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An Overview of Genus *Aesculus* L.: Ethnobotany, Phytochemistry, and Pharmacological Activities

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Abstract: The genus *Aesculus* L. (Hippocastanaceae) has 12 species distributed in eastern Asia, eastern and western North America, and Europe. In Europe and the United States, *A. hippocastanum* has been used for the treatment of chronic venous insufficiency, hemorrhoids, and postoperative edema. In China, *A. chinensis* var. *chinense* has been used as a stomachic and analgesic in the treatment of distention and pain in the chest and the abdomen, malaria, dysentery, and heart disease. The objective of this paper is to review the ethnobotany, phytochemistry, and pharmacological properties of the genus *Aesculus*. To date, more than 210 compounds have been isolated and identified primarily from the fruits or seeds of *Aesculus*, with polyhydroxylated triterpenoid saponins as the major active principles. Studies have confirmed that *Aesculus* seed extracts and isolates possess diverse activities, including anti-inflammatory, antitumor, antiviral, antifungal, antiangiogenic (vascular protection), anti-obesity, antioxidative, and antigenotoxic properties. Interestingly, detailed studies of the saponins from more eastern North American species and other uninvestigated *Aesculus* species are necessary to further confirm the chemotaxonomic value of the novel cytotoxic saponins discovered recently from *A. pavia*, an eastern North American species, and to fully understand the chemical profile of the genus. Animal investigations of these novel cytotoxic saponins on the potential for the treatment of cancer are also intriguing.

Keywords: *Aesculus*; horse chestnut; buckeyes; ethnobotany; phytochemistry; triterpenoid saponins; bioactivities; anti-tumor activity.

INTRODUCTION

Aesculus L. is a genus of the family Hippocastanaceae containing 12 species of deciduous trees and shrubs in the northern hemisphere, primarily in eastern Asia and eastern North America, with one species native to Europe, and two to western North America [1-4]. *Aesculus* have been cultivated as pharmaceutical crops for the production of Standardized Therapeutic Extracts (STEs). There are two Eurasian species commonly used in medicine: *A. hippocastanum* (common horse chestnut) and *A. chinensis* var. *chinensis* (Chinese horse chestnut). In Europe, the bark, leaves, horse chestnut seed extract (HCSE), and aescin (a saponin mixture) from *A. hippocastanum* have been used in the treatment of chronic venous insufficiency, hemorrhoids, and postoperative edema [5-9]. In China, the seeds of *A. chinensis* var. *chinensis* have been used as a stomachic and analgesic in the treatment of distention and pain in chest and abdomen, malaria, and dysentery [10] and tablets made from the seeds are also used for treating heart diseases [11]. Modern pharmacologic investigations have confirmed that HCSE, aescin and individual compounds isolated and identified from the two Eurasian species and other *Aesculus* species possess diverse activities, including anti-inflammatory, antitumor, antiviral,

antioxidative, and antigenotoxic properties. The chemical constituents of some *Aesculus* species have been well documented. To date, more than 210 compounds from different classes have been isolated and identified from the genus *Aesculus*. These compounds include triterpenoids, triterpenoid glycosides (saponins), flavonoids, coumarins, carotenoids, long fatty chain compounds, and some other classes of compounds. The present paper with 108 references reviews the research advances in the ethnobotany, phytochemistry and pharmacological activities of the genus *Aesculus*.

BOTANY, CULTIVATION, AND MEDICAL USES

To date, over a hundred species or varieties in *Aesculus* have been described because of great morphological variations due to natural pollination among the species. In his monograph of *Aesculus*, Koch (1857) recognized 13 species in four subgenera, *Hippocastanum*, *Pavia*, *Calothyrsus*, and *Macrothyrsus* [12]. Based on bud viscosity, fruit exocarp ornamentation, flower color, and petal morphology, the 13 species were grouped into five sections [1, 2, 13]. All new species discovered in the last several decades are not recognized and thus most authors recognized 13 species in the genus until *A. wilsonii* Rehder was recently treated as a variety under the species *A. chinensis* Bunge [3, 14]. The 12 currently recognized species in the genus *Aesculus* are grouped in the five sections: Section *Aesculus* in Europe and Japan (*A. hippocastanum* L. and *A. turbinata* Blume), Sec-

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tion *Calothyrsus* (Spach) K. Koch in Southeastern Asia and western United States (*A. assamica* Griffith, *A. californica* (Spach) Nuttall, *A. chinensis* Bunge var. *chinensis*, *A. chinensis* Bunge var. *wilsonii* ((Rehder) Turland & N.H. Xia, and *A. indica* (Camb.) Hook.), Section *Pavia* (Mill.) Persoon in the southeastern United States (*A. glabra* Willd. var. *glabra*, *A. glabra* Willd. var. *arguta* (Buckl.) Robinson, *A. flava* Sol., *A. pavia* L., and *A. sylvatica* Bartram), Section *Macrothyrsus* (Spach) K. Koch in the southeastern United States (*A. parviflora* Walter), and Section *Parryanae* Wiggins in Baja California, Mexico (*A. parryi* A. Gray) (Table 1).

The recent phylogenetic analysis of *Aesculus* by DNA sequences of chloroplast gene *matK* and internal transcribed spacers of nuclear ribosomal RNA genes supported Hardin's classification except for section *Calothyrsus* [4]. Some chemotaxonomical analysis has been conducted [15]. Interestingly, *A. pavia* in the Section *Pavia* has triterpenoid saponins with an oligosaccharide chain at C-3 of the aglycone with an α -arabinofuranosyl unit affixed to C-3 of the glucuronic acid while Eurasian species in Section *Aesculus* and Section *Calothyrsus* have saponins with a trisaccharide chain at C-3 of the aglycone with a β -glucopyranosyl unit attached to C-4 of the glucuronic acid [16] (see Triterpenoid Saponins for details). This significant chemical difference suggests that triterpenoid saponins may provide important clues in understanding the systematics and evolution of the genus *Aesculus*.

Aesculus are deciduous trees or shrubs with easily recognizable palmately compound leaves (5-11 leaflets), characteristic capsules and seeds, and attractive ornamental flowers. *Aesculus hippocastanum* has been cultivated since 1576 as an ornamental plant [17] while *A. chinensis* var. *chinensis* has been planted in temples and homes for several centuries. At present, all species are cultivated, with at least 27 commercial cultivars supplied by at least 95 nurseries in the United States [17]. Most species are propagated from seeds and the cultivars by budding or grafting. In Europe, HCSE and aescin are major pharmaceutical products from *Aesculus*.

Aesculus hippocastanum is a large deciduous tree, also known as Conker tree. This species is native to a small area in the mountains of the Balkans in southeast Europe, in small areas in northern Greece, Albania, the Republic of Macedonia, Serbia, and Bulgaria. It is now widely cultivated throughout the temperate world because of its large, beautiful flower clusters and its excellent resistance to environmental conditions. The leaves of this species are opposite and palmately compound with 5-7 leaflets. The sessile leaflets are green beneath with obtusely double-serrate. Its flowers are usually white with a small red spot. The fruit is a green, spiky capsule containing one (rarely two or three) brown seed, called chestnut or conker [2].

The common name "horse chestnut" may have come from the uses of seeds for horses to treat overexertion or coughs by Turks and Greeks [18]. Dated from the early 18th century, the horse chestnut has a therapeutic property for anti-fever [5]. In Europe, the bark and leaves of *A. hippocastanum* have been employed as an astringent to treat diarrhea and hemorrhoids [6]. The HCSE from *A. hippocastanum* has been used in the treatment of chronic venous insufficiency, hemorrhoids, and postoperative edema [7-9]. Aescin, also known

as β -escin, is the major active component in HCSE and has been shown to have a clinically significant activity in chronic venous insufficiency, hemorrhoids, and postoperative edema. The pharmacology, pharmacokinetics and therapeutic profile of aescin have been reviewed [5]. Based on the publication over the last two decades of numerous randomized controlled trials in prominent, peer-reviewed journals, *A. hippocastanum* extract is gaining wider acceptance as an effective therapy for venous disorders and edema in the United States [5].

Aesculus chinensis is native to China and is distributed mainly in the Hebei, Henan, and Shanxi provinces. The fruits of this species usually are smooth subglobose, truncate to slightly impressed at the apex, thickly walled with smaller seeds having proportionately larger hilum. The surface of its petiolulate leaflets is glabrous or sparingly pilose [2]. Its seeds, called "Sha Luo Zhi" in traditional Chinese medicine, have long been used as a stomachic and analgesic in the treatment of distention and pain in chest and abdomen, malaria, and dysentery [10]. Additionally, the tablets made from the seeds are used for treating heart diseases [11].

Aesculus turbinata, known as Japanese horse chestnut, is native only to Japan. It has been found on the island of Hokkaido and the central and northern parts of Honshu. This species has been widely cultured in England, the United States, and China. This species is similar to *A. hippocastanum*, differently mainly in the slightly smaller flowers, warty fruit surface, and in the larger leaves, which are glaucescent beneath and have more regularly crenate-serrate margins [2]. The seeds of the Japanese horse chestnut have been used as an emergency provision since ancient times and utilized traditionally in Japan as a confectionery ingredient in rice cakes and rice balls [19]. Its seed extract in combination with spirits have also been used as a folk medicine for the treatment of bruises and sprains in some regions of Japan [20].

Aesculus indica, known as Indian horse chestnut, is found on mountain slopes or in moist and shady valleys in the northwestern Himalayan forests. It is distributed from Nepal northwestward into the State of Kashmir in north India, and across the Indus River to West Pakistan and to northeastern Afghanistan. Its fruits are reddish brown, smooth ovoids. The petiolulate leaflets of this species are submembranaceous with finely serrate margin [2]. In some parts of Himachal Pradesh, the seeds are dried and grounded into flour, called *tattwakhar*. This flour is bitter and used for making *halwa*, which is taken as *phalahar* (non-cereal food) during fasts; the leaves are used as a fodder for cattle. India horse chestnut also has medicinal properties for animals and human beings. The fruits are given to horses suffering from colic. The oil extracted from the seeds is used to cure rheumatism [21].

Seeds of North American species (e.g., *A. pavia*, *A. flava*, *A. glabra*, and *A. californica*), known as buckeyes, were used by Native Americans to tranquilize fish to make them easier to catch [22]. Extracts from the seeds were used to treat earaches, sores, colic, sprains, and chest pains [23]. Powdered bark was sometimes used to alleviate toothaches and ulcer pain [24].

Table 1. Species List of *Aesculus* L

Taxa	Synonyms	Distribution (Native Range)	References for Chemical Studies
Sect. <i>Aesculus</i>		Europe, Japan	
<i>A. hippocastanum</i> L.	<i>A. asplenifolia</i> Loud. <i>A. castanea</i> Gilib. <i>A. heterophylla</i> Hort. ex Handl. <i>A. ohiotensis</i> Lindl. <i>A. hippocastanum</i> f. <i>beaumanii</i> (C.K.Schneid.) Dole <i>A. hippocastanum</i> f. <i>laciniata</i> (Jacq.) Schelle <i>A. hippocastanum</i> f. <i>pendula</i> (Puvill.) Rehder <i>A. hippocastanum</i> f. <i>pyramidalis</i> Henry <i>A. hippocastanum</i> var. <i>argenteovariegata</i> Loudon <i>A. hippocastanum</i> var. <i>aureovariegata</i> Loudon <i>A. hippocastanum</i> var. <i>asplenifolia</i> Hort. <i>A. hippocastanum</i> var. <i>beaumanii</i> Schneid. <i>A. hippocastanum</i> var. <i>dissecta</i> Hort. <i>A. hippocastanum</i> var. <i>flore-pleno</i> Loudon <i>A. hippocastanum</i> var. <i>incisa</i> Booth ex Loud. <i>A. hippocastanum</i> var. <i>laciniata</i> Jacq. <i>A. hippocastanum</i> var. <i>pendula</i> Puvill. <i>A. hippocastanum</i> var. <i>pumila</i> Dipp. <i>A. hippocastanum</i> var. <i>umbraculifera</i> Jaeg. <i>A. hippocastanum</i> var. <i>variegata</i> Loudon <i>A. incisa</i> hort. ex Handl. <i>A. memmingeri</i> C.Koch <i>A. procera</i> Salisb. <i>A. septenata</i> Stokes <i>Esculus hippocastanea</i> (L.) Rafinesque <i>Hippocastanum aesculus</i> Cav. <i>Hippocastanum vulgare</i> Gaertner <i>Pavia hippocastanum</i> (L.) Kuntze	S. Albania Bulgaria, N. Greece, former Yugoslavia	25, 26, 27, 28, 35, 36, 37, 38, 39, 40, 64, 65, 69, 70, 73, 75, 76
<i>A. turbinata</i> Blume	<i>A. dissimilis</i> Blume <i>A. sinensis</i> Hort. <i>A. turbinata</i> var. <i>pubescens</i> Rehder <i>A. turbinata</i> f. <i>pubescens</i> (Rehder) Ohwi ex Yas <i>Pavia dissimilis</i> (Blume) Kuntze <i>Pavia turbinata</i> (Blume) Kuntze	Japan	19, 20, 41, 42, 43, 44, 45, 71, 72, 74
Sect. <i>Calothyrsus</i> (Spach) K. Koch		SE Asia, W. USA	
<i>A. assamica</i> Griffith	<i>A. chuniana</i> Hu & W.P. Fang <i>A. coriaceifolia</i> Hu & W.P. Fang* <i>A. khassayana</i> C.R. Das & Majumdar.* <i>A. lantsangensis</i> Hu & W.P. Fang <i>A. megaphylla</i> Hu & W.P. Fang <i>A. polyneura</i> var. <i>dongchusnensis</i> Li & Yin <i>A. punduana</i> Wallich ex Hiern <i>A. rupicola</i> Hu & W.P. Fang <i>A. tsiangii</i> Hu & W.P. Fang* <i>A. wangii</i> Hu* <i>A. wangii</i> var. <i>rupicola</i> (Hu & W.P. Fang) W.P. Fang* <i>Pavia khassayana</i> Viogt.	SW China, Bangladesh, Bhutan, NE India, Laos, Myanmar, Thailand, N. Vietnam	32, 33, 54, 55, 56, 57
<i>A. californica</i> (Spach) Nuttall	<i>Calothyrsus californica</i> Spach <i>Hippocastanum californicum</i> Greene <i>Pavia californica</i> (Spach) Hartweg <i>Pavia californica</i> Kuntze	California, USA	68, 82
<i>A. chinensis</i> Bunge		China	
var. <i>chinensis</i>	<i>A. chekiangensis</i> Hu & W.P. Fang <i>A. chinensis</i> var. <i>chekiangensis</i> (Hu & W.P. Fang) W.P. Fang	Central China	46, 47, 48, 45, 50, 52, 53, 66

Table 1. contd....

