REVIEW ARTICLE

Horse Chestnut Seed Extract: An Opportunity for Creating Evidence-based New Natural Products

Poonam N. Chougule[1,2*], Kailasam Koumaravelou[1], Nilesh B. Chougule[3]

[1]*Department of Centre for Research and Development, PRIST University, Thanjavur, Tamil Nadu, India.*
[2]*Ashokrao Mane College of Pharmacy, Kolhapur, Maharashtra, India.*
[3]*Ashokrao Mane Institute of Pharmacy Ambap, Kolhapur, Maharashtra, India*

Received: 19th February, 2023; Revised: 04th April, 2023; Accepted: 25th May, 2023; Available Online: 25th June, 2023

ABSTRACT

For centuries, herbal remedies have been used to prevent and treat disease. Horse chestnut seeds are an abundant source of phytoconstituents containing triterpenoid saponins, with thirty molecules isolated and characterized. Escin, which is a combination of acylated triterpene glycosides composed of α- and β-escin, is the major biologically active component of horse chestnut seed extract. Escin is the most researched veno-active compound with anti-inflammatory, anti-oedematous, and antioxidant properties. Due to their unique three pharmacological actions, escin is a well-established molecule for the management of venous diseases such as chronic venous insufficiency (CVI) and hemorrhoids. The available treatments for CVI have some disadvantages, including severe side effects, expensive surgical options, long duration of treatment, and limited accessibility to allopathic drugs. As a result, phytoconstituents and lifestyle changes can be seen as safe and effective therapy options for CVI management because they have no major side effects and can simultaneously function on several targets. This review article focuses on the chemical components of horse chestnut extract, particularly the phytoconstituents escin, and proanthocyanidins, which have been extensively studied in preclinical and clinical studies. The review article provides a comprehensive overview of their potential therapeutic benefits in managing symptoms of CVI and their regulatory status.

Keywords: Escin, Anti-inflammatory, Anti-edema, Antioxidant, Horse chestnut extract, Varicose veins.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.2.33

How to cite this article: Chougule PN, Koumaravelou K, Chougule NB. Horse Chestnut Seed Extract: An Opportunity for Creating Evidence-based New Natural Products. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):446-456.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

For centuries, herbal remedies have been used to prevent and treat disease. The usage of herbal medicinal plants for therapeutic and nutraceutical purposes is a long-standing tradition in many civilizations. Herbal medicines are becoming more popular in India and the Western world. Herbal remedies embrace herbs, herbal ingredients, herbal preparations, and finished herbal dosage forms. They are manufactured through a series of processes such as extraction, purification, concentration, and important physiochemical as well as some biological procedures.[1,2] A few of them are herbal formulations made by immersing or heating herbal plant materials in alcoholic as well as hydro-alcoholic solvents or honey. Herbal products in their finished dosage forms are natural medicines made from one or more herbs. The term "mixture herbal product" is used when multiple herbs are used. Traditional medicine and therapies such as Chinese herbal medicine, Ayurvedic preparations, the Unani system, naturopathy, and homeopathic preparations make extensive use of herbal medications.[3] In India, herbal medicine has a long history and tradition. Herbal treatments were once employed in a similar way to how pharmaceuticals are used today. Ayurveda is an ancient Indian medical system that focuses on both curing and preventing illness.[4] Herbal medicine contains various chemicals that have a pharmacological effect, but in some cases, the specific phytoconstituent responsible for the therapeutic effect is not yet identified. Many herbalists and academics believe that an isolated component is less effective than the whole plant extract. Herbal remedies are complicated due to the presence of multiple chemicals and the unique qualities of each ingredient.[5] Due to disparities in the convenience, accessibility, and cost of ultra-modern health care, herbal medications are most popular in rural and urban areas.[6] In their daily lives, the majority of Indians use herbal medications as spices, home treatments, and healthy cuisine. Many ancient societies used plants and herbs as medicine to treat a variety of ailments. This herbal medicine is a tradition and practice that includes almost all constituents and opens up new possibilities.[7] Fortunately, despite the government's advertising and brainwashing campaigns, this behavior has

**Author for Correspondence: poonamchougule@amcoph.org*

Table 1: Name of horse chestnut in India

Language	Common/Regional Name
Himachal Pradesh (Pahadi)	Khanor, Tatwokhar
Hindi	Kanor, Bankhor, Fangar, Gugu, Pankor
Kannada	Kanor, Kanur
Kashmiri	Hane, Hanudun
Kumaon	Kishing, Pangar
Nepali	Karu, Ghodepangro
Punjabi	Kanur, Gun, Khanor

taken hold in India, with more than half of the population participating, according to numerous agencies.[8] People have used herbal medicine in some form or another since ancient times.

The small natural range of the horse chestnut (*Aesculus hippocastanum l.*, Hippocastanaceae) is found in the southeastern part of Europe and the southern part of the Balkan peninsula.[8, 9] It is a plant that is widely grown in small, medium, and large towns with moderate climates throughout the northern hemisphere. It is also somewhat tolerant of many urban conditions.[10] Many parts of this plant have been used medicinally for centuries, including seeds, leaflets, bark, and flowers. A seed of this plant has a position in European phytotherapy and is used to take steps to reduce inflammation. Turkish tea made from horse chestnut is a popular beverage. Horse chestnut seeds are commonly used to alleviate stomach distress, pass kidney stones, and treat hemorrhoids, among other conditions. Additionally, these seeds are also utilized as a food source.[11]

The Indian variety of horse chestnuts is popularly known as the Himalayan chestnut and natively known as khanor in most parts of Himachal Pradesh and Kashmir. It is a tree that grows primarily in the temperate regions of Asia. The tree thrives in wet, shady ravines and can be found in the Indian states of Jammu & Kashmir, Himachal Pradesh, and Uttar Pradesh.[12] The name "horse chestnut" is thought to have originated from the tree's brown conkers, which bear a resemblance to chestnuts, as well as the horseshoe-shaped mark that remains on the twig after the leaves shed in autumn, accompanied by dots resembling horseshoe nails.[13] It is identified by different names throughout India. Details are given in Table 1.

Pharmacognostic Features of Plant

The scales of the tree are smooth when young, but as the tree ages, they develop into long, delicate scales that separate at the ends before falling. Strong, glabrous, light grey or brown twigs with a few mild lenticels can be observed. Massive, ovoid, deep reddish-brown, resinous, and sticky buds measure 2.5–5 cm in diameter. The gigantic (60 cm wide), opposing leaves are palmate and have five to seven leaflets, each measuring 8–25 cmin length, with the in a pinnately compound leaf, the leaflet at the end of the rachis being the biggest. The leaves also have a long (7–20 cm) terete petiole.[14]

The branch has hermaphroditic flowers at the bottom and male flowers at the top. Large, terminal, erect, conical to cylindrical panicles, andromonoeic inflorescences with hermaphroditic flowers next to the base and male flowers at the apex. Flowers are zygomorphic, about 2 cm wide, with five sepals forming a serrated or tubular calyx and four to five petals that are white with yellow and eventually pink basal patches. Five to nine stamens of various lengths, most of which are longer than the petals and curved downward, are present within a single flower; the pollen is red. The ovary has three cells, two ovules in each cell, a single short, straightforward style, and a diminutive stigma. Male flowers feature an undeveloped ovary and a tiny, useless pistil. Nectar is a lobed disc. At the base of the panicle, only 2–5(8) blooms yield fruit. Fruit has a 5–8 cm diameter, is subglobose, spiky, and contains one (sometimes two or three) conkers. Hilum gigantic; white; or round elliptic; seed large; glabrous; smooth; lustrous; glabrous; soft; red-brown nuts, 34–48 mm in diameter, elliptical, with the radicular lobe discernible as low, broad dark ridges, particularly in dried nuts.[15] Every portion of the horse chestnut tree has its unique set of benefits and actions in the biological system. It is utilized for food, feed, wood, and a decorative tree in the home. It offers a wide range of therapeutic and nutritional qualities.

