulite massages, breast-firming and soothing applications, as well as creams, gels, lotions, and compresses for the legs and feet to prevent and alleviate issues such as swelling, venous dilation, heaviness, and fatigue. Esculin, on the other hand, plays a role in improving skin trophism and is effective in addressing cellulite. When used topically, esculin improves the morphology of the smallest blood vessels and increases "capillary density," or the quantity of capillaries open to blood flow per unit of skin surface. It is particularly recommended for various conditions characterized by chronic deficiencies in localized skin microcirculation. It has been successfully used to treat aging skin, cellulite, and reduce hair loss, seborrhoea, and alopecia. The primary use of the extract is in the production of creams and sun gels for these purposes (1994, Proserpio *et al*. 1980) [120]. Applications for cosmetic purposes include escin, the cholesterol/escina complex, and extracts from horse chestnuts (*Aesculus hippocastanum*). (Proserpio *et al*. 1986) [120].

8. Industrial and technological

Various parts of the horse chestnut plant have industrial and technological applications. The dextrin's found in the seeds are used in liquor production and for preparing glues used in bookbinding, weaving, and box manufacturing (Hegi, G. 1906-1931) [56]. Boiling the raw flour in water produces a long-lasting glue that can be used for herbarium sticky boards and labelling. Powdered horse chestnut seed can be used to prepare concrete and act as a foaming agent in fire extinguishers.The oil that is produced from the seeds is used to make lubricating oils and soap, among other industrial uses, as an emulsifying agent. It can also be utilised in the textile dyeing industry and in insecticidal and anti-parasitic compounds. (De Philippis, A. 1941, Hegi, G. 1906-1931) [31, 56].

8.1 Wood

The wood of the horse chestnut tree appears yellowish-white, occasionally reddish. It possesses a soft and lightweight quality and has a waxy texture. To the naked eye, it can be easily mistaken for conifer wood. The wood showcases a fine, compact, and consistently even grain, occasionally featuring a shiny surface. While many consider horse chestnut wood to be one of the least desirable timbers for both industrial and heating purposes, some argue that its reputation is primarily due to its relative obscurity (Lieutaghi, P. 1975) [80]. This wood is relatively easy to work with but tends to produce uneven cuts due to its susceptibility to twisting, warping, and rapid decay when exposed to the elements. Although the wood burns vigorously, it does so for only a brief duration. Consequently, horse chestnut wood is rarely used for a variety of applications, such as crafting furniture interiors, making trelliswork, manufacturing cooking utensils, and constructing pianos. Instead, it finds more common use in producing boxes for storing vegetables and fruits and for pyrography. The wood does, however, possess the ability to absorb a black stain easily and accept a good polish. This property makes it suitable for imitating ebony. Historically, horse chestnut wood was employed in sculpture because of the white paint that was used as a protective layer prior to gilding could conceal any imperfections in the wood (Allegri, E. 1935, Lieutaghi, P. 1975) [2, 80].

8.2 Animal nutrition

Horse chestnut seeds serve as valuable fodder for various animals, including cattle, game animals, pigs, goats, sheep, and fish. In Eastern regions, they are also used as feed for horses and cattle. When introduced gradually, this fodder proves to be beneficial for cattle, potentially contributing to increased milk production due to its high nutritional value. However, pigs notably refuse to consume these seeds. Chickens, although not poisoned by the seeds, tend to lose weight and stop laying eggs when fed with them, while ducks may even perish. Deer, on the other hand, do not have a preference for horse chestnut seeds (Lieutaghi, P. 1975) [80]. To prepare these seeds for use as animal feed, they undergo a process involving soaking in lime-water to eliminate their bitter taste. Subsequently, they are ground into a meal and mixed with regular animal feed (Allegri, E. 1935., Hänsel *et al*. 1992) [2, 52]. An even more efficient way is to soak the partially crushed seeds in cold water for an entire night, and then boil the mixture for around 1.5 hours. After the water is removed, the residue can be dried, partially peeled, and processed into flour. Despite of slight bitterness, this flour has a pleasant taste and look, making it a better option for animal consumption. (Grieve, F.R.H.S. 1967) [49].

9. Conclusion

In conclusion, horse chestnut's remarkable properties have positioned it as a pivotal player among medicinal plants, with potential future roles in both ornamental and pharmaceutical domains. Horse chestnut extracts and escin-based chemicals are currently used in pharmaceutical goods to treat disorders including edoemas and peripheral chronic venous insufficiency, while they are used in cosmetics to protect skin, reduce cellulitis, and treat hair loss. However, gaps exist in our understanding of cultural practices, genetic diversity, and the factors governing escin accumulation. Further research is imperative to elucidate these aspects, optimize escin quantification methods, and establish sustainable cultivation practices. Such endeavours promise not only to improve the effectiveness of breeding programmes, but also to encourage ecologically friendly agriculture systems, helping both the rural economy and the environment while delivering additional income to farmers.

13. References

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