Taxa	Synonyms	Distribution (Native Range)	References for Chemical Studies
<i>var. wilsonii</i> (Rehder) Turland & N.H. Xia	<i>A. wilsonii</i> Rehder <i>Actinotinus sinensis</i> Oliver	Central and SW China	32, 33, 34, 79, 83
<i>A. indica</i> (Camb.) Hook.	<i>A. indica</i> var. <i>concolor</i> Browicz <i>Pavia indica</i> Wall. ex Cambess	Himalayas	29, 58, 59, 60, 62, 63, 76, 77, 78
<i>Sect. Pavia</i> (Mill.) Persoon		SE USA	
<i>A. glabra</i> Willd.			
<i>var. glabra</i>	<i>A. ohioensis</i> De Candolle <i>Nebroopsis glabra</i> (Willd.) Rafinesque <i>Pavia glabra</i> (Willd.) Spach <i>Pawia glabra</i> (Willd.) Kuntze		31
<i>var. arguta</i> (Buckl.) Robinson	<i>A. arguta</i> Buckl.		
<i>A. flava</i> Sol.	<i>A. flava</i> Aiton <i>A. flava</i> f. <i>vestita</i> (Sargent) Fernald <i>A. flava</i> var. <i>virginica</i> (Sargent) Fernald <i>A. flava</i> var. <i>purpurascens</i> A. Gray <i>A. flava</i> var. <i>purpurea</i> Hort <i>A. flava</i> var. <i>rosea</i> Hort <i>A. flava</i> var. <i>sanguinea</i> Hort <i>A. lutea</i> Wangh. <i>A. maxima</i> D. Drake <i>A. octandra</i> Mrshall <i>A. octandra</i> f. <i>vestita</i> (Sargent) Fernald <i>A. octandra</i> f. <i>virginica</i> (Sargent) Fernald <i>A. octandra</i> var. <i>purpurascens</i> (A.Gray) C.K.Schneid <i>A. octandra</i> var. <i>vestita</i> Sargent <i>A. octandra</i> var. <i>virginica</i> Sargent <i>Pavia bicolor</i> Rafinesque <i>P. flava</i> (Sol.) Moench <i>P. fulva</i> Rafinesque <i>P. lutea</i> Poir. <i>Pavia reticulata</i> Rafinesque <i>Paviana flava</i> (Sol.) Rafinesque <i>Pawia octandra</i> (Marshall) Kuntze <i>P. octandra</i> var. <i>flava</i> (Sol.) Kuntze		
<i>A. pavia</i> L.	<i>A. austrina</i> Small <i>A. bicolor</i> Pursh <i>A. florida</i> Bartram <i>A. humilis</i> Loddiges ex Lindley <i>A. octandra</i> var. <i>discolor</i> (Pursh) Rehder <i>A. pavia</i> var. <i>atrosanguinea</i> Kirchner <i>A. pavia</i> var. <i>discolor</i> (Pursh) Torrey & A. Gray <i>A. pavia</i> var. <i>nana</i> Dippel <i>A. pavia</i> var. <i>pendula</i> (Loudon) Rehder <i>A. pavia</i> var. <i>whitleyi</i> Rehder <i>A. splendens</i> Sargent		16, 30, 61, 67, 74, 75
<i>A. pavia</i> L. (continued)	<i>Pavia americana</i> Saint-Hilaire <i>P. atropurpurea</i> Spach <i>P. glauca</i> Rafinesque <i>P. humilis</i> (Loddiges ex Lindley) G. Don ex Loudon <i>P. intermedia</i> Spach <i>P. lindleyana</i> Spach <i>P. livida</i> Spach <i>P. longiflora</i> Rafinesque <i>P. lucida</i> Spach		

Table 1. contd....

Taxa	Synonyms	Distribution (Native Range)	References for Chemical Studies
	<i>P. michauxii</i> Spach <i>P. mollis</i> Rafinesque <i>P. octandra</i> Miller <i>P. parviflora</i> Rafinesque <i>P. punctata</i> Rafinesque <i>P. rubra</i> Lamarck <i>P. versicolor</i> Spach <i>P. willdenowiana</i> Spach		
<i>A. sylvatica</i> Bartram	<i>A. georgiana</i> Sargent <i>A. georgiana</i> var. <i>lanceolata</i> Sargent <i>A. georgiana</i> var. <i>pubescens</i> Sargent <i>A. neglecta</i> var. <i>georgiana</i> Sargent <i>A. neglecta</i> var. <i>lanceolata</i> Sargent <i>A. neglecta</i> var. <i>tomentosa</i> Sargent <i>A. sylvatica</i> var. <i>lanceolata</i> (Sargent) Bartr.		
<i>Sect. Macrothyrsus</i> (Spach) K. Koch		SE USA	
<i>A. parviflora</i> Walter	<i>A. alba</i> (Poir.) Rafinesque <i>A. macrostachya</i> Michx. <i>A. macrostachys</i> Pers. <i>A. odorata</i> F. Dietr. <i>A. parviflora</i> f. <i>serotina</i> Rehder <i>Macrothyrsus discolor</i> Spach <i>M. odorata</i> (F. Dietr.) Rafinesque <i>Nebropsis alba</i> (Poir.) Rafinesque <i>Pavia alba</i> Poir. <i>P. edulis</i> Poit. ex DC. <i>P. macrostachya</i> (Michx.) DC. <i>P. macrostachys</i> (Pers.) Loisel. <i>P. parviflora</i> (Walter) Rafinesque <i>Pawia parviflora</i> (Walter) Kuntze	SE USA	74, 80, 81
<i>Sect. Parryanae</i> Wiggins		Baja California, Mexico	
<i>A. parryi</i> A. Gray	<i>Pawia parryi</i> (A. Gray) Kuntze		

Note: * The taxon was not validly published.

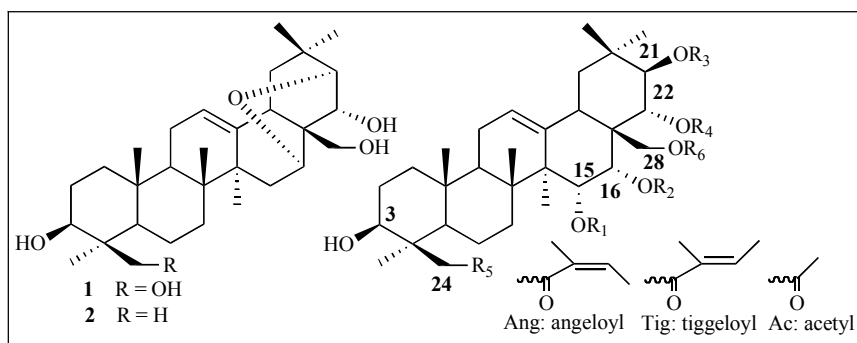
PHYTOCHEMISTRY

Seeds of *Aesculus* are the plant materials often used for medicine in Eurasia and North America, and thus the seeds and fruits are the main targets for chemical investigations in the last several decades. To date, more than 210 compounds have been isolated and identified from *Aesculus*. These compounds include triterpenoids, triterpenoid glycosides (saponins), flavonoids, coumarins, carotenoids, long fatty chain compounds, and some other classes of compounds.

Triterpenoids

Triterpenoids from the genus *Aesculus* belong to pentacyclic triterpenoids and have multiple hydroxyl groups at C-3, C-15, C-16, C-21, C-22, C-24, and C-28. Their structures vary based on the substituents of hydroxyl at C-15 and C-24 as well as the number and type of acyl groups at C-16, C-21, C-22, and C-28. To date, six triterpenoids (**1-6**) and 14 acyl-triterpenoids (**7-20**) (Table 2) have been isolated and identified from the hydrolysate of the saponin mixtures extracted

from the seeds of *Aesculus*. Aescigenin (**1**), a product of the acid hydrolysis of the saponin aescin from the seeds of *A. hippocastanum*, was the first triterpenoid reported [25]. Later, a number of triterpenoids (sapogenins) and acyl-triterpenoids (prosapogenins) were isolated and identified from the acid and alkaline hydrolysates of the *Aesculus* saponin mixtures. These hydrolytic products include barringtogenol-D (**2**) [26], R₁-barrigenol (**4**) [27], barringtogenol-C (**6**) [27], 21-*O*-tigloyl-22-*O*-angeloyl-R₁-barrigenol (**10**) [27], 16-*O*-acetylprotoaescigenin (**12**) [28], 21-*O*-angeloylprotoaescigenin (**13**) [28], 28-*O*-angeloylprotoaescigenin (**15**) [28], and 21-*O*-angeloylbarringtogenol-C (**18**) [27] from *A. hippocastanum*; aescigenin (**1**), protoaescigenin (**5**), barringtogenol-C (**6**), 22-angeloyl-R₁-barrigenol (**9**), 21-angeloylbarringtogenol-C (**18**), 21,22-diangeleylbarringtogenol-C (**20**) from *A. indica* [29]; 24-hydroxy-R₁-barrigenol (**3**) [16], R₁-barrigenol (**4**) [16], barringtogenol-C (**6**) [30], 21-*O*-angeloyl-24-hydroxy-R₁-barrigenol (**7**) [30], 21-*O*-angeloylprotoaescigenin (**13**) [30], 22-*O*-angeloylprotoaescigenin (**14**) [30], 21, 22-*O*-diangeleylprotoaescigenin (**16**) [30], 21-*O*-



Number	Name	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
3	24-Hydroxy-R ₁ -barrigenol	OH	H	H	H	OH	H
4	R ₁ -barrigenol	OH	H	H	H	H	H
5	Protoaescigenin	H	H	H	H	OH	H
6	Barringtogenol-C	H	H	H	H	H	H
7	21-O-angeloyl-24-hydroxy-R ₁ -barrigenol	OH	H	Ang	H	OH	H
8	21-O-angeloyl-R ₁ -barrigenol	OH	H	Ang	H	H	H
9	22-O-angeloyl-R ₁ -barrigenol	OH	H	H	Ang	H	H
10	21-O-tigloyl-22-O-angeloyl-R ₁ -barrigenol	OH	H	Tig	Ang	H	H
11	21, 22-O-diangeloyl-R ₁ -barrigenol	OH	H	Ang	Ang	H	H
12	16-O-acetylprotoaescigenin	H	Ac	H	H	OH	H
13	21-O-angeloylprotoaescigenin	H	H	Ang	H	OH	H
14	22-O-angeloylprotoaescigenin	H	H	H	Ang	OH	H
15	28-O-angeloylprotoaescigenin	H	H	H	H	OH	Ang
16	21, 22-O-diangeloylprotoaescigenin	H	H	Ang	Ang	OH	H
17	21-O-tigloylbarringtogenol-C	H	H	Tig	H	H	H
18	21-O-angeloylbarringtogenol-C	H	H	Ang	H	H	H
19	28-O-tigloylbarringtogenol-C	H	H	H	H	H	Tig
20	21, 22-O-diangeloylbarringtogenol-C	H	H	Ang	Ang	H	H

Table 2. The Triterpenoids and Triterpenoid Saponins Reported in Various Taxa of *Aesculus*

No.	Name	Sect. <i>Aesculus</i>		Sect. <i>Calothyrsus</i>				Sect. <i>Pavia</i>	
		AH ^a	AT ^a	AA ^a	AC ^a	ACW ^a	AI ^a	AG ^a	AP ^a
1	Aescigenin	25 ^b				34 ^b	29 ^b		
2	Barringtogenol-D	26							
3	24-Hydroxy-R ₁ -barrigenol								16 ^b
4	R ₁ -barrigenol	27							16
5	Protoaescigenin			32 ^b		34	29		
6	Barringtogenol-C	27		33			29		30
7	21-O-angeloyl-24-hydroxy-R ₁ -barrigenol								30
8	21-O-angeloyl-R ₁ -barrigenol							31	
9	22-O-angeloyl-R ₁ -barrigenol						29	31	

Table 2. contd....

No.	Name	Sect. <i>Aesculus</i>		Sect. <i>Calothyrsus</i>				Sect. <i>Pavia</i>	
		AH ^a	AT ^a	AA ^a	AC ^a	ACW ^a	AI ^a	AG ^a	AP ^a
10	21- <i>O</i> -tigloyl-22- <i>O</i> -angeloyl-R ₁ -barrigenol	27							
11	21, 22- <i>O</i> -diangeloyl-R ₁ -barrigenol							31	
12	16- <i>O</i> -acetylprotoaescigenin	28							
13	21- <i>O</i> -angeloylprotoaescigenin	28		32		34			30
14	22- <i>O</i> -angeloylprotoaescigenin								30
15	28- <i>O</i> -angeloylprotoaescigenin	28							
16	21, 22- <i>O</i> -diangeloylprotoaescigenin								30
17	21- <i>O</i> -tigloylbarringtogenol-C								30
18	21- <i>O</i> -angeloylbarringtogenol-C	28					29	31	30
19	28- <i>O</i> -tigloylbarringtogenol-C								30
20	21, 22- <i>O</i> -diangeloylbarringtogenol-C						29	31	
21	Escin Ia	26, 38, 39	41		46	51, 52			
22	Escin Ib	35, 38, 39	41		46	51, 52			
23	Escin IIa	38, 39	42						
24	Escin IIb	38, 39	42						
25	Escin IIIa	38, 39				51			
26	Escin IIIb	40							
27	Escin IV	40							
28	Escin IVc		44		47	52			
29	Escin IVd				47	52			
30	Escin IVe				47	53			
31	Escin IVf				47				
32	Escin IVg				48				
33	Escin IVh				48	53			
34	Escin V	40							
35	Escin VI	40							
36	Escin VIb				48				
37	Deacetylescins Ia		43						
38	Deacetylescins Ib		43			53	58		
39	Deacetylescins IIa		43						
40	Deacetylescins IIb		43						
41	Desacetylescins I		43		48				
42	Desacetylescins II		43						
43	Isoescins Ia	40	44		46	51, 52			
44	Isoescins Ib	40	44	60	46	51, 52			
45	Isoescins IIa				49	51			
46	Isoescins IIb				49	51			
47	Isoescins IIIa				49	51			
48	Isoescins IIIb				49				
49	Isoescins V	40							
50	Isoescins VIa		45						

Table 2. contd....