Important Chemical Constituents of Horse Chestnut Seed Extract

Primarily triterpene saponin is present in the seeds. Horse chestnut seeds have a good composition of saponin in the range of 3 to 5%, with 30 molecules isolated and distinguished.[16] Escin (Figure 1**)** was first isolated in 1953, primarily composed of acylated triterpene glycosides, including isomers α- and β-escin, as the most important biologically dynamic horse chestnut seed extract constituent. The escin mixture primarily consists of two major glycosides, glucuronic acid, two glucose molecules, and the aglycone protoescigenin. The only difference between the two aglycones is the acylation at the C21 position (r), which can be by angelic acid or tigilic acid. Other compounds, such as proanthocyanidin A2 (Figure 2) and esculin (Figure 3), were discovered alongside escin. Escin is assumed to be involved in the anti-edema,[17] anti-exudative, and vasoprotective effects. Proanthocyanidin A2 has wound-healing properties when administered orally or topically. Escin combines α-escin, β-escin, and cryptoaescin as the major glycoside.[18] Escin is divided into two polymorphic forms, α-escin and β-escin, which can be distinguished by their melting points, hemolytic indices, water solubilities, and specific rotations.[19] Carbohydrates make up the majority of a seed's composition. Other important phytoconstituents include such as cellulose, lignin, hemicellulose, water-soluble (6.6%) and non-water-soluble saccharides (18.1%). Ashes are identified at 3%, whereas proteins and lipids are found at levels of 34.4 and 13.1%, respectively.[20] Anthocyanins, flavonoids, and proanthocyanins are antioxidant content present in various parts of the plant. The seed also contains fatty acid glycerides.[21] The triglycerides of fatty acids are also detected in the seed oils of *A. hippocastanum*. Oleic acid (43.2–59.4%) and linoleic acids (35.2%) are the predominant components.[22,23]

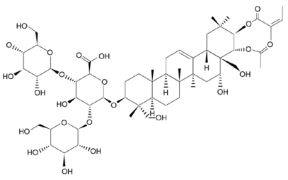

Figure 1: Structure of escin

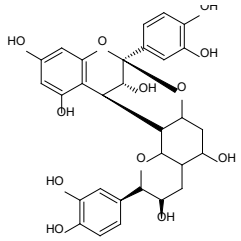

Figure 2: Structure of proanthocyanidin A2

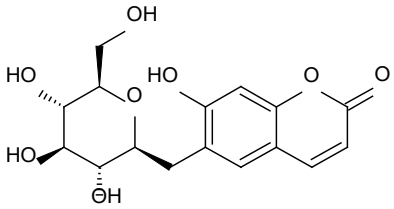

Figure 3: Structure of aesculin

Coumarins like esculin, esculetin, scopoletin, and fraxetin can be found in flowers. Esculetin, scopoletin, and fraxetin are anti-inflammatory agents inhibiting the pro-inflammatory pathways of cyclooxygenase (COX) and lipoxygenase (LOX) involved in arachidonic acid metabolism.[24] In addition to fatty acids like oleic and linoleic acid, tannins, amino acids, flavonoids, phytosterol, uric acid, resins, leucocyanidine, epicatechin, and derivatives of kaempferol like quercetin, 3-o-rhamnopyranoside, 3-o-glucopyranoside, 3-o-rhamnopyranoside-l6-o-glucopyranoside, and 3-o-arabino furanoside.[25-28]

α-aescin and β-aescin can be distinguished based on various parameters such as water solubility, hemolytic index, melting point, and specific rotation. Heating α-aescin to 100°C can convert it to β-aescin.[29] During the 1960s, Lorenz and colleagues discovered that horse chestnut seeds contain a fraction of triterpenic sapogenins that can be separated chemically without denaturation. When protoescigenin and barringtogenol, two pentacyclic triterpenic sapogenins, were discovered, the proportion was given the following escin (and later β--escin).[30] Escin has a 3-OH residue connected to a trisaccharide (glucose, xylose, and galactose), and so this organic acid is used to esterify the C21 and C22 domains (e.g., tiglinic, angelic, or acetic acid). α-Escin and kryptoescin are the two main isomers of escin. Unlike kryptoescin, this is rather water-insoluble but much less active than α-escin and is water soluble. Escin has the chemical formula $C_{55}H_{86}O_{24}$ and a molecular weight of 1131.27 da. It has been established that escin primarily accounts for the effectiveness of horse chestnut seed extract in decreasing edema and inflammation.[31]

Coveted Pharmacological Properties of Horse Chestnut Extracts

According to numerous scientific investigations, *A. hippocastanum* has major antioxidant,[32-34] antiinflammatory,[35-37] and anti-edematous[38] properties. The escin acts as an antioxidant via inhibiting hyaluronidase enzyme, but Wilkinson and brown state that this is insufficient to explain the potent venotonic effects of horse chestnut extract on the microvasculature. But it was reported that other enzymes such as, collagenase, d-glucuronidase, and elastase, which work together to keep the extravascular matrix solid, are also involved in the mechanism of action for venotonic activity. Escin impacts hypoxia in endothelial cells, leading to a decrease in ATP levels. These factors contribute to the development of inflammation and edema. Phospholipase A2 and PAF (platelet-activating factor) trigger the release of prostaglandins, which are signaling molecules involved in the inflammatory response. This response leads to edema formation and neutrophil adhesion to the endothelium. After the inflammatory response is initiated, neutrophils are recruited to the site and release enzymes like elastase. These enzymes can weaken the vein wall and stimulate the production of fibroblast growth factors (FGFs), leading to vein enlargement. Additionally, the adhesion of neutrophils to the endothelium decreases blood flow and promotes hypoxia. Figure 4 illustrates the biological activity of horse chestnut extracts.

Protection against Capillary Hyperpermeability

CVI, or deprived flow of blood in the veins of the legs, is a common health concern, especially as people age.[39] The symptoms of CVI mostly include leg edema, itching (pruritus), apprehension, skin hardening (dermatosclerosis), and fatigue.[40,41] Researchers have discovered aesculin as an additional active component inside the extract and have further identified the anti-edematous, venotonic, and vasoprotective properties of horse chestnut seed extract.[42] The triterpene saponin component of escin is the primary component responsible for anti-edematous, anti-inflammatory, and venotonic activities. According to animal studies, escin enhances the sensitivity of ion channels in the walls of venous capillary vessels, specifically to calcium ions, which results in venoprotective and venotonic effects.[43] The antioxidant