No.	Name	Sect. <i>Aesculus</i>		Sect. <i>Calothyrsus</i>				Sect. <i>Pavia</i>	
		AH ^a	AT ^a	AA ^a	AC ^a	ACW ^a	AI ^a	AG ^a	AP ^a
92	Aesculoside IIIe								61
93	Aesculoside IIIf								61
94	Xanifolia-Y								61
95	Aesculoside IVa								16, 61
96									16, 61
97	Aesculoside IVb								16, 61
98									16, 61
99	Aesculoside IVc								16, 61

^a AH: *A. hippocastanum*, AT: *A. turbinata*; AA: *A. assamica*; AC: *A. chinensis*; ACW: *A. chinensis* var. *wilsonii*; AI: *A. indica*; AG: *A. glabra*, AP: *A. pavia*. ^b reference number.

tigloylbarringtogenol-C (17) [30], 21-*O*-angeloylbarringtogenol-C (18) [30], and 28-*O*-tigloylbarringtogenol-C (19) [30] from *A. pavia*; 21-angeloyl-R₁-barrigenol (8), 22-*O*-angeloyl-R₁-barrigenol (9), 21,22-*O*-diangeloyl-R₁-barrigenol (11), 21-*O*-angeloylbarringtogenol-C (18), and 21,22-*O*-diangeloylbarringtogenol-C (20) from *A. glabra* [31]; protoaescigenin (5) [32], barringtogenol-C (6) [33], and 21-*O*-angeloylprotoaescigenin (13) [32] from *A. assamica*; and aescigenin (1), protoaescigenin (5), and 21-*O*-angeloylprotoaescigenin (13) from *A. chinensis* var. *wilsonii* [34].

Triterpenoid Glycosides (Triterpenoid Saponins)

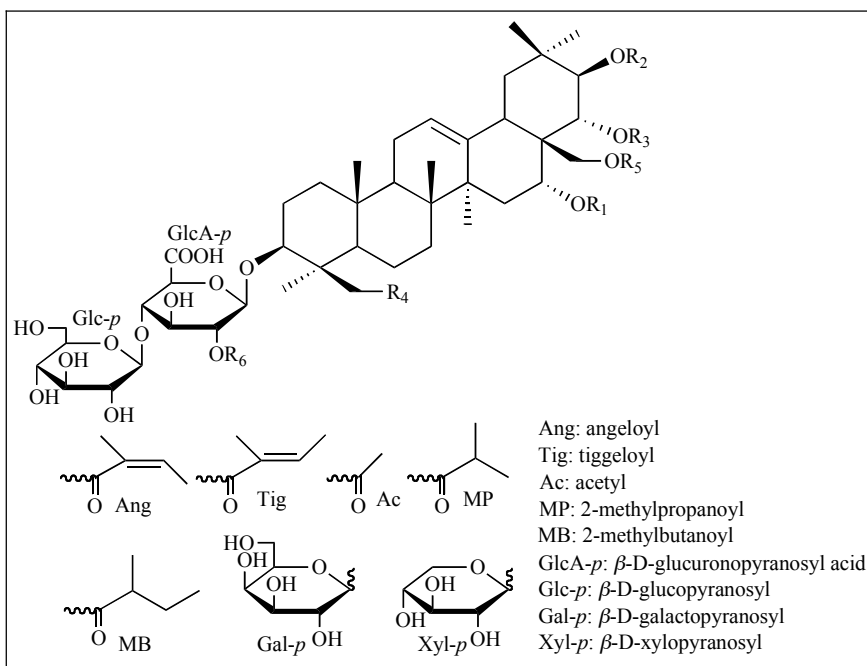
Triterpenoid glycosides or saponins are one of the main groups of chemical constituents from *Aesculus*. They are classified as polyhydroxylated triterpenoid glycosides based on four different aglycones (sapogenins), including 24-hydroxy-R₁-barrigenol (3), R₁-barrigenol (4), protoaescigenin (5), and barringtogenol-C (6), due to the different substituents of hydroxyl at C-15 and C-24. To date, about 79 saponins have been isolated and identified from the genus *Aesculus* (Table 2). Most of the *Aesculus* saponins have a trisaccharide chain with a glucuronopyranosyl unit attached to the C-3 position of the aglycone. A few saponins have a disaccharide chain at C-3 of the sapogenin, such as assamicins V (69), VIII (71), aesculosides Ie (76), and IId (80) while assamicins II (68), III (60), IV (61), and VI (62) are bisdesmosidic glycosides. The *Aesculus* type of saponins usually possesses acyl functions at C-21, C-22, and C-28 positions, some rarely, at C-16, such as escin VIb (36). The acyl functions include angeloyl (Ang), tigloyl (Tig), acetyl (Ac), 2-methylbutanoyl (MB), and 2-methylpropanoyl (MP) groups.

The isolation and the structural determination of the individual ingredients in the saponin mixtures from the genus *Aesculus* have been the target of many investigations. The structures of two major saponins in aescin from the seeds of *A. hippocastanum* were first presumed as escins Ia (21) and Ib (22) on the basis of chemical and physicochemical evidences obtained by using the saponin mixture [26, 35]. Then, the two major saponins were isolated from the commercial β-escin [36], and their MS data were reported based on the previous presumed structures [37]. The detailed phytochemical investigation of the saponins from *A. hippocastanum* was

conducted by Yoshikawa's group [38-40]. A total of 12 saponins were isolated from the seeds of this species. The structures of these saponins were determined by extensive NMR and HRMS analysis as well as chemical degradation. These saponins are escins Ia (21) [38, 39], Ib (22) [38, 39], IIa (23) [38, 39], IIb (24) [38, 39], IIIa (25) [38, 39], IIIb (26) [40], IV (27) [40], V (34) [40], and VI (35) [40] and isoescins Ia (43) [40], Ib (44) [40], and V (49) [40].

The seeds of *A. turbinata* also contain rich triterpenoid saponins as the main components. A number of investigations on the seeds of Japanese horse chestnut resulted in the isolation and structure determination of 16 polyhydroxylated triterpenoid saponins. These saponins are escins Ia (21) [41], Ib (22) [41], IIa (23) [42], and IIb (24) [42]; deacetylescins Ia (37), Ib (38), IIa (39), and IIb (40) [43]; desacylescins I (41) and II (42) [43]; escin IVc (28) [44]; isoescin Ia (43) [44], Ib (44) [44], VIa (50) [45], VIIa (51) [45], and VIIIa (52) [45].

Of the section *Calothyrsus*, *A. chinensis* var. *chinensis* has been well investigated. The triterpenoid saponins, occurring as a complex mixture in the seeds of *A. chinensis* var. *chinensis*, had not been investigated until 1999, when two groups of researchers reported a number of novel saponins. The first group reported four pair of new geometrical isomer saponins from the plant, named aesculosides A-H (53-56, 63-66), with two pair of known compounds, escins Ia (21), and Ib (22) and isoescins Ia (43) and Ib (44) [46]. The second group reported four new saponins escins IVc (28), IVd (29), IVe (30), and IVf (31) [47]. The structures of these novel saponins were determined by extensive NMR, HRMS, and x-ray analyses, as well as chemical degradation. Later, from 2001 to 2004, more *Aesculus* polyhydroxylated triterpenoid saponins were isolated and identified from the seeds of *A. chinensis* var. *chinensis*. These saponins include escins IVg (32), IVh (33), and VIb (36) [48]; desacylescins I (41) [48]; isoescins IIa (45), IIb (46), IIIa (47), and IIIb (48) [49]; and aesculoside A (58) [50]. Escins Ia (21), Ib (22), IIIa (25), isoescins Ia (43), Ib (44), IIa (45), IIb (46), and IIIa (47) were isolated and identified from *A. chinensis* var. *wilsonii* [51]. *Aesculus chinensis* Bunge var. *chekiangensis* (Hu & W.P. Fang) W.P. Fang was described based on cultivated plants and is now recognized as the synonym of *A. chinensis* var. *wilsonii* [14]. Its seeds, also called "Sha Luo Zhi" in



No.	Name	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
21	Escin Ia	H	Tig	Ac	OH	H	Glc-p
22	Escin Ib	H	Ang	Ac	OH	H	Glc-p
23	Escin IIa	H	Tig	Ac	OH	H	Xyl-p
24	Escin IIb	H	Ang	Ac	OH	H	Xyl-p
25	Escin IIIa	H	Tig	Ac	H	H	Gal-p
26	Escin IIIb	H	Ang	Ac	H	H	Gal-p
27	Escin IV	H	Ac	Ac	OH	H	Glc-p
28	Escin IVc	H	H	Tig	OH	Ac	Glc-p
29	Escin IVd	H	H	Ang	OH	Ac	Glc-p
30	Escin IVe	H	H	H	OH	Tig	Glc-p
31	Escin IVf	H	H	H	OH	Ang	Glc-p
32	Escin IVg	H	H	Tig	OH	H	Glc-p
33	Escin IVh	H	H	Ang	OH	H	Glc-p
34	Escin V	H	MP	Ac	OH	H	Glc-p
35	Escin VI	H	MB	Ac	OH	H	Glc-p
36	Escin VIb	Ang	Ac	H	OH	H	Glc-p
37	Deacetylescins Ia	H	Tig	H	OH	H	Glc-p
38	Deacetylescins Ib	H	Ang	H	OH	H	Glc-p
39	Deacetylescins IIa	H	Tig	H	OH	H	Xyl-p
40	Deacetylescins IIb	H	Ang	H	OH	H	Xyl-p
41	Desacylescin I	H	H	H	OH	H	Glc-p
42	Desacylescin II	H	H	H	OH	H	Xyl-p
43	Isoescin Ia	H	Tig	H	OH	Ac	Glc-p

Compound 21-62. contd....

No.	Name	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
44	Isoescsin Ib	H	Ang	H	OH	Ac	Glc- <i>p</i>
45	Isoescsin IIa	H	Tig	H	OH	Ac	Xyl- <i>p</i>
46	Isoescsin IIb	H	Ang	H	OH	Ac	Xyl- <i>p</i>
47	Isoescsin IIIa	H	Tig	H	H	Ac	Gal- <i>p</i>
48	Isoescsin IIIb	H	Ang	H	H	Ac	Gal- <i>p</i>
49	Isoescsin V	H	MP	H	OH	Ac	Glc- <i>p</i>
50	Isoescsin VIa	H	MB	H	OH	Ac	Glc- <i>p</i>
51	Isoescsin VIIa	H	Tig	H	OH	Ac	Gal- <i>p</i>
52	Isoescsin VIIIa	H	Ang	H	H	Ac	Glc- <i>p</i>
53	Aesculoside A	H	Tig	H	OH	H	Glc- <i>p</i>
54	Aesculoside B	H	Ang	H	OH	H	Glc- <i>p</i>
55	Aesculoside C	H	Tig	Tig	OH	H	Glc- <i>p</i>
56	Aesculoside D	H	Tig	Ang	OH	H	Glc- <i>p</i>
57		H	Ang	Ang	OH	H	Glc- <i>p</i>
58	Aesculoside A	H	Ac	H	OH	Ac	Glc- <i>p</i>
59	Aesculoside B	H	H	H	OH	H	Glc- <i>p</i>
60	Assamicin III	H	4- <i>O</i> -ang-6-deoxy-glc- <i>p</i>	H	OH	Ac	Glc- <i>p</i>
61	Assamicin IV	H	4- <i>O</i> -ang-6-deoxy-glc- <i>p</i>	H	OH	H	Glc- <i>p</i>
62	Assamicin VI	H	3,4- <i>O</i> -diang-6-deoxy-glc- <i>p</i>	H	OH	Ac	Glc- <i>p</i>

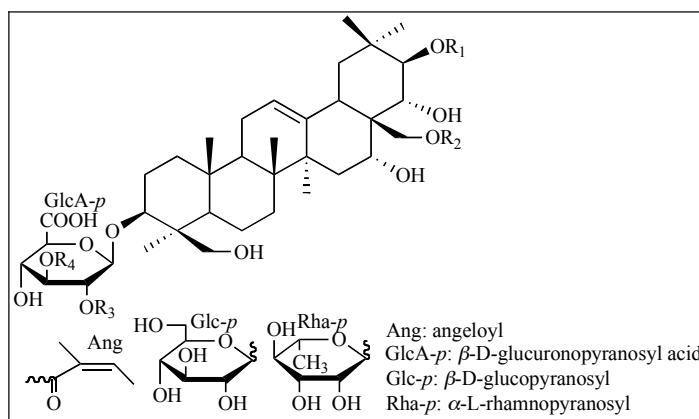
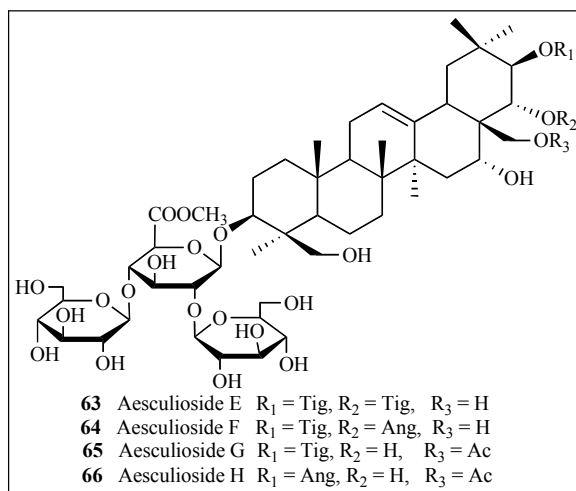
traditional Chinese medicines, have the same medicinal value [11]. A number of saponins including escins Ia (21) [52], Ib (22) [52], IVc (28) [52], IVd (29) [52], IVe (30) [53], IVh (33) [53], aesculoside A (deacetylescins Ib, 38) [53], isoescsins Ia (43) [52] and Ib (44) [52] were isolated and identified from the seeds of this variety. Except aesculoside A (38), all saponins isolated from *A. chinensis* var. *chekiangensis* were also found in the seeds of *A. chinensis* var. *chinensis*.

The saponins from other species of the section *Calothyrsus* were also reported. Interestingly, some unique saponins including assamicins I (67) [54], II (68) [54], III (60) [55], IV (61) [55], V (69) [56], VI (62) [57], VII (70) [57], and VIII (71) [57] were isolated and identified from *A. assamica*, together with a known saponin isoescsin Ib (44). In addition, aesculosides A (deacetylescins Ib, 38) [58], B (59) [59], and 3-*O*-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)]-β-D-glucuronopyranosyl-21,22-*O*-diangeloyl-3β,16α,21β,22α,24β,28-heptahydroxyolean-12-ene (57) [56] were isolated from *A. indica*.

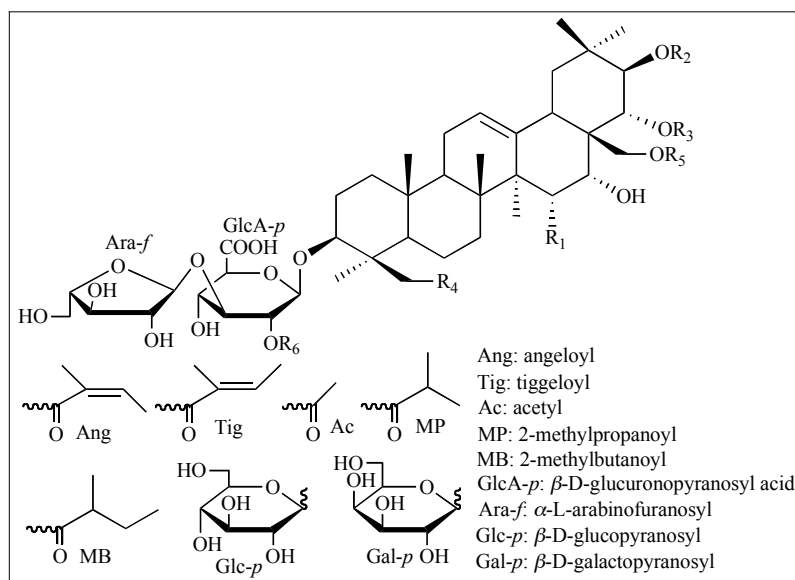
The investigation of saponins from section *Pavia* is limited to *A. pavia*. Recently, a detailed phytochemical investigation of the saponins occurring as a very complex mixture from the fruits of *A. pavia* located in Nacogdoches (Texas, USA) resulted in the isolation and discovery of 25 new saponins, named as aesculosides Ia–Ie (72–76), IIa–IIk (77–

87), IIIa–IIIf (88–93), and IVa–IVc (95, 97, 99), together with three known saponins 3-*O*-[β-D-galactopyranosyl-(1→2)]-[α-L-arabinofuranosyl-(1→3)]-β-D-glucuronopyranosyl-21,22-*O*-diangeloyl-3β,15α,16α,21β,22α, 28-hexahydroxyolean-12-ene (*xanifolia*-Y, 94), 3-*O*-[β-D-glucopyranosyl-(1→2)]-[α-L-arabinofuranosyl-(1→3)]-β-D-glucuronopyranosyl-21,22-*O*-diangeloyl-3β,16α,21β,22α,24β,28-hexahydroxyolean-12-ene (96), and 3-*O*-[β-D-galactopyranosyl-(1→2)]-[α-L-arabinofuranosyl-(1→3)]-β-D-glucuronopyranosyl-21,22-*O*-diangeloyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene (98) [16, 61].

The polyhydroxylated triterpenoid saponins from the genus *Aesculus* share a common oligosaccharide chain with a glucuronopyranosyl unit attached to C-3 of the aglycone, although the components and sequences of the oligosaccharides are different. Interestingly the type of the monosaccharide linked to C-2 of the glucuronopyranosyl unit in the oligosaccharide chain for the most of saponins (21–50, 53–68) is related to the substituent at C-24 of aglycone. Specifically, the saponins possessed the aglycones of 24-hydroxy-R₁-barrigenol (3) or protoaescigenin (5) with a hydroxyl group at C-24 had a glucopyranosyl or xylopyranosyl unit attached to C-2 of the glucuronopyranosyl unit while a galactopyranosyl unit linked to C-2 of the glucuronopyranosyl unit was found in the saponins with the aglycones of R₁-barrigenol (4) or barringtogenol-C (6) without the hydroxyl group at C-24. Isoescsins VIIa (51), VIIIa (52), and assamicin



No.	Name	R ₁	R ₂	R ₃	R ₄
67	Assamicin I	Ang	Ac	Glc- <i>p</i>	Rha- <i>p</i>
68	Assamicin II	3,4- <i>O</i> -diangeloyl-6-deoxy-glc- <i>p</i>	Ac	D-Glc- <i>p</i>	Rha- <i>p</i>
69	Assamicin V	Ang	H	H	Rha- <i>p</i>
70	Assamicin VII	Ang	H	Rha- <i>p</i>	Glc- <i>p</i>
71	Assamicin VIII	Ang	H	H	Glc- <i>p</i>



Compounds 72-99. contd....

No.	Name	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
72	Aesculoside Ia	OH	H	H	H	H	Gal- <i>p</i>
73	Aesculoside Ib	OH	H	H	OH	H	Glc- <i>p</i>
74	Aesculoside Ic	H	H	H	H	H	Gal- <i>p</i>
75	Aesculoside Id	H	H	H	OH	H	Glc- <i>p</i>
76	Aesculoside Ie	H	H	H	OH	H	H
77	Aesculoside IIa	OH	Ang	H	H	H	Gal- <i>p</i>
78	Aesculoside IIb	H	Ang	H	OH	H	Glc- <i>p</i>
79	Aesculoside IIc	H	Ang	H	H	H	Gal- <i>p</i>
80	Aesculoside IId	H	Ang	H	OH	H	H
81	Aesculoside IIe	OH	Tig	Ac	OH	H	Glc- <i>p</i>
82	Aesculoside IIIf	OH	Tig	Ac	H	H	Gal- <i>p</i>
83	Aesculoside IIg	OH	Ang	Ac	H	H	Gal- <i>p</i>
84	Aesculoside IIh	H	Tig	Ac	OH	H	Glc- <i>p</i>
85	Aesculoside IIi	H	Ang	Ac	OH	H	Glc- <i>p</i>
86	Aesculoside IIj	H	Tig	Ac	H	H	Gal- <i>p</i>
87	Aesculoside IIk	H	Ang	Ac	H	H	Gal- <i>p</i>
88	Aesculoside IIIa	H	Ang	H	OH	Ac	Glc- <i>p</i>
89	Aesculoside IIIb	OH	Ang	MP	OH	H	Glc- <i>p</i>
90	Aesculoside IIIc	OH	Tig	MP	H	H	Gal- <i>p</i>
91	Aesculoside IIId	OH	Tig	Tig	OH	H	Glc- <i>p</i>
92	Aesculoside IIIe	OH	Tig	Ang	OH	H	Glc- <i>p</i>
93	Aesculoside IIIIf	OH	Tig	Tig	H	H	Gal- <i>p</i>
94	Xanifolia-Y	OH	Ang	Ang	H	H	Gal- <i>p</i>
95	Aesculoside IVa	OH	Ang	MB	H	H	Gal- <i>p</i>
96		H	Ang	Ang	OH	H	Glc- <i>p</i>
97	Aesculoside IVb	H	Ang	MB	OH	H	Glc- <i>p</i>
98		H	Ang	Ang	H	H	Gal- <i>p</i>
99	Aesculoside IVc	H	Ang	MB	H	H	Gal- <i>p</i>

VII (70) are the only three saponins that exhibit exceptions to this structural configuration.

The saponins from the five Eurasian taxa, including *A. hippocastanum*, *A. chinensis* var. *chinensis*, *A. chinensis* var. *wilsonii*, *A. turbinata*, and *A. indica* discussed above (Table 2), were structurally based on two aglycones of protoaescigenin (5) and barringtonenol-C (6) with a trisaccharide chain at C-3 position of the aglycone. This trisaccharide chain has a glucopyranosyl unit attached to C-4 of the glucuronopyranosyl unit, which is linked to C-3 of the aglycone. The aglycones of the saponins from the North America species *A. pavia* are more variable with four different aglycones, in-

cluding 24-hydroxy-R₁-barrigenol (3), R₁-barrigenol-C (4), protoaescigenin (5) and barringtonenol-C (6). These saponins (72-99) from *A. pavia* have an oligosaccharide chain at C-3 of the aglycone with an arabinofuranosyl unit affixed to C-3 of the glucuronopyranosyl unit instead. This significant chemical difference represents a variable chemotaxonomic feature and may be the basis for different medicinal uses between North American and Eurasian *Aesculus* species. This result also supports a conclusion drawn from phenotypic and DNA sequence analyses that eastern North American species of *Aesculus* represent a different evolutionary lineage that diverged early from their Eurasian counterparts [1, 4, 13].

It is also worth mentioning that assamicins I (67), II (68), III (60), IV (61), V (69), VI (62), VII (70), and VIII (71) from *A. assamica* have different components and sequences of the oligosaccharide chain at C-3 from the saponins isolated from the other *Aesculus* species. Some of assamicin saponins (60, 61, 62, and 68) have two oligosaccharide moieties attached to C-3 and C-21 of the aglycone.

Flavonoids

Flavonoids and their derivatives are also one of the main components of the genus *Aesculus*. A total of 49 flavonoids including flavonols and their glycosides (100-126), flavanones, and flavanone derivatives (127-148) were isolated and identified from the seeds of *Aesculus*. The glycosides of quercetin (100) and kaempferal (116) with an oligosaccharide chain at C-3 or two oligosaccharide chains at C-3 and C-3' are types of flavonol glycosides while flavanone derivatives are characterized as one of two types of proanthocyanidin and polymerized epicatechin.

A few early investigations led to the isolation and identification of rutin (111), quercitrin (113), and astragaline (122) from *A. indica* [62, 63] and quercetin (100) and kaempferal (116) from *A. assamica* [33].

The flavonols and their glycosides from the seeds of *A. hippocastanum* have been well documented. Two investigations on the seeds of this species resulted in the isolation and structural determination of 15 flavonols and their glycosides [64, 65]. These compounds are quercetin 3-*O*-[β -D-xylopyranosyl-(1 \rightarrow 2)]-[*O*- β -D-glucopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranosyl-3'-*O*- β -D-glucopyranoside (101), tamarixetin 3-*O*-[β -D-xylopyranosyl-(1 \rightarrow 2)]-[*O*- β -D-glucopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranoside (102), quercetin 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)-*O*- β -D-glucopyranosyl-3'-*O*- β -D-glucopyranoside (104), quercetin 3-*O*-[β -D-xylopyranosyl-(1 \rightarrow 2)]-[*O*- β -D-glucopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranoside (106), quercetin 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)-*O*- β -D-glucopyranosyl-(1 \rightarrow 3)-*O*- β -D-glucopyranoside (107), tamarixetin 3-*O*-[β -D-xylopyranosyl-(1 \rightarrow 2)]-[*O*- β -D-glucopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranoside (108), quercetin 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)-*O*- β -D-glucopyranoside (109), quercetin 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 4)-*O*- α -L-rhamnopyranoside (multinoside A, 110), quercetin 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)-*O*- β -D-glucopyranosyl-3'-*O*-[nicotynoyl-(1 \rightarrow 6)]- β -D-glucopyranoside (124), quercetin 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)-*O*- β -D-glucopyranosyl-3'-*O*-[indolin-2-on-3-hydroxy-3-acetyl-(1 \rightarrow 6)]- β -D-glucopyranoside (125), quercetin 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)-*O*- β -D-glucopyranosyl-3'-*O*-[indolin-2-on-3-acetyl-(1 \rightarrow 6)]- β -D-glucopyranoside (126), kaempferal 3-*O*-[β -D-xylopyranosyl-(1 \rightarrow 2)]-[*O*- β -D-glucopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranoside (118), kaempferal 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)-*O*- β -D-glucopyranosyl-(1 \rightarrow 3)-*O*- β -D-glucopyranoside (119), kaempferal 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)-*O*- β -D-glucopyranoside (leucoside, 120), and kaempferal 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 4)-*O*- α -L-rhamnopyranoside (multiflorine B, 121).

The seeds of *A. chinensis* var. *chinensis* are rich in flavonoids. A number of flavonoid glycosides were isolated from the seeds of this species. These flavonoids were identified as quercetin 3-*O*-[β -D-xylopyranosyl-(1 \rightarrow 2)]-[α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranosyl-3'- β -D-glu-

copyranoside (aescuflavoside, 103), quercetin 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl-3'- β -D-glucopyranoside (104), tamarixetin 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl-3'-*O*- β -D-glucopyranoside (aescuflavoside A, 105), quercetin 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (109), quercetin 3-*O*- β -D-glucopyranoside (isoquercitrin, 112), kaempferal 3-*O*-[β -D-xylopyranosyl-(1 \rightarrow 2)]-[β -D-glucopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranoside (117), kaempferal 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (leucoside, 120), kaempferal 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranoside (multiflorine B, 121), kaempferal-3-*O*- β -D-glucopyranoside (astragaline, 122), and kaempferal-3-*O*- β -D-galactopyranoside (trifoline, 123) [66].

Three known flavonoid glycosides quercetin 3-*O*- α -L-rhamnopyranoside (quercitrin, 113), quercetin 3-*O*- α -L-arabinopyranoside (114), and isorhamnetin 3-*O*- α -L-arabinoside (distichin, 115) were recently isolated and identified from the leaves of *A. pavia*, grown in Italy [67].

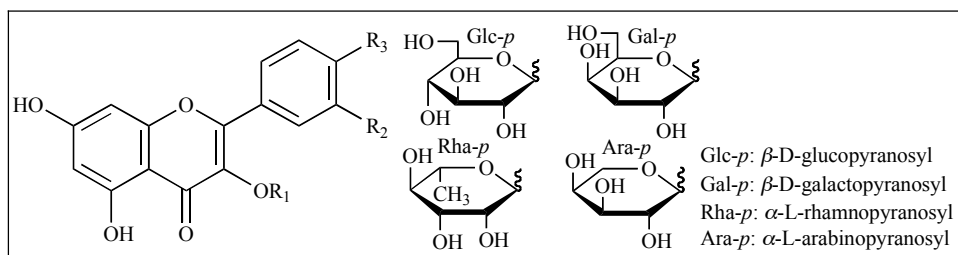
In addition, some flavanone derivatives were also identified from *Aesculus* species. These compounds include (-)-epicatechin (127) from the seeds of *A. californica* [68]; proanthocyanidins A-2 (131), A-4 (132), A-6 (134), A-7 (135), B-2 (130), B-5 (133), C-1 (137), epicatechin-(4 β \rightarrow 6)-epicatechin-(4 β \rightarrow 6)-epicatechin (139), cinnamtannins B₁ (136), B₂ (138), aesculitannins A (140), B (141), C (142), D (143), E (144), F (145), G (146) [69], and leucocyanidine (128) [70] from the seeds of *A. hippocastanum*; and three docyl sulfide derivatives of (-)-epicatechin (129) and proanthocyanidins (147, 148) from the seeds of *A. turbinata* [19].