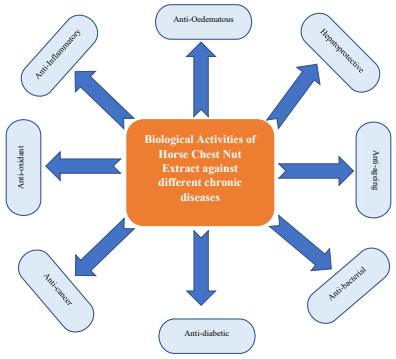

Figure 4: Therapeutic potential and biological activities of horse chestnut extract against different chronic diseases

proanthocyanidin A2 can be found in the triterpene saponin of escin.[35,44-46] It has been demonstrated that the proteoglycan breakdown enzyme hyaluronidase is inhibited by escin.[47] In animal studies, it was discovered that increased release of prostaglandin F2 in the outer wall of veins inhibited the vasodilator effects of serotonin (5-hydroxytryptamine) and histamine. The authors hypothesized that this finding may also help preserve the integrity of connective tissue by reducing the breakdown of mucopolysaccharide components within the tissue. In the context of chronic venous insufficiency (CVI), the gathering of white blood cells in the affected limbs, along with subsequent enzyme activation and release, are recognized as crucial pathophysiological mechanisms contributing to the condition.[48-50] A study conducted by Kucukkurt *et al.* aimed to explore the *in-vivo* effects of horse chestnut tea enriched with aescin on the antioxidant defense system in the blood and tissues of male rats. The study was based on the efficacy of aescin in treating hemorrhoidal symptoms in the Turkish population. The male rats were given a dose of 100 mg/kg of aescin extract and either standard or high-fat for about 5 weeks.[51] The researchers then recorded the levels of oxidative stress markers such as malonyl dialdehyde, decreased levels of glutathione, and superoxide dismutase in blood and tissue samples from these male rats and it was noted that in rats on high-fat diets and subjected to high levels of oxidative stress, escin was also proven to protect against oxidative damage. The research work by Güney *et al.* (2013) aimed to investigate the potential anticancer effects of escin on the H-Ras transformed 5RP7 cell line. The study discovered that the administration of escin effectively suppressed cell growth and triggered apoptosis in the 5RP7 cell line. The researchers observed notable alterations in cell morphology and DNA fragmentation, which are well-known indicators of apoptosis. Additionally, the investigation showcased that escin treatment reduced anti-apoptotic protein expression and heightened pro-apoptotic protein expression. Overall, the study suggests that escin may have potential as an anticancer agent, particularly in the treatment of Ras-driven cancers.[52] Escin has antioxidant and apoptotic properties that are significant in the context of inflammation, as oxidative stress and apoptosis play important roles in it. The findings of a study indicate that the utilization of escin extract can enhance the sensitivity of human pancreatic cancer cells to chemotherapy by suppressing the nuclear-factor kappa B signaling pathway. *In-vivo* pharmacokinetic investigations show that aescin's active ingredient has a half-life of 17 hours after oral administration and remains present in the body for at least 24 hours at concentrations exceeding 5 µg/mL.[53] The horse chestnut extract was also formulated into extended-release tablets where the action of the drug and its antioxidant, anti-edematous, and anti-inflammatory activity was mainly beneficial in post-hemorrhoid surgery.[17]

Guillaume and Padioleau conducted a study to evaluate the efficacy of orally administered horse chestnut extract in preventing capillary hyperpermeability induced by serotonin and histamine in rats. The researchers used a 70% extract of horse chestnut and administered it orally to the rats. The degree of hyperpermeability was measured by the amount of Evans blue dye that leaked out of the capillaries and into the surrounding tissue. The results showed that the rats treated with the horse chestnut extract had significantly less capillary hyperpermeability than the control group. This suggests that the extract was effective in protecting against the effects of serotonin and histamine on capillary permeability. Overall, this study highlighted the potential of horse chestnut extract as a natural remedy for conditions that involve increased capillary permeability, such as edema and inflammation.[35] The protective effects of both inflammatory mediators were discovered to be dosage-dependent, with a 200 mg/kg extract dose producing an 80% increase in dye appearance time. Aescin's modes of action in *CVI* is presented in Figure 5.

The German commission approved the use of *A. hippocastanum* products for managing CVI due to strong

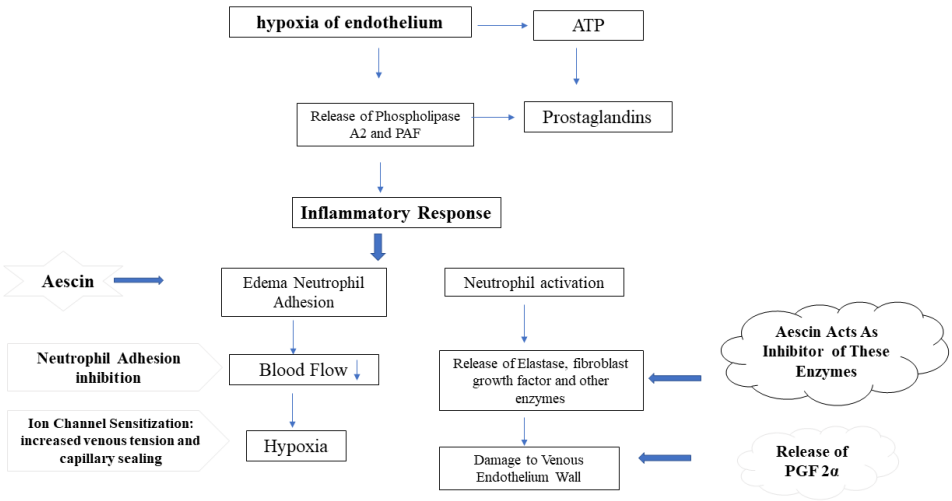

Figure 5: Aescin's modes of action in CVI

scientific evidence.[54] As a result, *A. hippocastanum* seed extract is widely used in Europe and gaining popularity in North America for treating CVI. Topical management with escin-containing external preparations can also be used for CVI. Multiple clinical studies have demonstrated that horse chestnut seed extract is a safe and effective short-term therapy for CVI.

The study conducted by Ruffini *et al.* in 2004 aimed to investigate the effects of escin gel on skin perfusion in the treatment of CVI. The researchers used a topical gel containing escin, which is known for its venotonic and anti-inflammatory properties. A group of 40 patients diagnosed with CVI participated in the study, undergoing a 60 day treatment with escin gel. The findings indicated a noteworthy enhancement in skin perfusion and nutrition among the patients who received the escin gel compared to the control group. This suggested that the topical application of escin gel can improve the microcirculation and tissue oxygenation in patients with CVI, which can contribute to the overall management of the condition. Overall, this study highlighted the potential of escin gel as a safe and effective topical treatment for CVI. The improvement in skin perfusion and nutrition observed in this study suggested that the gel can help alleviate the symptoms associated with CVI, such as leg swelling, pain, and skin changes.[55]

Esraerolu *et al*. investigated the *in-vitro* skin permeation of escin, using a gel containing 1% escin, and tested its permeation through human skin samples using a Franz diffusion cell. The results showed that the new gel formulation significantly increased the permeation of escin through the skin compared to a control solution of escin. The cumulative amount of escin that permeated through the skin after 24 hours was also significantly higher with the gel formulation.