Coumarins

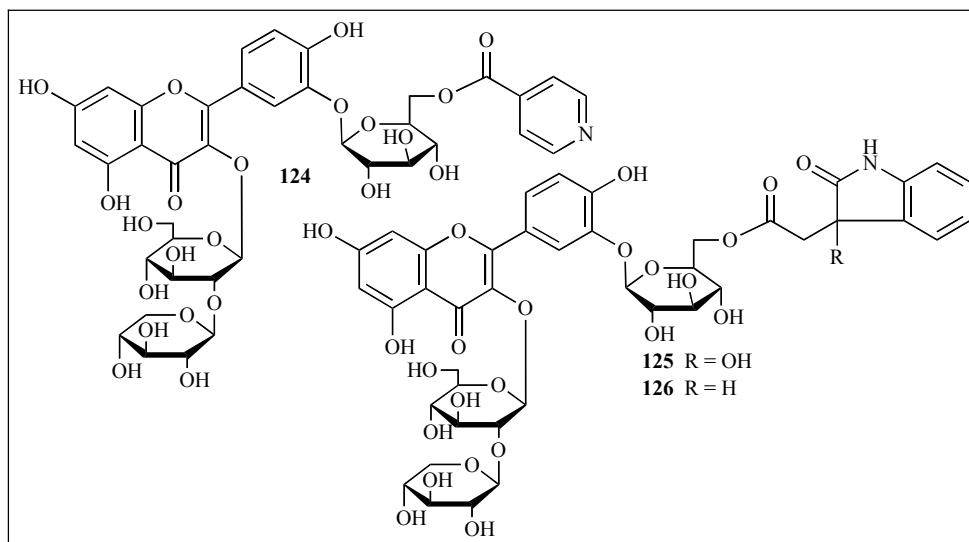
Coumarins isolated from *Aesculus* usually have simple structures. The main coumarins are esculetin (150), fraxetin (151), fraxin (156), and aesculin (157) from *A. turbinata* [71, 72]; umbelliferone (149), esculetin (150), scopoletin (152), isoscooletin (153), skimin (154), chikhorin (155), fraxin (156), aesculin (157), scopolin (158), and isoscopolin (159) from *A. hippocastanum* [73]; and scopolin (158) and 5-methoxyscopolin (160) from *A. pavia* [61]. Recently, a new prenylated coumarin with antifungal activity, pavietin (161), was isolated and identified from the leaves of *A. pavia* [67].

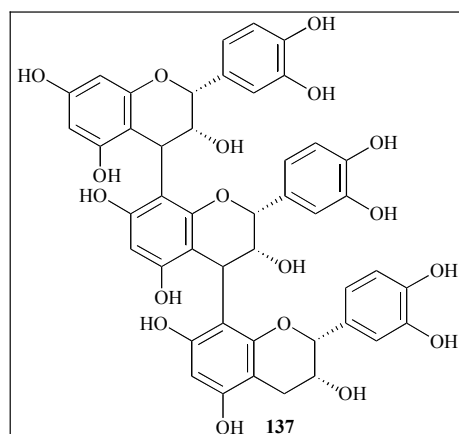
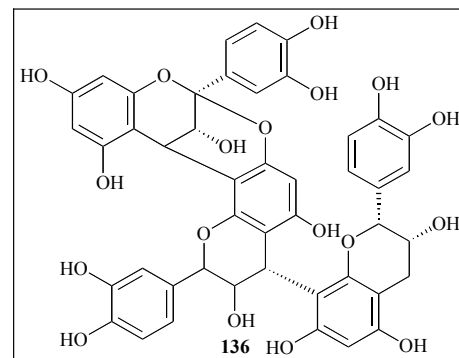
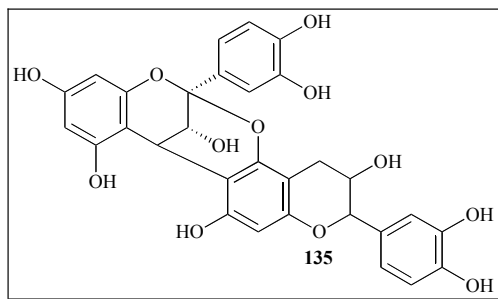
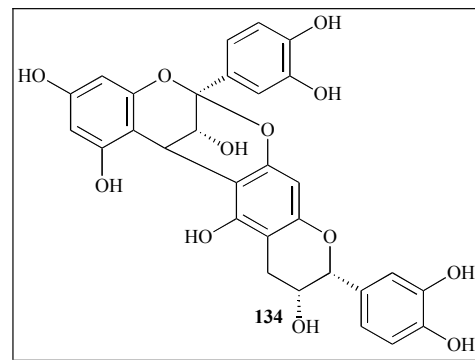
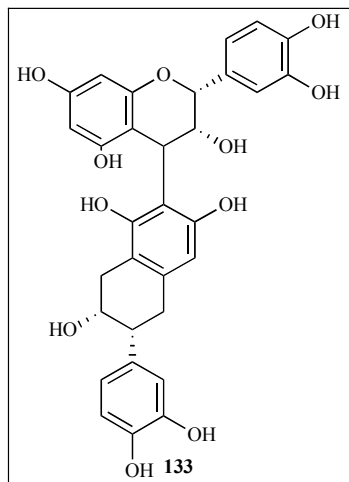
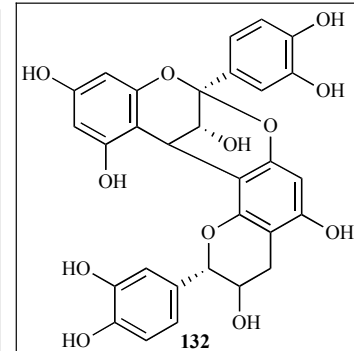
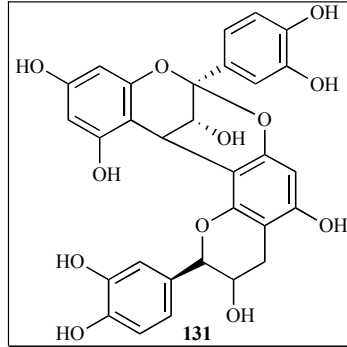
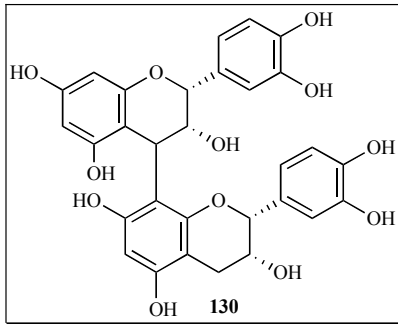
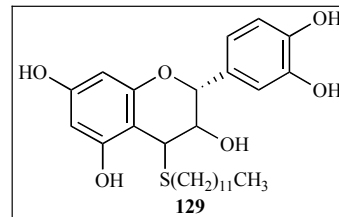
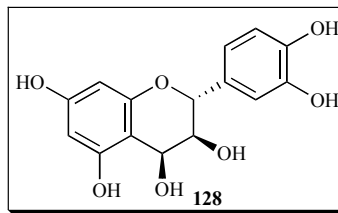
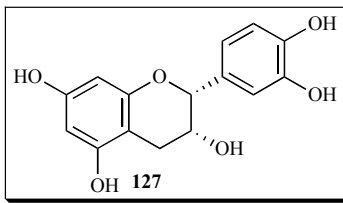
Carotenoids

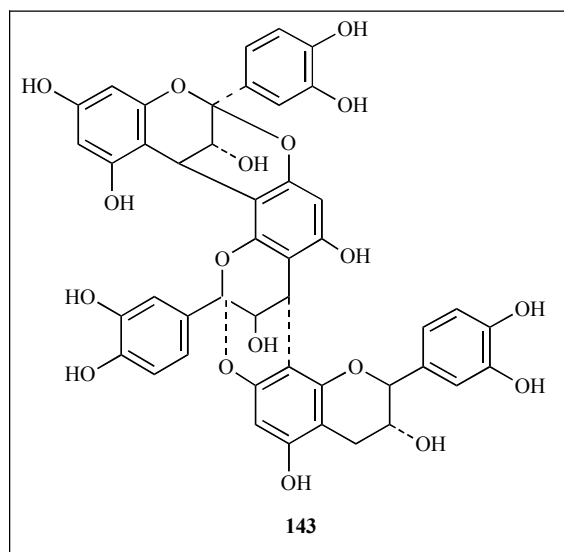
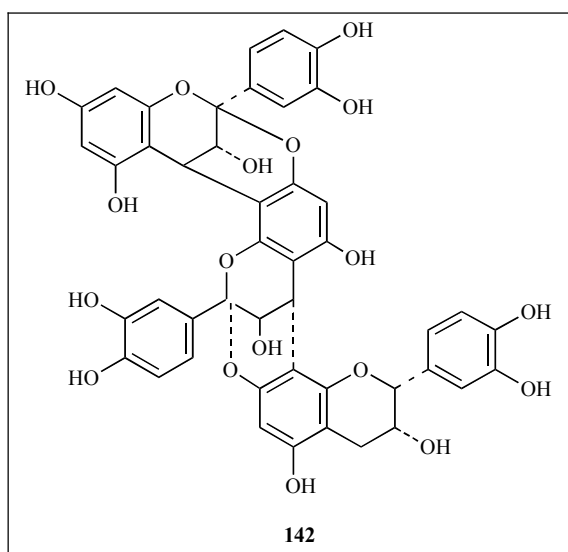
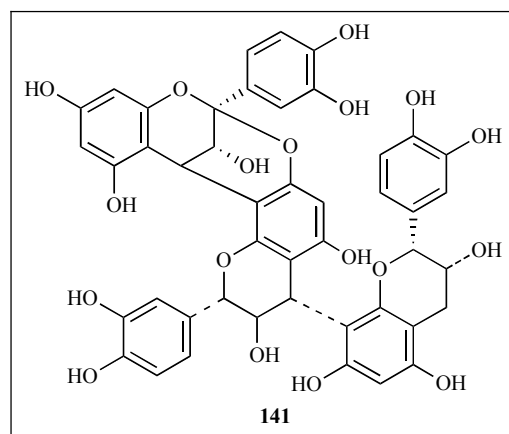
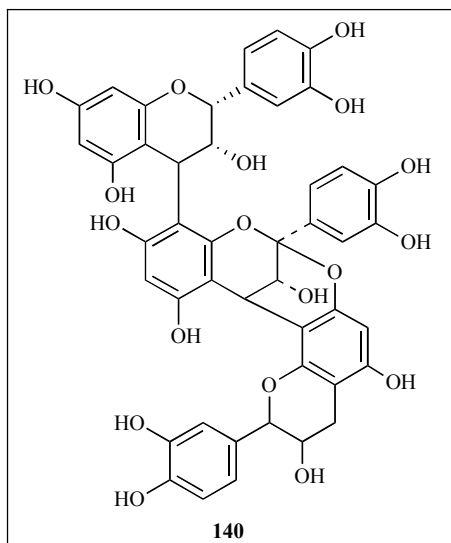
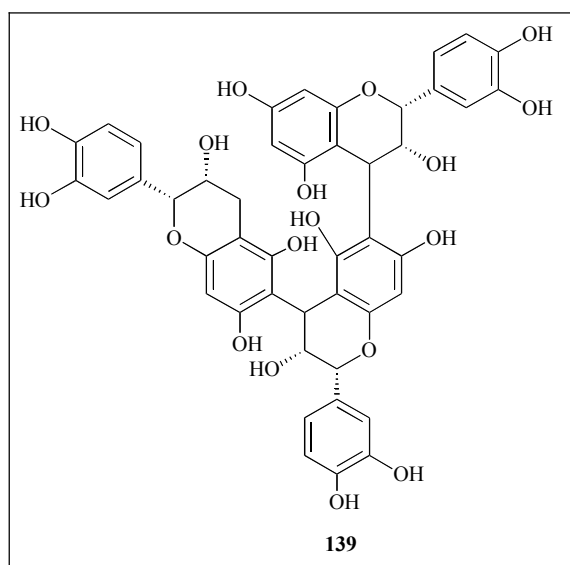
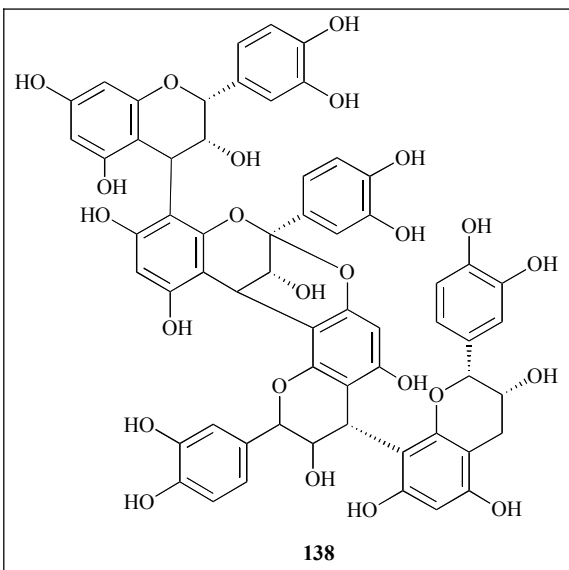
Twenty-one carotenoids have been identified from *Aesculus*. An early investigation on the leaves and pollen of the three species *A. turbinata*, *A. pavia*, and *A. parviflora* by HPLC analysis indicated the presence of seven carotenoids β -carotene (162), zeaxanthin (163), α -carotene (167), lutein (168), capsanthin (176), capsanthin 5,6-epoxide (177), and capsorubin (178) [74]. A high concentration of α -carotene was found in the leaves and keto hydroxyl carotenoids with a pentanuclear ring structure (capsanthin, capsanthin 5,6-epoxide, and capsorubin) were identified in the pollen. These keto hydroxyl carotenoids were proposed to have special chemotaxonomic significance for the genus *Aesculus*. Later, in 2000, 16 carotene derivatives were detected in buds, pollen, and petals of *A. hippocastanum* and *A. pavia* by HPLC analysis with a diode array detector utilizing authentic samples as the references. These carotenoids are β -carotene (162), β -cryptoxanthin (164), violaxanthin (165), 9-cis-