The researchers concluded that the new gel formulation has the potential to increase the skin permeation of escin, which could enhance its therapeutic effects in the treatment of CVI and other related conditions.[56] Recent studies have shown that using escin extract can reduce the likelihood that human pancreatic cancer cells would become resistant to treatment. By interfering with the nuclear-factor kappa B signalling pathway, this effect is produced.[57]

Anti-inflammatory Activity

It has been discovered that horse chestnut possesses anti-inflammatory effects. Horse chestnut extract's ability to reduce inflammation in both *in-vivo* and *in-vitro* has been the subject of several investigations. It is thought that aescin, which has been demonstrated to block the production of inflammatory cytokines and chemokines *in-vitro*, is the active substance in charge of this function. Horse chestnut extract has been demonstrated in animal experiments to lessen inflammation in a number of scenarios, including adjuvant-induced arthritis, cotton pellet-induced granuloma, and carrageenan-induced paw edoema. Horse chestnut extract has been shown in human tests to lessen inflammation in people with *CVI*. Overall, these findings suggest that HCSE and its active component aescin have significant anti-inflammatory effects.[16] Horse chestnut exhibits anti-inflammatory activity through several mechanisms. Leukotrienes and prostaglandins, two pro-inflammatory mediators, are mostly inhibited from being released as the main mechanism. Aescin, an ingredient in horse chestnut, has been demonstrated to suppress the activity of the enzymes needed to produce these mediators. Horse chestnut has also been demonstrated to reduce the activity of neutrophils, which are important players in the inflammatory response. Lastly, it has been shown that horse chestnut possesses antioxidant qualities that can assist to lower oxidative stress and inflammation in the body. Overall, these mechanisms contribute to horse chestnut's anti-inflammatory activity in the management of arthritis and venous insufficiency.[16,17,58] Researchers tested seeds from the Japanese horse chestnut to look into their potential anti-inflammatory effects, both *in-vivo* and *in-vitro*. The results showed that the seed extract had a protective effect on the mouse concha, significantly reducing swelling brought on by croton oil exposure. The COX-1 and COX-2 enzymes were suppressed by seed extract, whereas the enzymes 15-lipoxygenase or phospholipase A2 were unaffected. Compared to COX-1 inhibition, COX-2 inhibition happened at a lower dose of seed extract. Japanese horse chestnut seeds included coumarins and saponin; however, they did not affect COX activity. These results suggest that COX inhibition is supposed to be accountable for the anti-inflammatory action of Japanese horse chestnut seeds. This seed may contain a chemical or chemicals besides coumarins and saponin that inhibit COX.[37,59-62] G. Belcaro *et al*. conducted several studies on the pharmacological actions of escin. These studies investigated the effects of escin on various diseased conditions, including CVI, edema, and inflammation. One of the notable findings of Belcaro *et al*.'s research was that escin can improve the symptoms of *CVI*, such as leg swelling, pain, and fatigue. Another study by Belcaro et al. investigated the effects of a topical gel containing 2% escin on edema and inflammation. After the application of the gel to the skin of patients with post-surgical edema, it significantly reduced swelling and improved skin elasticity compared to the control group. Belcaro *et al*.'s research provides compelling evidence that escin possesses robust anti-inflammatory and antioxidant properties, positioning it as a promising natural remedy for a wide range of health conditions.[63] Venous microcirculatory alterations of the lower extremities, *CVI* as well as varicose veins can be treated locally using "essaven gel" (EG). Essaven gel is also used to treat the signs and symptoms of superficial inflammatory disorders in the lower limbs, such as spider veins. Early EG treatment is beneficial for sporting injuries like bruising and swelling brought on by sprains and contusions. The active components in essaven gel are ascinate, sodium heparin, and phospholipids (EPL). The doses of 1-g aescinate, 10,000 IU sodium heparin, and 1 g EPL are used in a 100 g EG formulation. Unless specifically shown in specific, particular uses, EG is normally utilized multiple times per day into a thin film that is squarely applied above the pretentious area. The

fact that EG has been confirmed to be effectual for the listed symptoms and should be readily available for therapeutic usage for many years is why the study emphasized its importance. Because of its low cost and elevated level of protection, the use of EG in minor circulatory disorders to constrain symptoms and signs also precludes the utilization of extra costly and unsafe medications.[63-65]

Anti-edema Properties

Horse chestnut has been traditionally used to treat edema, which is the accumulation of fluid in the body tissues. Scientific studies have shown that the plant's active ingredient, escin, has anti-edema properties. Escin has been shown to reduce capillary permeability, which helps to prevent fluid leakage from the capillaries into the surrounding tissue. It also increases venous tone and decreases venous distensibility, which improves blood flow and reduces blood pooling in the veins. These effects of escin can help to reduce edema and related symptoms, such as swelling and pain. Several clinical studies have investigated the effectiveness of horse chestnut extract in treating edema associated with CVI. Horse chestnut extract has been shown to improve symptoms of CVI, including edema, leg pain, and heaviness. In addition to CVI, horse chestnut extract may also be effective in treating edema caused by other conditions, such as lymphedema and post-surgical swelling.[66]

In models of inflammation that resemble the first exudative phase, such as paw edoema brought on by a group of irritating substances (dextran, ovalbumin, carrageenan, and others), aescin is useful in inhibiting the development of edoema. highly severe formalin-induced peritonitis in rats and carrageenan-induced edoema in mice.[11,66] Aescin is ineffective in reducing edema in anti-inflammatory models that predominantly mimic the belatedly proliferative stage, which includes pocket-granuloma and formalin-induced edema in rat paw models. Horse chestnut extract's ability to reduce edoema has been connected to both an increase in calcium ion sensitivity and a decrease in the activation of human endothelial cells brought on by hypoxia, which causes a "closing effect" on narrow, water-permeable arteries. Inflammatory conditions and blood stasis can lead to reduced mitochondrial oxidative phosphorylation, resulting in a decrease in ATP concentration. This triggers a series of metabolic processes that lead to the release of prostaglandins and PAF, as well as neutrophil recruitment and activation, in varicose disease. These processes contribute to venous stasis and edema. The rise in phospholipase A2 and decrease in ATP content, which causes the release of inflammatory mediator precursors, can be successfully countered by aescin.[14] Additionally, there is less neutrophil adhesion/activation, which protects veins and lessens edema. The effect of horse chestnut seed extract HCSE on edema caused by carrageenan in rats was studied. The HCSE was administered orally to the rats and the edema was induced by injecting carrageenan into their hind paws. The degree of edema was measured by the increase in paw volume. According to the findings, carrageenan-induced paw edoema was greatly reduced by the HCSE. This suggests that HCSE has the potential as an anti-edema agent in the management of edematous conditions.[67]

Antibacterial Activity

It has been discovered that horse chestnut seed has antibacterial properties against a variety of bacterial strains, including *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Escherichia coli*. The seed extract contains compounds such as saponins and tannins, which have been shown to have antimicrobial properties. In a study, horse chestnut seed extract was found to inhibit the growth of *S. aureus* and *S. pyogenes*, which are known to cause infections such as skin infections, pneumonia, and sepsis. Another study found that horse chestnut seed extract was effective against *E. coli*, which can cause urinary tract infections and other infections. These findings suggest that horse chestnut seed may have the potential as a natural antimicrobial agent for the treatment of bacterial infections. Ten clinical strains of bacteria that were isolated from the urine of individuals with urinary tract infections are susceptible to the antibacterial effects of aqueous and ethanolic horse chestnut bark extracts. Using the microdilution method, and the Kirby Bauer disc diffusion method, the test strains' susceptibility to antibiotics was evaluated.[68] According to researchers from the University of Bristol, the antioxidant action is considered to support the antibacterial properties of horsetail seeds. The oxygen radical absorbance capacity (ORAC) of the terrestrial outer theca was 531 mmol trolox equivalents (TG)/g.[69] *A. hippocastanum* (horse chestnut) leaf extract was used to synthesise silver nanoparticles, which Fatma ztürk Küp *et al.* then tested for antibacterial, antioxidant, and drug release system properties. By reducing silver nitrate with horse chestnut leaf extract, the silver nanoparticles were created. *Staphylococcus aureus* and *Escherichia coli* bacteria were used to investigate the antibacterial activity of the synthesised silver nanoparticles. The outcomes demonstrated that both types of bacteria were significantly inhibited by the nanoparticles' antibacterial activity. Utilising the DPPH and ABTS assays, the antioxidant activity of the synthesised nanoparticles was assessed. The nanoparticles showed good antioxidant activity in both assays, indicating their potential as an antioxidant agent. Overall, the study suggests that horse chestnut leaf extract can be used as a natural reducing agent for the synthesis of silver nanoparticles with significant antibacterial and antioxidant activities. The synthesized nanoparticles also have the potential as a sustained drug release system.[68] One study examined the antibacterial and antifungal properties of several extracts from the leaf, seed, seed coat, and fruit capsule of the *A. hippocastanum* plant. The extracts were examined for their ability to fight against various bacterial and fungi species. The outcomes demonstrated that the plant extracts exhibited substantial antibacterial activity against the examined microorganisms in all of their sections. In instance, the fruit capsule extract was the most efficient against *E. coli* whereas the seed coat extract was most active against *S. aureus*. Additionally, the strongest antifungal action against