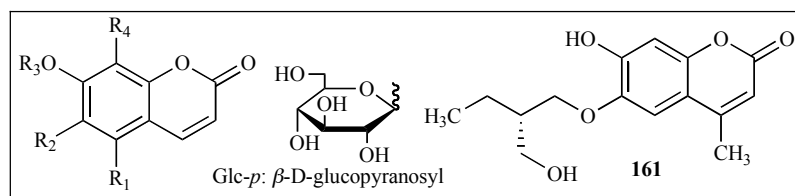
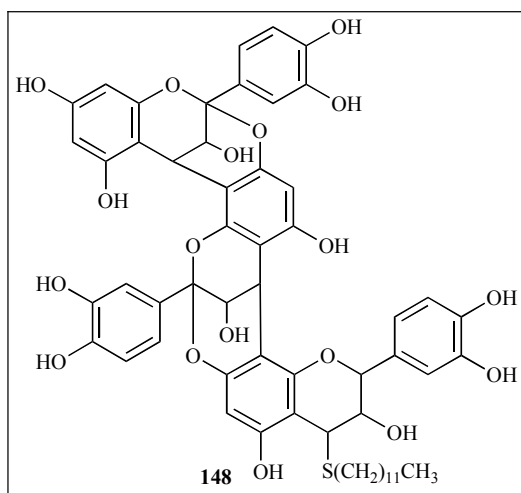
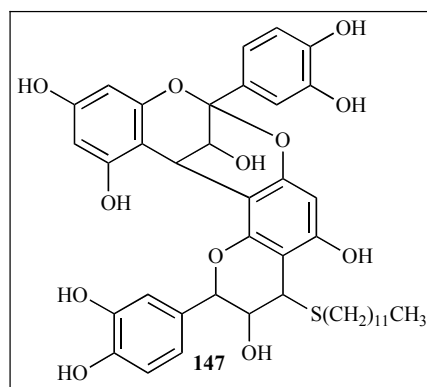
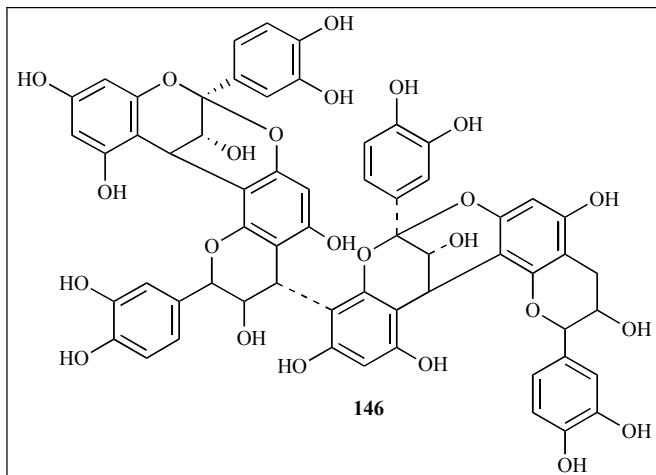
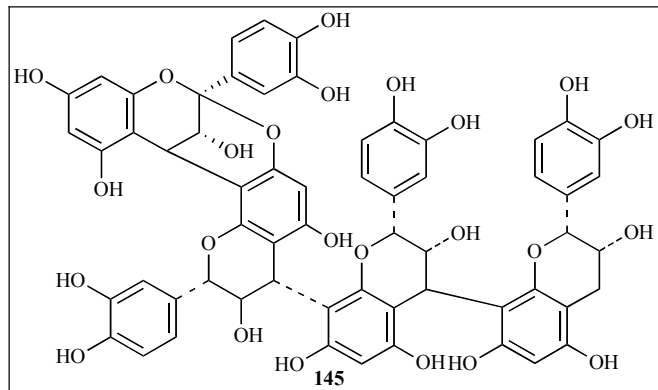
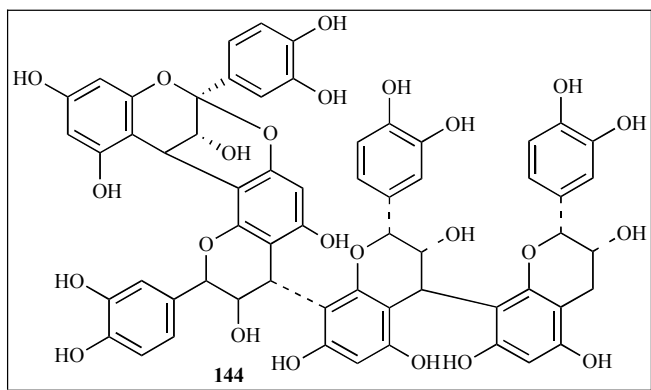


Number	Name	R ₁	R ₂	R ₃
100	Quercetin	H	OH	OH
101		[Xyl (1→2)] [Glc (1→3)] Glc	O-Glc	OH
102		[Xyl (1→2)] [Glc (1→3)] Glc	O-Glc	OCH ₃
103	Aescuflavoside	[Xyl (1→2)] [Rha (1→6)] Glc	O-Glc	OH
104		Xyl (1→2) Glc	O-Glc	OH
105	Aescuflavoside A	Xyl (1→2) Glc	O-Glc	OCH ₃
106		[Xyl (1→2)][Glc (1→3)] Glc	OH	OH
107		Xyl (1→2) Glc (1→3) Glc	OH	OH
108		[Xyl (1→2)] [Glc (1→3)] Glc	OH	OCH ₃
109	Quercetin 3-O-sambubioside	Xyl (1→2) Glc	OH	OH
110	Muitinoside A	Glc (1→4) Rha	OH	OH
111	Rutin	Rha (1→2) Glc	OH	OH
112	Isoquercitrin	Glc	OH	OH
113	Quercitrin	Rha	OH	OH
114	Quercetin 3-ara	Ara	OH	OH
115	Distichin	Ara	CH ₃	OH
116	Kaempferal	H	H	OH
117		[Xyl (1→2)] [Glc (1→6)] Glc	H	OH
118		[Xyl (1→2)] [Glc (1→3)] Glc	H	OH
119		Xyl (1→2) Glc (1→3) Glc	H	OH
120	Leucoside	Xyl (1→2) Glc	H	OH
121	Multiflorin B	Glc (1→4) Rha	H	OH
122	Astragalinalin	Glc	H	OH
123	Trifolin	Gal	H	OH





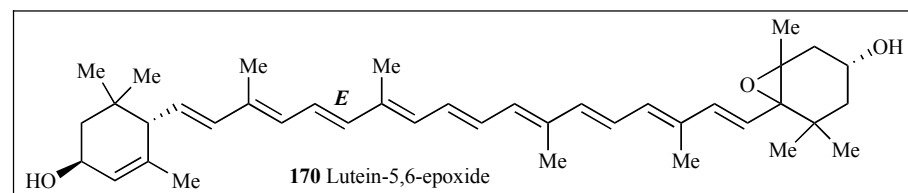
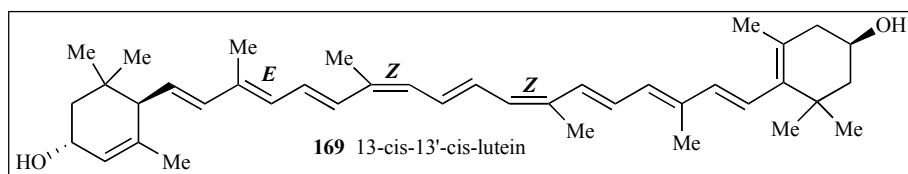
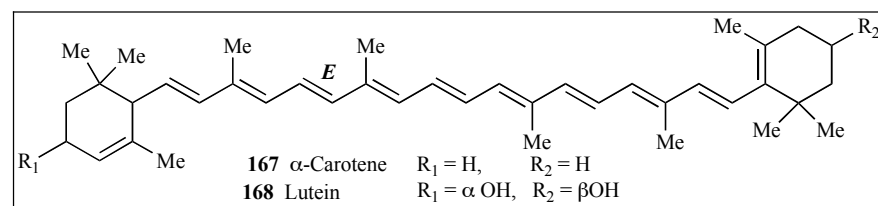
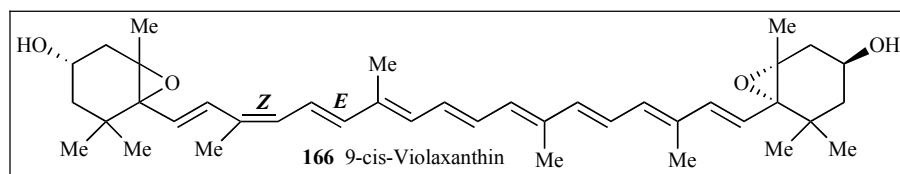
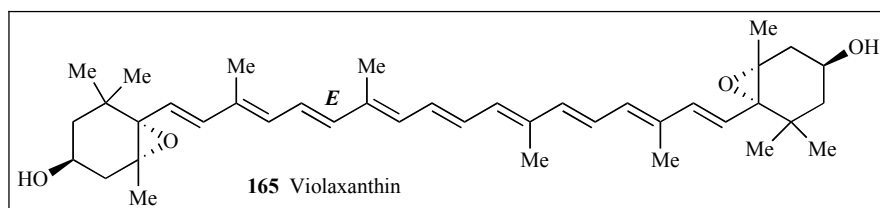
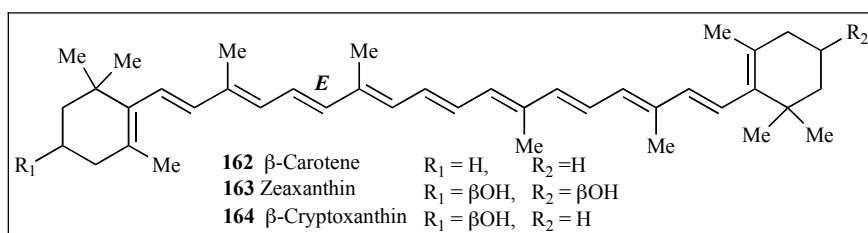


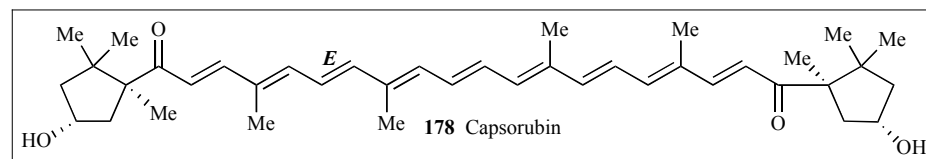
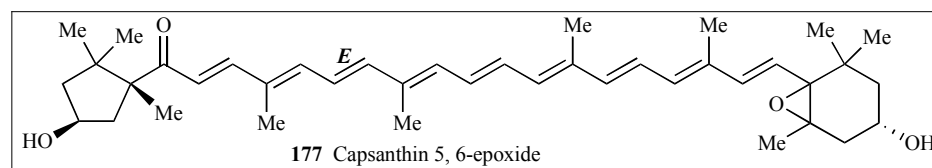
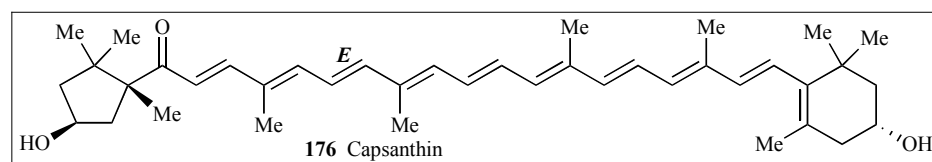
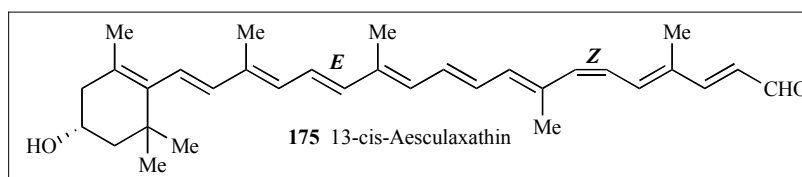
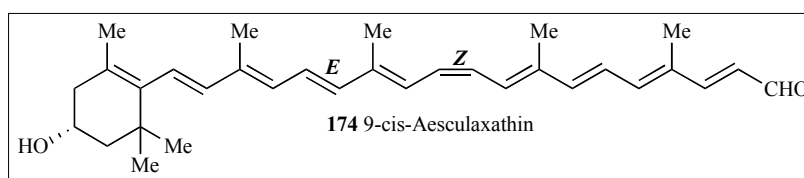
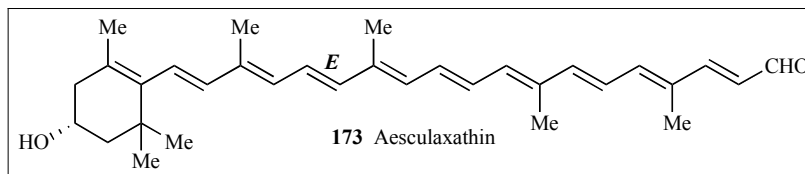
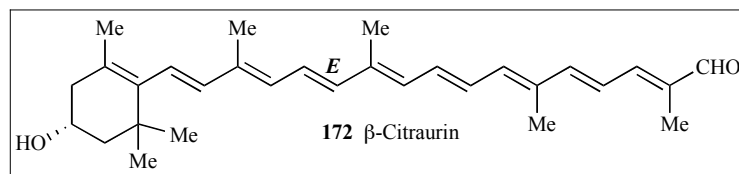
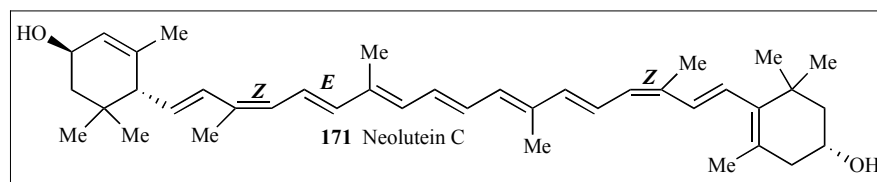


No.	Name	R ₁	R ₂	R ₃	R ₄
149	Umbelliferone	H	H	H	H
150	Esculetin	H	OH	H	H
151	Fraxetin	H	OCH ₃	H	OH

Compounds 149-160 contd....