Candida albicans was demonstrated by the seed coat extract. The study also revealed that the methanol extract of the seed coat had higher content of phenolic and flavonoid contents, which are known to possess antimicrobial and antioxidant properties. This suggests that the observed antimicrobial activity of the plant extracts may be due to their high content of these bioactive compounds. Overall, the results indicate that *A. hippocastanum* plant extracts have the potential as natural antimicrobial agents, which could be further developed as a novel source of antimicrobial compounds for pharmaceutical and industrial applications.[70]

Hepatoprotective Effect

Numerous research have suggested that HCSE has hepatoprotective properties. It is thought that HCSE's anti-inflammatory and antioxidant qualities help to explain why it has hepatoprotective benefits. One study conducted on rats to find out the effect of HCSE on carbon tetrachloride (CCl_4) induced liver damage. The rats were pretreated with HCSE for 7 days before being exposed to CCl_4. The results showed that HCSE significantly reduced the CCl_4-induced liver damage and oxidative stress markers, suggesting its hepatoprotective effect. Another study investigated the protective effects of HCSE on liver damage induced by acetaminophen (APAP), a common analgesic and antipyretic drug known to cause liver toxicity at high doses. The rats were treated with HCSE for 7 days before being exposed to high doses of APAP. The results showed that HCSE significantly reduced APAP-induced liver damage, oxidative stress, and inflammation, suggesting its potential use as a hepatoprotective agent.[69,71] Hepatoprotective effects of AHSE were studied in Con A induced acute liver injury mice. The levels of liver enzymes, inflammatory cytokines, and oxidative stress markers were measured, and a histological examination of liver tissues was conducted. The findings demonstrated that AHSE significantly decreased the Con A-induced increase in inflammatory cytokines like TNF-alpha and IL-6 as well as liver enzymes like ALT and AST. Histological analysis also showed that the AHSE-treated group had less liver tissue damage than the Con A group did. In addition, AHSE reduced the Con A-induced activation of the JNK signaling pathway and the formation of reactive oxygen species (ROS) in liver tissues, which play key roles in the development of liver damage and inflammation. The study suggests that AHSE may have the potential as a natural hepatoprotective agent against liver injury and inflammation by inhibiting ROS and JNK signaling pathways.[72]

Anti-obesity Effect

There is limited research on the anti-obesity effect of HCSE, but some studies have suggested its potential in this area. Adipocyte differentiation, a process involved in the development of obesity, was examined for the impacts of HCSE. The results showed that HCSE significantly inhibited the differentiation of adipocytes and decreased the expression of genes related to adipogenesis. Furthermore, a study on human subjects found that HCSE supplementation for 12 weeks resulted in a significant reduction in BMI, weight, circumference of waist as well as improvements in lipid profiles and inflammatory markers. While these studies suggest the potential of HCSE in reducing obesity, yo completely comprehend its mode of action and its efficacy in people, further study is required.[73] Studies conducted *in-vitro* have demonstrated that the edible seeds of Japanese horse chestnuts (JHC) contain saponins that can inhibit pancreatic lipase while also limiting the gain in body weight. The production of nutraceutical foods with anti-obesity and decreased bitterness may make use of the saponin fractions from the stem bark and seed. A study on the ethanol extract of JHC stem bark found that it could effectively inhibit lipid accumulation without affecting cell viability, as demonstrated by the downregulation of PPAR and C/EBP expression, which are crucial adipogenic indicators. In a research on rats given a high-fat diet, it was shown that adding HCSE to their diets significantly reduced their body weight, visceral fat mass, and triglyceride levels, indicating an anti-obesity effect.[74]

Effect on Diabetic Neuropathy

HCSE was administered to the rats orally for eight weeks and animals were sacrified to examine the kidneys for the signs of diabetic nephropathy, including glomerular basement membrane thickness, mesangial matrix expansion, and tubular interstitial fibrosis. The results showed that the rats with diabetes had very high blood glucose level, creatinine, and urea nitrogen compared to the control group. The diabetic rats also exhibited pathological changes in their kidneys consistent with diabetic nephropathy. However, treatment with HCSE significantly reduced the levels of blood glucose, creatinine, and urea nitrogen and attenuated the pathological changes in the kidneys. These findings suggest that HCSE have potent therapeutic effects for diabetic nephropathy.[75] The potential therapeutic effect of escin, on cardiac autonomic neuropathy (CAN) in diabetic rats, was studied. The escin was administered orally to the rats and evaluated its effects on CAN were by measuring heart rate variability and other parameters. As compared to the control group, the diabetic rats treated with escin had considerably better heart rate variability and other CAN indicators. The researchers concluded that escin may have a protective effect against the development and progression of CAN in diabetic individuals, possibly through its anti-inflammatory and antioxidant properties.[76] In one of the studies ith streptozotocin-induced diabetic rats, HCSE was administered orally for 8 weeks. The results showed that HCSE significantly reduced hyperglycemia and improved nerve conduction velocity, indicating its potential in the treatment of diabetic neuropathy. Another study on diabetic rats found that HCSE improved antioxidant status and reduced lipid peroxidation in nerve tissue, further supporting its potential as a therapeutic agent for diabetic neuropathy. To completely comprehend the processes of HCSE in diabetic neuropathy and its potential application in human patients, additional study is necessary.

Anticancer Activity

HCSE has been widely studied for its therapeutic effects against inflammation and edema, and more recent research

has explored its potential anti-carcinogenic properties. Despite the numerous benefits associated with β-escin, there has been relatively limited research conducted on its efficacy in cancer models. Researchers investigated the pharmacological potential of β-escin on NNK (a potent tobacco-specific carcinogen)-induced lung adenocarcinoma in A/J mice and the growth of H460 human lung cancer cell lines. The results showed that β-escin inhibited NNK-induced lung adenocarcinoma in A/J mice, with a decrease in tumor size and number, and a decrease in the expression of ALDH1A1 (a cancer stem cell marker) and RhoA/Rock. Additionally, β -escin reduced cell viability and migration while inhibiting the development of H460 human lung cancer cells. These results imply that β -escin may have the ability to act as a lung cancer preventive and therapeutic agent.[77] In one study, natural clinoptilolite zeolite was modified with pharmacologically active escin and horse chestnut extract, and their anticancer effects were then assessed *in-vitro*. The modified versions of the zeolite exhibited high efficacy in inducing apoptosis in human alveolar basal epithelial cancer cells. The anticancer properties of the modified zeolites were also observed in various other cancer cells types such as leukemia, hepatocellular carcinoma, and lung adenocarcinoma. Escin's potential as an adjuvant treatment was further demonstrated by the discovery that it might enhance the effectiveness of currently used chemotherapy medicines. To learn more about the structural and chemical alterations of the modified zeolites, spectral analyses were carried out.[78,79] Overall, the results point to the potential of naturally occurring modified zeolites as strong anticancer agents.

Cosmetics

Horse chestnut extract is commonly used in cosmetics due to its various beneficial properties for skin and hair. It is rich in saponins, flavonoids, and tannins, that can protect the skin from UV radiations, pollution as well as can avoid the early wrinkles. Horse chestnut extract has been shown to improve hair growth and reduce hair loss, making it a popular ingredient in hair care products. Its ability to improve blood circulation can also help reduce the appearance of dark circles and puffiness around the eyes. Overall, the use of horse chestnut extract in cosmetics is supported by scientific research, and its benefits for skin and hair health are well established.[80]

Future Scope of HCSE in Drug Delivery as Potential Phytoconstituent

The future of horse chestnut seed extract (HCSE) in drug delivery systems and pharmacology looks promising. HCSE contains various bioactive phytoconstituents having anti-inflammatory, antioxidant, and anticancer properties. These properties make HCSE a potential candidate for drug development. In recent years, studies have explored the use of HCSE in novel delivery technologies including nanocarriers, neosomes, microemulsions, and lipidic liposomes. These technologies can enhance the bioavailability as well as efficacy of HCSE, leading to better therapeutic outcomes. Moreover, research is ongoing to explore the potential of HCSE in various pharmacological applications such as diabetes, cardiovascular diseases, and cancer. In addition, the use of HCSE in cosmeceuticals has also been gaining popularity due to antioxidant as well as anti-inflammatory actions.

HCSE has already been studied extensively due to its therapeutic properties and potential health benefits. It contains a variety of phytochemicals like flavonoids, saponins, tannins, and triterpenoids that have been shown to have antioxidant, antimicrobial, anticancer, hepatoprotective and anti-inflammatory properties. As research in the field of herbal medicine continues to grow, it is possible that horse chestnut seed extract and its phytoconstituents may become more widely recognized and utilized in traditional and modern medicine. With advancements in technology and drug delivery systems, it may be possible to develop more effective and targeted treatments using horse chestnut seed extract as a natural therapeutic agent. To completely comprehend the mechanisms of action of horse chestnut seed extract and its phytoconstituent as well as any potential adverse effects further research is still required. Overall, the potential of HCSE in drug delivery systems and pharmacology is vast and promising, and continued research will likely reveal more applications and benefits in the future.

CONCLUSION

Horse chestnut extract, containing aescin, has shown promising results in CVI and hemorrhoidal disease because of its venotonic, vasoprotective, and anti-inflammatory characteristics. Recent was also directed to its antioxidant and apoptotic characteristics, which suggest the potential for further research. Essavan gel, a naturally occurring herbal substance containing horse chestnut extract and sodium heparin, has been approved for the treatment of microcirculatory problems, poor venous circulation, hematomas, edema, sprains, and sports injuries. The approval of Essavan gel has increased confidence among scientific communities to conduct further pharmacological properties of escin. Overall, the future of escin as a venoactive compound looks bright, and this review article attempts to synthesize the available scientific data on horse chestnut extract and its important phytoconstituent aescin.

ACKNOWLEDGEMENTS

All authors are thankful to department of centre for research and development, Prist University, Vallam, Thanjavur 613 403, Tamilnadu, India for providing facility for the work. Also thankful to management and principal of Ashokrao Mane College of Pharmacy, Peth-Vadgaon for guidance and support for work.

REFERENCES

1. Targhotra M, Chauhan MK. An overview on various approaches and recent patents on buccal drug delivery systems. Current Pharmaceutical Design. 2020 Nov 1;26(39):5030-9.
2. Barreto FS, Sousa EO, Rodrigues FF, Costa JG, Campos AR. Antibacterial activity of Lantana camara linn lantana montevidensis brig extracts from cariri-ceara, Brazil. Journal of young pharmacists. 2010 Jan 1;2(1):42-4.