152	Scopoletin	H	OCH ₃	OH	H
153	Isoscapoletin	H	OH	OCH ₃	H
154	Skimin	H	H	Glc- <i>p</i>	H
155	Chikhorin	H	OH	Glc- <i>p</i>	H
156	Fraxin	H	OCH ₃	H	<i>O</i> -Glc- <i>p</i>
157	Aesculin	H	<i>O</i> -Glc- <i>p</i>	H	H
158	Scopolin	H	OCH ₃	Glc- <i>p</i>	H
159	Isoscopolin	H	Glc- <i>p</i>	OCH ₃	H
160	5-Methoxyscopolin	OCH ₃	OCH ₃	Glc- <i>p</i>	H



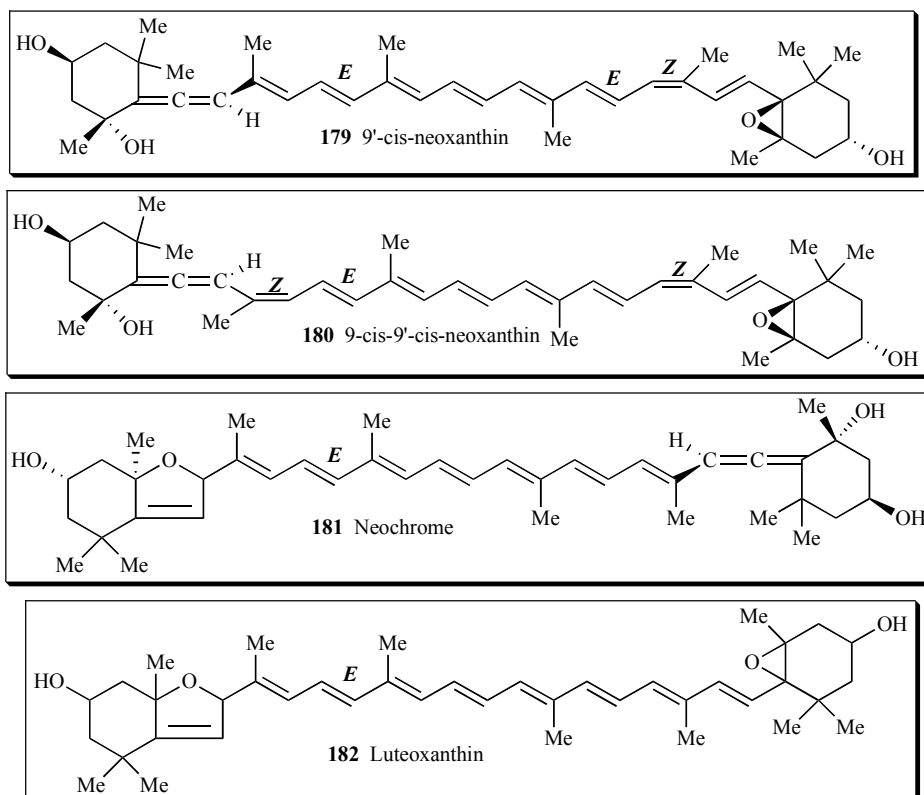


violaxanthin (166), lutein (168), 13-cis-13'-cis-lutein (169), lutein 5, 6-epoxide (170), neolutein C (171), β -citraurin (172), aesculaxanthin (173), 9-cis-aesculaxanthin (174), 13-cis-aesculaxanthin (175), 9'-cis-neoxanthin (179), 9-cis-9'-cis-neoxanthin (180), neochrome (181), and luteoxanthin (182) [75].

Long Fatty Chain Compounds

The seeds of *Aesculus* also contain a number of long fatty chain compounds. These compounds include lauric acid

(183), myristic acid (184), palmitic acid (185), stearic acid (189), arachic acid (190), and oleic acid (191) from *A. hippocastanum* and *A. indica* [76]; linoleic acid (192) from *A. hippocastanum* [70]; oleic acid (191), linoleic acid (192), and linolenic acid (193) from the seeds of *A. turbinata* [20]; heptadecanoic acid (186), methyl heptadecanoate (187), ethyl heptadecanoate (188), methyl octadec-(13Z)-enoate (194), ethyl octadec-(13Z)-enoate (195) [77], palmitone (197), n-hentriacontanol (198), and n-hentriacontane (199)



from *A. indica* [78]; and tianshic acid (**196**) from *A. chinensis* var. *wilsonii* [79].

Other Compounds

Some other classes of components were isolated and identified from some species of *Aesculus*. These compounds are ester of indole-3-acetic acid and myo-inositol (**200**), ester of indole-3-acetic acid and the disaccharide rutinose (**201**), three cyclopropane amino acids of cis- α -(carboxycyclopropyl)-glycine (**202**), trans- α -(carboxycyclopropyl)-glycine (**203**), and exo-3,4-methanoproline (**204**) from *A. parviflora* [80, 81]; 2-amino-4-methylhexanoic acid (**205**), 2-amino-4-methylhex-4-enoic acid (**206**), *N*-(1-carboxy-3-methyl-3-pentenyl)-glutamine (**207**), α -amino- β -methyl-2-methylene-cyclopropanepropionic acid (**208**), 2-amino-6-hydroxy-4-methylhex-4-enoic acid (**209**), and arbutin (**210**) from *A. californica* [68, 82]; and wilsonic acid (**211**), *N*-acetyl glutamic acid (**212**), and fumaric acid (**213**) from *A. chinensis* var. *wilsonii* [79, 83].

BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES

Anti-inflammatory Activities

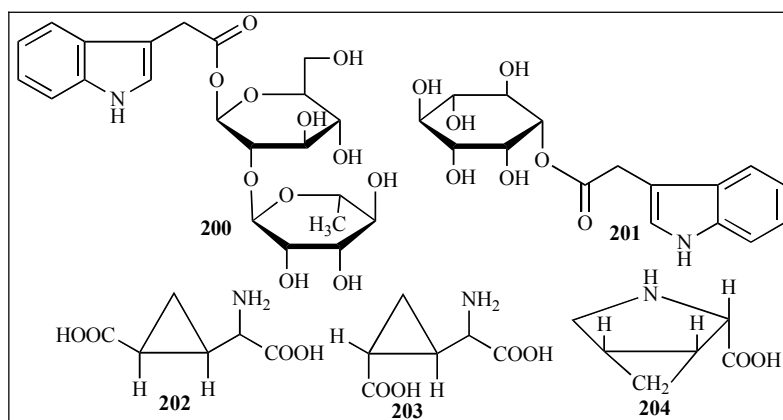
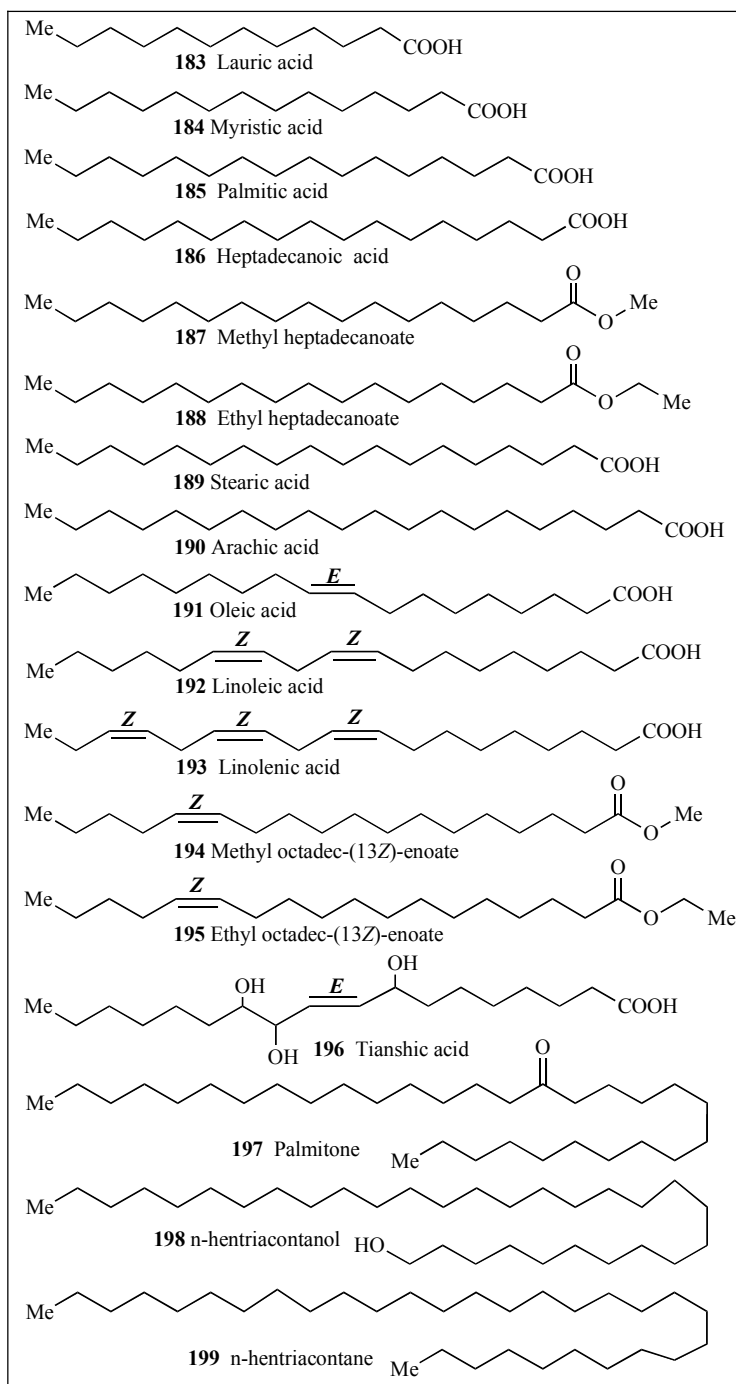
The therapeutic benefits of HCSE and aescin for the treatment of chronic venous insufficiency, hemorrhoids, and postoperative edema are due to their significant anti-inflammatory properties, which were demonstrated in animal models [5, 50, 84, 85]. The anti-inflammatory activities of HCSE or aescin are related to the molecular mechanism of the agents, which include the improved entry of ions into channels [86] thus raising venous tension [87], the release of prostaglandin-F_{2a} from veins [88], the release of inflamma-

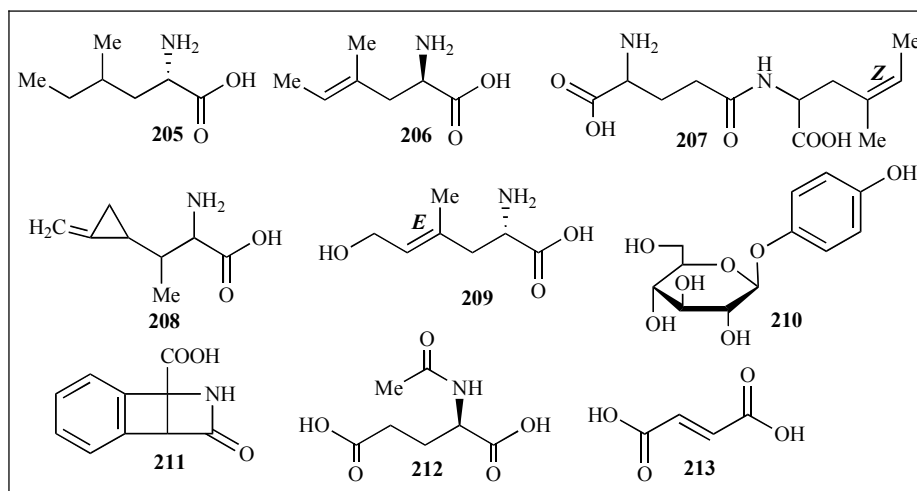
tory mediators by decreasing leukocyte activation and adhesiveness [84, 89], the antagonism to the pro-inflammatory 5-HT receptors and histamine [90], and the decrease in the activity of tissue hyaluronidase [91].

Individual compounds isolated and identified from the genus *Aesculus* also showed anti-inflammatory properties. Escins Ia (**21**), Ib (**22**), IIa (**23**), and IIb (**24**) from *A. hippocastanum* prevented the increase of vascular permeability induced by both acetic acid in mice and histamine in acute inflammatory rats. Escins Ia, Ib, IIa, and IIb inhibited hind paw edema induced by carrageenin and scratching behavior in mice. However, desacylescins I (**41**) and II (**42**) without acyl groups had no effects in the animal model tested, which suggests that acyl groups in escins were essential for their activities [92]. Escins Ia, Ib, isoescins Ia (**43**), and Ib (**44**) isolated from the seeds of *A. chinensis* var. *chinensis* markedly inhibited dimethyl benzene induced inflammation in mice at a dose of 30 mg/kg. The inhibitory effects of the four pure saponins were nearly equal to each other, but had more potent activity than a total saponin extract and dexamethasone, a commercially available anti-inflammatory drug [50]. Oleic acid (**191**), linoleic acid (**192**), and linolenic acid (**193**) from a hexane seed extract of *A. turbinata* showed an inhibition of the activity of cyclooxygenase (COX-1 and COX-2), the important target for anti-inflammatory drugs. Linolenic acid and linoleic acid had a high selectivity toward COX-2 [20].

Anti-tumor Activities

Recent studies *in vivo* and *in vitro* indicate that aescin (β -escin) has significant antitumor activities. β -escin from *A. hippocastanum* inhibited chemically induced colon carcinogenesis in rats, and *in vitro* exhibited cytotoxicity at 30





$\mu\text{mol/L}$ or above concentrations in colon cancer cell lines. β -Escin at $5 \mu\text{mol/L}$ also inhibited HT-29 colon cancer cell proliferation. β -escin induced cell cycle arrest at G_1 -S phase in part mediated by induction of p21WAF1/CIP1 and/or associated with reduced levels of Cdk2 and cyclins A and E complex; additionally, there was a lower phosphorylation of Rb [93].

β -escin isolated from the seeds of *A. chinensis* var. *chinensis* induced apoptosis and caused a significant inhibition of HL-60 human acute myeloid leukemia cell and K562-human chronic myeloid leukemia cell proliferation in dose- and time-dependent fashion. Morphological evidence of apoptosis, a significant increase of annexin V+ and PI- cells (early apoptotic) and apoptotic DNA fragmentation, were observed in K562 cells treated with β -escin. Flow cytometry analysis indicated that β -escin induced G_1 -S arrest and led to a significant accumulation of the sub- G_1 population in HL-60 and K562 cells [94, 95].

Aescin isolated from the seeds of *A. chinensis* var. *wilsonii* at a dose of 2.8 mg/kg had a rather high inhibition ratio (43.5 %) on mice H22 tumor growth *in vivo*. Aescin could induce significant concentration- and time-dependent inhibition of HepG (2) cell viability and induce cell cycle checkpoint arrest and caspase-independent cell death in HepG (2) cells [96].

An early investigation showed that two prosapogenins of 21-*O*-tigloyl-22-*O*-angeloyl- R_1 -barrigenol (**10**) and 21-*O*-angeloylbarringtogenol-C (**18**) from the acid hydrolysates of the HCSE of *A. hippocastanum* exhibited significant *in vitro* cytotoxicity with ED_{50} of $3.6 \mu\text{g/mL}$ and $3.0 \mu\text{g/mL}$, respectively, in the human nasopharyngeal carcinoma 9-KB cell culture assay [27].