3. Izzo AA, Ernst E. Interactions between herbal medicines and prescribed drugs: an updated systematic review. Drugs. 2009 Sep;69:1777-98.
4. World Health Organization. Obesity: preventing and managing the global epidemic 2000.
5. Mukherjee PK, Harwansh RK, Bahadur S, Banerjee S, Kar A. Evidence-based validation of Indian traditional medicine: Way forward. InFrom Ayurveda To Chinese Medicine 2017 (pp. 137-167).
6. Schulz V, Hänsel R, Tyler VE. Rational phytotherapy: a physician's guide to herbal medicine. Psychology Press; 2001.
7. Payyappallimana U. Role of traditional medicine in primary health care. Yokohama Journal of Social Sciences. 2010;14(6):57-75.
8. Gautam V, Raman RM, Kumar A. Exporting Indian Healthcare: Export Potential of Ayurveda and Siddha Products and Services. Export Import Bank of India; 2003.
9. Jovanović B. Dendrology and Elements of Phytosociology. Naučna Knjiga, Belgrade. 1971.
10. Šedivá J, Vlašínová H, Mertelík J. Shoot regeneration from various explants of horse chestnut (*Aesculus hippocastanum* L.). Scientia Horticulturae. 2013 Sep 24;161:223-7.
11. Brune M. Urban trees under climate change. Potential impacts of dry spells and heat waves in three German regions in the 2050s. Report. 2016 May;24
12. Rose JO, Treadway SC. Herbal support for a healthy cardiovascular system. Clinical Nutrition Insight1999;6:1-6.
13. Parmar C, Kaushal MK. Aesculus indica. In wild fruits. (1982): 6-9.
14. Özel HB, Bilir N. Effects Of Light And Moisture On Growth And Morphological Characteristics Of Horsechestnut (*Aesculus hippocastanum* L.) seedlings in the western blacksea region in turkey. Poljoprivreda i Sumarstvo. 2016;62(1):65.
15. Weryszko-Chmielewska E, Chwil M. Structure of floral nectaries in *Aesculus hippocastanum* L. Acta Botanica Croatica. 2017 Apr 1;76(1):41-8.
16. Weryszko-Chmielewska E, Tietze M, Michonska M. Ecological features of the flowers of *Aesculus hippocastanum* L. and characteristics of Aesculus L. pollen seasons under the conditions of central-eastern Poland. Acta agrobotanica. 2012;65(4).
17. Lichota A, Gwozdzinski L, Gwozdzinski K. Therapeutic potential of natural compounds in inflammation and chronic venous insufficiency. European Journal of Medicinal Chemistry. 2019 Aug 15;176:68-91.
18. Gallelli L. Escin: a review of its anti-edematous, anti-inflammatory, and venotonic properties. Drug design, development and therapy. 2019 Sep 27:3425-37.
19. Edwards SE, da Costa Rocha I, Williamson EM, Heinrich M. Phytopharmacy: An evidence-based guide to herbal medicinal products. John Wiley & Sons; 2015 Feb 17.
20. Sirtori CR. Aescin: pharmacology, pharmacokinetics and therapeutic profile. Pharmacological Research. 2001 Sep 1;44(3):183-93.
21. Marton G, Bakan A. Research and development on an integrated and wastefree utilization of horse chestnut (Aesculus hippocastaneum) seeds. Bioresource technology. 1995 Jan 1;54(2):197-200.
22. Kapusta I, Janda B, Szajwaj B, Stochmal A, Piacente S, Pizza C, Franceschi F, Franz C, Oleszek W. Flavonoids in horse chestnut (*Aesculus hippocastanum*) seeds and powdered waste water byproducts. Journal of agricultural and food chemistry. 2007 Oct 17;55(21):8485-90.
23. Baraldi C, Bodecchi LM, Cocchi M, Durante C, Ferrari G, Foca G, Grandi M, Marchetti A, Tassi L, Ulrici A. Chemical composition and characterisation of seeds from two varieties (pure and hybrid) of *Aesculus hippocastanum*. Food chemistry. 2007 Jan 1;104(1):229-36.
24. Čukanović J, Tešević V, Jadranin M, Ljubojević M, Mladenović E, Kostić S. Horse chestnut (*Aesculus hippocastanum* L.) seed fatty acids, flavonoids and heavy metals plasticity to different urban environments. Biochemical Systematics and Ecology. 2020 Apr 1;89:103980.
25. Paterska M, Bandurska H, Wysłouch J, Molińska-Glura M, Moliński K. Chemical composition of horse-chestnut (Aesculus) leaves and their susceptibility to chestnut leaf miner Cameraria ohridella Deschka & Dimić. Acta physiologiae plantarum. 2017 Apr;39:1-6.
26. Duke JA. Handbook of medicinal herbs. CRC press; 2002 Jun 27.
27. Robbers JE, Tyler VE. Tyler's herbs of choice. The therapeutic use of phytomedicinals. Haworth Press Inc.; 1999.
28. Newall CA, Anderson LA, Phillipson JD. Herbal medicines. A guide for health-care professionals. The pharmaceutical press; 1996.
29. Dudek-Makuch M, Matławska I. Coumarins in horse chestnut flowers: isolation and quantification by UPLC method. Acta Poloniae Pharmaceutica. 2013 May 1;70(3):517-22.
30. Lorenz D, Marek ML. The active therapeutic principle of horse chestnut (*Aesculus hippocastanum*). Part 1. Classification of the active substance. Arzneimittel-Forschung. 1960 Apr;10:263-72.
31. Pietta P, Mauri P, Facing RM, Carini M. High-performance liquid chromatographic analysis of β-escin. Journal of Chromatography A. 1989 Jan 1;478:259-63.
32. Zhang Z, Li S, Lian XY. An overview of genus Aesculus L.: ethnobotany, phytochemistry, and pharmacological activities. Pharmaceutical Crops. 2010 Oct 12;1(1).
33. Ogawa S, Kimura H, Niimi A, Katsube T, Jisaka M, Yokota K. Fractionation and structural characterization of polyphenolic antioxidants from seed shells of Japanese horse chestnut (Aesculus turbinata BLUME). Journal of agricultural and food chemistry. 2008 Dec 24;56(24):12046-51.
34. Kimura H, Ogawa S, Ishihara T, Maruoka M, Tokuyama-Nakai S, Jisaka M, Yokota K. Antioxidant activities and structural characterization of flavonol O-glycosides from seeds of Japanese horse chestnut (Aesculus turbinata BLUME). Food Chemistry. 2017 Aug 1;228:348-55.
35. Bielarska AM, Jasek JW, Kazimierczak R, Hallmann E. Red Horse Chestnut and Horse Chestnut Flowers and Leaves: A Potential and Powerful Source of Polyphenols with High Antioxidant Capacity. Molecules. 2022 Mar 31;27(7):2279.
36. Guillaume M, Padioleau F. Veinotonic effect, vascular protection, anti-inflammatory and free radical scavenging properties of horse chestnut extract. Arzneimittel-forschung. 1994 Jan 1;44(1):25-35.
37. Yoshikawa M, Matsuda H. Chemical and pharmacological studies on triterpene saponins, escins, from horse chestnut seeds. Saponins in Food, Feedstuffs and Medicinal Plants. 2000:189-203.
38. Sato I, Kofujita H, Suzuki T, Kobayashi H, Tsuda S. Anti-inflammatory effect of Japanese horse chestnut (Aesculus turbinata) seeds. Journal of veterinary medical science. 2006;68(5):487-9.

39. de Siqueira Patriota LL, de Souza Lira CB, de Souza Aguiar LM, da Silva Barros BR, Paiva PMG, de Melo CML, et al. Purification, characterization, and immunomodulatory activity of a lectin from the seeds of horse chestnut (*Aesculus hippocastanum* L.). Current Research in Biotechnology 2022; 4:203-10.
40. Bradbury W, Pappas J. Chronic venous insufficiency, varicose veins, lymphedema, and arteriovenous fistulas. Vascular Surgery, 2006;105-124.
41. Callam M. Prevalence of chronic leg ulceration and severe chronic venous disease in western countries. Phlebology. 1992 Jan 1;7:6-12.
42. Kerrigan DC, Frates EP, Rogan S, Riley PO. Hip hiking and circumduction: quantitative definitions. American journal of physical medicine & rehabilitation. 2000 May 1;79(3):247-52.
43. Hippocastanum HH. *Aesculus hippocastanum* (Aescin, Horse Chestnut) in the Management of Hemorrhoidal Disease. Turkish Journal of Colorectal Disease. 2018;28:54-7.
44. Domanski D, Zegrocka-Stendel O, Perzanowska A, Dutkiewicz M, Kowalewska M, Grabowska I, Maciejko D, Fogtman A, Dadlez M, Koziak K. Molecular mechanism for cellular response to β-escin and its therapeutic implications. PloS one. 2016 Oct 11;11(10):e0164365.
45. Bombardelli E, Morazzoni P, Griffini A. *Aesculus hippocastanum* L. Fitoterapia (Milano). 1996;67(6):483-511.
46. Lorenz D, Marek ML. The active therapeutic principle of horse chestnut (*Aesculus hippocastanum*). Part 1. Classification of the active substance. Arzneimittel-Forschung. 1960 Apr;10:263-72.
47. Schrader E, Schwankl W, Sieder CH, Christoffel V. Comparison of the bioavailability of beta-aescin after single oral administration of two different drug formulations containing an extract of horse-chestnut seeds. Die Pharmazie. 1995 Sep 1;50(9):623-7.
48. Facino RM, Carini M, Stefani R, Aldini G, Saibene L. 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. 1995;328(10):720-4.
49. Moyses C, Cederholm-Williams SA, Michel CC. Haemoconcentration and accumulation of white cells in the feet during venous stasis. International Journal of Microcirculation, Clinical and Experimental. 1987 Jan 1;5(4):311-20.
50. Thomas PR, Nash GB, Dormandy JA. White cell accumulation in dependent legs of patients with venous hypertension: a possible mechanism for trophic changes in the skin. British Medical Journal (Clinical research ed.).1988 Jun 18;296(6638):1693-5.
51. Pittler MH, Ernst E. Rosskastaniensamen-Extrakt zur Behandlung der chronisch. Phytopharmaka V: Forschung und klinische Anwendung. 2013 Jul 2;5:127.
52. Küçükkurt I, Ince S, Keleş H, Akkol EK, Avcı G, Yeşilada E, Bacak E. Beneficial effects of *Aesculus hippocastanum* L. seed extract on the body's own antioxidant defense system on subacute administration. Journal of ethnopharmacology. 2010 May 4;129(1):18-22.
53. Güney G, Kutlu HM, Işcan A. The Apoptotic Effects of Escin in The H-Ras Transformed 5RP7 Cell Line. Phytotherapy Research. 2013 Jun;27(6):900-5.
54. Omi K, Matsuo Y, Ueda G, Aoyama Y, Kato T, Hayashi Y, Imafuji H, Saito K, Tsuboi K, Morimoto M, Ogawa R. Escin inhibits angiogenesis by suppressing interleukin-8 and vascular endothelial growth factor production by blocking nuclear factor-κB activation in pancreatic cancer cell lines. Oncology reports. 2021 May 1;45(5):1-9.
55. Yang L, Sherali A CHAPTER 67 - Nephrotoxicity of Herbal Remedies. In: Geary DF, Schaefer F, editors. Comprehensive Pediatric Nephrology. Philadelphia: Mosby; 2008, 1027-1043.
56. Ruffini I, Belcaro G, Cesarone M, Dugall M. Efficacy of topical treatment with aescin essential phospholipids gel in venous insufficiency and hypertension. 2004, Angiology.55 Suppl 1:S19-21.
57. Özkan EE, Mesut B, Polat A, Nur TA. In vitro skin permeation of escin in the new gel formulation of *Aesculus hippocastanum* (Horse Chestnut). Journal of Faculty of Pharmacy of Istanbul University. 2016 Dec 1;46(2):79-88.
58. Owczarek A, Kolodziejczyk-Czepas J, Woźniak-Serwata J, Magiera A, Kobiela N, Wąsowicz K, Olszewska MA. Potential activity mechanisms of *Aesculus hippocastanum* bark: Antioxidant effects in chemical and biological in vitro models. Antioxidants. 2021 Jun 22;10(7):995.
59. Felixsson E, Persson IA, Eriksson AC, Persson K. Horse chestnut extract contracts bovine vessels and affects human platelet aggregation through 5-HT2A receptors: an in vitro study. Phytotherapy Research. 2010 Sep;24(9):1297-301.
60. Idris S, Mishra A, Khushtar M. Phytochemical, ethanomedicinal and pharmacological applications of escin from *Aesculus hippocastanum* L. towards future medicine. Journal of Basic and Clinical Physiology and Pharmacology. 2020 Sep 1;31(5).
61. Maghsoudi H, Hallajzadeh J, Rezaeipour M. Evaluation of the effect of polyphenol of escin compared with ibuprofen and dexamethasone in synoviocyte model for osteoarthritis: an in vitro study. Clinical rheumatology. 2018 Sep;37:2471-8.
62. Li M, Lu C, Zhang L, Zhang J, Du Y, Duan S, Wang T, Fu F. Oral administration of escin inhibits acute inflammation and reduces intestinal mucosal injury in animal models. Evidence-Based Complementary and Alternative Medicine. 2015 Jan 1;2015.
63. Yoshikawa M, Matsuda H. Chemical and pharmacological studies on triterpene saponins, escins, from horse chestnut seeds. Saponins in Food, Feedstuffs and Medicinal Plants. 2000:189-203.
64. Belcaro G, Rulo A, Candiani C. Evaluation of the microcirculatory effects of Venoruton in patients with chronic venous hypertension by laserdoppler flowmetry, transcutaneous PO2 and PCO2 measurements, leg volumetry and ambulatory venous pressure measurements. VASA. Zeitschrift fur Gefasskrankheiten. 1989 Jan 1;18(2):146-51.
65. Belcaro G, Cesarone MR, De Sanctis MT, Incandela L, Laurora G, Fevrier B, Wargon C, De Gregoris P. Laser Doppler and transcutaneous oxymetry: modern investigations to assess drug efficacy in chronic venous insufficiency. International Journal of Microcirculation. 1995;15(Suppl. 1):45-9.
66. Belcaro G, Rosaria Cesarone M, Ledda A, Cacchio M, Ruffini I, Ricci A, Ippolito E, Di Renzo A, Dugall M, Corsi M, Marino Santarelli AR. O-(β-hydroxyethyl)-rutosides systemic and local treatment in chronic venous disease and microangiopathy: an independent prospective comparative study. Angiology. 2008 Feb;59(1_suppl):7S-13S.
67. Xin W, Zhang L, Sun F, Jiang N, Fan H, Wang T, Li Z, He J, Fu F. Escin exerts synergistic anti-inflammatory effects with low doses of glucocorticoids in vivo and in vitro. Phytomedicine. 2011 Feb 15;18(4):272-7.
68. Khar'kov Yuriy K, Arsene MM, Martynenkova Aliya V, Podoprigora Irina V, Volina Elena G. Assessment of

antimicrobial activity of ethanolic and aqueous extracts of *Aesculus hippocastanum* L.(horse chestnut) bark against bacteria isolated from urine of patients diagnosed positive to urinary tract infections. bioRxiv. 2021 Aug 25:2021-08.

69. Küp FÖ, Çoşkunçay S, Duman F. Biosynthesis of silver nanoparticles using leaf extract of *Aesculus hippocastanum* (horse chestnut): Evaluation of their antibacterial, antioxidant and drug release system activities. Materials Science and Engineering: C. 2020 Feb 1;107:110207.
70. Singh H, Sidhu S, Chopra K, Khan MU. The novel role of β-aescin in attenuating CCl4-induced hepatotoxicity in rats. Pharmaceutical biology. 2017 Jan 1;55(1):749-57.
71. ERTÜRK Ö. Antibacterial and antifungal effects of the leaf, seed, seed coat and fruit capsule of *Aesculus hippocastanum* (Sapindaceae) extracts. Acta Biologica Turcica. 2017 Feb 10;30(1):20-3.
72. Kumar GS. An emphasis on global use of traditional medicinal system and herbal hepatoprotective drugs. Journal of Pharmacy Research2014 Jan;8:28-37.
73. WU SJ, SA RN, GU ZR, Zhao P, YU J, Wang YH, GE B. The hepatoprotective effect of *Aesculus hippocastanum* seed extract against concanavalin A-induced acute liver injury in mice via inhibition of ROS and JNK pathway. Chinese Pharmacological Bulletin. 2018:412-8.
74. Kucukkurt I, Akkol EK, Karabag F, Ince S, Süntar I, Eryavuz A, Sözbilir NB. Determination of the regulatory properties of Yucca schidigera extracts on the biochemical parameters and plasma hormone levels associated with obesity. Revista Brasileira de Farmacognosia. 2016 Apr;26:246-50.
75. Kimura H, Ogawa S, Katsube T, Jisaka M, Yokota K. Antiobese effects of novel saponins from edible seeds of Japanese horse chestnut (Aesculus turbinata BLUME) after treatment with wood ashes. Journal of agricultural and food chemistry. 2008 Jun 25;56(12):4783-8.
76. Elmas O, Erbas O, Yigitturk G. The efficacy of *Aesculus hippocastanum* seeds on diabetic nephropathy in a streptozotocin-induced diabetic rat model. Biomedicine & Pharmacotherapy. 2016 Oct 1;83:392-6.
77. Suryavanshi SV, Kulkarni YA. Attenuation of cardiac autonomic neuropathy by escin in diabetic rats. Pharmacology. 2021;106(3-4):211-7.
78. Patlolla JM, Qian L, Biddick L, Zhang Y, Desai D, Amin S, Lightfoot S, Rao CV. β-Escin Inhibits NNK-Induced Lung Adenocarcinoma and ALDH1A1 and RhoA/Rock Expression in A/J Mice and Growth of H460 Human Lung Cancer CellsPrevention of Lung Cancer by β-Escin. Cancer Prevention Research. 2013 Oct 1;6(10):1140-9.
79. Tomečková V, Reháková M, Mojžišová G, Wadsten T, Zelenáková K, Komanický V. Spectral study of modified natural clinoptilolite with pharmacologically active escin and horse chestnut extract. Spectroscopy Letters. 2016 Feb 7;49(2):63-72.
80. Cheong DH, Arfuso F, Sethi G, Wang L, Hui KM, Kumar AP, Tran T. Molecular targets and anticancer potential of escin. Cancer letters. 2018 May 28;422:1-8