More recently, 28 individual saponins (**72-99**) from the fruits of *A. pavia* and six prosapogenins produced from these saponins were assayed *in vitro* for their cytotoxicity and inhibition of DNA topoisomerase I (TOP1) [61, 97]. Saponins **94**, **96** and **98** with two acyl groups at C-21 and C-22 showed activity with GI_{50} of 0.175 – $8.71 \mu\text{M}$ against most of 59 cell lines tested, which were from nine different human cancers including leukemia, non-small cell lung, colon, central nervous system (CNS), melanoma, ovarian, renal, prostate, and breast tumor cell lines. Aesculosides Iic (**79**) and

Ild (**80**) with only one acyl group at C-21 showed less activity while aesculosides Ia-e (**72-76**) without acyl group showed no or weak activity [61]. Aesculosides IIa-k (**77-87**), IIIa-f (**88-93**), IVa (**95**), IVb (**97**), IVc (**99**), saponins **94**, **96**, **98** and six prosapogenins were also tested for their inhibition of DNA topoisomerase I (TOP1) and their activities against A549, PC-3, HL-60, PANC-1, and MRC cell lines. Most of the tested saponins and prosapogenins with acyl groups showed cytotoxic activity with different GI_{50} value. Sixteen cytotoxic aesculosides **77-80** and **88-99** inhibited TOP1 catalytic activity by interacting directly with the free enzyme and preventing the formation of the DNA-TOP1 complex. Interestingly, six prosapogenins, including 21-*O*-angeloylproaescigenin (**13**), 21,22-*O*-diangeloylprotoaescigenin (**16**), 21,22-*O*-diangeloylbarringtogenol-C (**20**), 21-*O*-angeloyl-22-*O*-2-methylbutanoyl- R_1 -barrigenol, 21-*O*-angeloyl-22-*O*-2-methylbutanoylprotoaescigenin, and 21-*O*-angeloyl-22-*O*-2-methylbutanoylbarringtogenol-C prepared from the acid hydrolysates of saponins **78**, **96**, **98**, **95**, **97**, and **99**, respectively, showed no TOP1 inhibitory activity, but had stronger cytotoxicity when compared to the related saponins [97].

Antiviral and Antifungal Activities

Isoescins Ia (**43**) and Ib (**44**) and escins Ia (**21**), Ib (**22**), IVc (**28**), IVd (**29**), IVe (**30**), and IVf (**31**) isolated from the seeds of *A. chinensis* var. *chinensis* showed activity against HIV-1 protease. Escins Ia (**21**) and Ib (**22**) inhibited the enzyme's activity by $86.1 \pm 0.2\%$ at $100 \mu\text{M}$ with IC_{50} values of 35 and $50 \mu\text{M}$, respectively, while saponins **28-31**, **43**, and **44** showed weaker antiviral activity [47].

Three flavonol glycosides of aescuflavoside (**103**), aescuflavoside A (**105**), and leucoside (**120**) from *A. chinensis* var. *chinensis* showed significant antiviral activities against respiratory syncytial virus with IC_{50} values of 4.5 , 6.7 , and $4.1 \mu\text{g/mL}$, and selective index values of 15.8 , 32 , and 63.8 , respectively. Astragalin (**122**) demonstrated significant antiviral activity against influenza virus type A with an IC_{50} of $24.5 \mu\text{g/mL}$ and a selective index of 16.0 [66].

Pavietin (**161**), a new prenylated coumarin, isolated from the leaves of a genotype of *A. pavia*, showed antifungal activity. In a preliminary antifungal screening by filter disk

method, pavietin (15 mg/dish) possessed significant activity against an *Aesculus*-specific fungal parasite, *Guignardia aesculi*, and weaker activity against the generalist polyphagous *Pythium ultimum* and the *Aesculus* nonpathogenic *Fusarium basilici*. More detailed antifungal screening by adding pavietin to mycelial growth medium of different fungal pathogens showed that pavietin exhibits appreciable antimicrobial properties against several pathogens including *Alternaria alternata*, *A. dianthi*, *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium fulvum*, *Fusarium oxysporum dianthi*, *Guignardia aesculi*, *Penicillium expansum*, *Polyporus marginatus*, *Rhizopus stolonifer*, and *Trichoderma viride* [67].

Antiangiogenic Activities (Vascular Protection)

β -escin sodium inhibited angiogenesis in chick chorioallantoic membrane [98] and in aortic disk assay [99]. Further study indicated that β -escin sodium (10, 20, and 40 $\mu\text{g/mL}$) dose-dependently inhibited endothelial cells (ECs) proliferation in human umbilical vein endothelial cells and ECV304 cells. β -escin sodium also induced ECs apoptosis at 40 $\mu\text{g/mL}$ and suppressed ECs migration and cell motility. Western blot results suggest that β -escin sodium acts on ECs possibly by increasing expression of thrombospondin-1, decreasing expression of PKC- α and activation of p44/42 mitogen-activated protein (MAP) kinase and p38 mitogen-activated protein kinase (p38 MAPK) [100].

Aescin from *A. hippocastanum* was found to improve endothelial dysfunction in rat aortic rings subjected to oxidative stress generated by pyrogallol and to induce contraction in these preparations. These apparently contradicting effects could be due to the well-known ability of escin to enhance cellular permeability to calcium which, on one hand, would increase endothelial nitric-oxide synthase (eNOS) activity and nitric oxide production and would lead to vascular smooth muscle contraction. Endothelial protection could contribute to the therapeutic usefulness of escin, while arterial vasoconstriction could represent a limiting side effect in susceptible patients [101].

When HUVECs (human vascular endothelial cells) were exposed to CoCl_2 as an *in vitro* model of hypoxia, aescin from *A. hippocastanum*, in a dose-dependent fashion (0.1-1.0 μM), prevented the action of CoCl_2 on the vascular cell adhesion molecule (VCAM-1) and platelet endothelial cell adhesion molecule (PECAM-1) and preserved endothelial cell morphology. In an *in vitro* model of inflammation induced by *Escherichia coli* lipopolysaccharide (LPS), aescin reduced IL-6 release from LPS-activated vascular endothelium [102].

HCSE from *A. hippocastanum* dose-dependently contracted both bovine mesenteric veins and arteries, with veins being the more sensitive. Contraction of both veins and arteries was significantly inhibited by the 5-HT(2A) receptor antagonist ketanserin. HCSE also significantly reduced ADP-induced human platelet aggregation. These findings may, at least partly, account for the mechanism of action of HCSE in the treatment of chronic venous insufficiency [103].

Anti-obesity Effects

Escins Ia (21), Ib (22), IIa (23), IIb (24), deacetylescins Ia (37), Ib (38), IIa (39), IIb (40), and desacylescins I (41)

and II (42) had inhibitory effects on the elevation of blood glucose levels by means of the oral glucose tolerance test in mice. Escins (21-24) with two acyl groups had stronger activity than deacetylescins (37-40) with only one acyl group and desacylescins (41, 42) without acyl group [38, 39, 43]. These compounds also exhibited the activity of pancreatic lipase with very different IC_{50} values. Escins (21-24) had low IC_{50} values of 14-61 $\mu\text{g/mL}$, while deacetylescins Ia and IIa had very high IC_{50} values of 345, and >400 $\mu\text{g/mL}$, respectively [43]. Escins (21-24) with two acyl groups at C-21 and C-22 showed potent inhibitory activity on ethanol absorption in the dose of 50-100 mg/kg [38, 39]. An analysis comparing the structure and activity suggested that the acyl groups at C-21 and C-22 in these saponins were important for their activities.

Testing by using 4-methylumbelliferyl oleate as a substrate, escins (21-24), deacetylescins (37-40), and desacylescins (41, 42) isolated from the natural and edible seeds of *A. turbinata* exhibited inhibitory effect on lipase activity. The potency was in the order of escins > desacylescins > deacetylescins. Escins Ib and IIb as well as deacetylescins Ib and IIb with the angeloyl moiety were more potent than the corresponding Ia and IIa series with the tigloyl moiety. Saponin fractions from natural seeds (SFNS) and edible seeds (SFES) of *A. turbinata* effectively inhibited fat digestion in an *in vivo* anti-obesity experiment in mice fed high-fat diets. SFNS (0.1% or 0.5%) and SFES (0.5%) both significantly attenuated the elevation in body weight, the mass of peritoneal adipose tissues, and plasma triacylglycerol, which was accompanied by higher contents of undigested fats in feces without changes in food intake [104]. Escins Ib and IIa also inhibited pancreatic lipase activity with dose-dependence (0-0.5 mg/mL) in an *in vitro* assay system using triolein emulsified with lecithin [105].

In addition, assamicins I (67, 100 $\mu\text{g/mL}$) and II (68, 25 $\mu\text{g/mL}$) almost completely inhibited release of free fatty acids from epinephrine-treated rat adipocytes. Both compounds enhanced glucose uptake into 3T3-L1 adipocytes as insulin does. When the cells were incubated in a medium containing [3H]-2-deoxyglucose, the uptake of the radioisotope into the cells was enhanced 2.5- and 3.5-fold by addition of assamicins I and II, respectively, at the concentration of 25 $\mu\text{g/mL}$. Assamicin II showed stronger activity than assamicin I in both assay systems tested [57].

Antioxidant and Antigenotoxic Activities

HCSE from the seeds of *A. turbinata* dose-dependently inhibited the autooxidation of linoleic acid (IC_{50} of 0.2 mg/mL) and the inhibition was almost complete at a concentration of 1 mg/mL. The HCSE scavenged DPPH radicals and superoxide anions with IC_{50} of 0.65 and 0.21 mg/mL, respectively. The polyphenols (21 mg/g) in the HCSE were the main active compounds [106]. This HCSE also inhibited the genotoxicities induced by furylfuramide, *N*-methyl-*N*-nitrosourea, methyl methanesulfonate, mitomycin C, 2-aminoanthracene and aflatoxin B₁ at a concentration of 1 mg/mL or more [106].

Aescin from the seeds of *A. hippocastanum* increased the antioxidative defense system of the body and prevented high fat diet (HFD)-induced lipid peroxidation in male mice fed either standard pellet diet (SPD) or HFD at 100mg/kg doses

daily for 5 weeks. Aescin mixture prohibited the adverse effects of oxidative stress and showed a protective effect on the liver architecture both in SPD and HFD consumed male mice. Combined administration of high-fat diet with escin mixture significantly increased blood and liver reduced glutathione GSH levels in mice and decreased the level of malondialdehyde in blood, liver, kidney, heart, the level of liver superoxide dismutase, and the catalase activity [107].

Three docyl sulfide derivatives of (-)-epicatechin (**129**) and proanthocyanidins (**147**, **148**) isolated from the seeds of *A. turbinata* showed potent antioxidative activity by assaying with DPPH (1,1-diphenyl-2-picrylhydrazyl). Compounds **147** and **148** exhibited similar antioxidative activities with IC₅₀ of 2.4–2.6 µg/mL. Their inhibitory actions were slightly more potent than that of the monomer of (-)-epicatechin or (+)-catechin [19].

CONCLUSIONS

The genus *Aesculus* has 12 species, but only *A. hippocastanum* and *A. chinensis* var. *chinensis*, two Eurasian species, are officially recognized sources of herbal products in traditional medicine. HCSE or aescin from *A. hippocastanum* has shown satisfactory evidence for clinically significant activity in chronic venous insufficiency, hemorrhoids, and post-operative oedema, largely due to its anti-inflammatory properties, which were well demonstrated by *in vitro* and *in vivo* assay. However, the mechanism of action of the Chinese horse chestnut is poorly understood and further investigation is necessary.

Although other constituents may have some bioactivity, the polyhydroxylated triterpenoid glycosides (saponins) in *Aesculus* extract are considered to be the major active principles. To date, the saponins from the seeds of six species including *A. hippocastanum* (AH), *A. chinensis* var. *chinensis* (ACC), *A. chinensis* var. *wilsonii* (ACW), *A. turbinata* (AT), *A. assamica* (AA), and *A. pavia* (AP) have been well documented. Very interestingly, the saponins from four Eurasian species (AH, ACC, ACW, and AT) have very similar structures but are different from the novel cytotoxic saponins from an eastern North American species *A. pavia*. This chemical difference suggests a chemotaxonomic significance between the two groups [13, 61]. More studies of the saponins from the seeds of other North American species are necessary to further confirm the chemotaxonomic value of these novel cytotoxic saponins. Detailed investigations on the saponins from the seeds of other *Aesculus* species, which have not been conducted phytochemically, are also needed to fully understand the chemical profile of the genus *Aesculus*. These studies, which are now being conducted at Stephen F. Austin State University, may not only identify more bioactive saponins but could also provide chemotaxonomic data for the genus *Aesculus* and better understanding of its evolution.

The biological and pharmacological activities of HCSE, aescin, and individual components from the genus *Aesculus* have received much interest in recent years. Accumulated studies indicate that HCSE, aescin, and individual compounds have anti-inflammatory, antitumor, antiviral, antifungal, antiangiogenic (vascular protection), anti-obesity antioxidative, and antigenotoxic properties. One of the most interesting activities is the antitumor property of some novel

saponins discovered recently from *A. pavia*. This type of novel saponins has showed potent cytotoxicity and inhibition of TOP1 by *in vitro* assays [61, 97, 108]. Further animal studies are required to evaluate the potential for the treatment of cancer as anticancer agents.

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ABBREVIATIONS

A549	=	Human lung adenocarcinoma epithelial cell line
Ac	=	Acetyl
Ang	=	Angeloyl
CNS	=	Central nervous system
COX	=	Cyclooxygenase
DPPH	=	1,1-diphenyl-2-picrylhydrazyl
EC	=	Inhibited endothelial cell
eNOS	=	Endothelial nitric-oxide synthase
HCSE	=	Horse chestnut seed extract
HFD	=	High fat diet
HL-60	=	Human promyelocytic leukemia cell line
HUVEC	=	Human vascular endothelial cell
LPS	=	Lipopolysaccharide
MAP	=	Mitogen-activated protein
MB	=	2-methylbutanoyl
MP	=	2-methylpropanoyl
MARC-5	=	Human normal lung cell line
p38 MAPK	=	p38 mitogen-activated protein kinase
PANC-1	=	Human pancreatic carcinoma, epithelial-like cell line
PECAM-1	=	Platelet endothelial cell adhesion molecule
SFES	=	Saponin fractions from edible seed
SFNS	=	Saponin fractions from natural seed
SPD	=	Standard pellet diet
STE	=	Standardized therapeutic extract
Tig	=	Tigloyl
TOP1	=	DNA topoisomerase I
VCAM-1	=	Vascular cell adhesion molecule

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