Regular article

Phytochemical investigation of the branches of *Viburnum awabuki* K. Koch and its chemotaxonomic significance

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Abstract

Phytochemical investigation of the branches of *Viburnum awabuki* K. Koch led to the isolation of thirteen known compounds, including five lignans (1-5), one phytosterol (6), two phenylpropanoids (7-8), one chromone derivative (9), three pentacyclic triterpenoids (10-12), and one glyceride (13). The structures of these compounds were elucidated through extensive spectroscopic analyses and comparison of experimental data with literature data. Additionally, the chemotaxonomic significance of the isolated secondary metabolites was also discussed.

Keywords: *Viburnum awabuki*; spectroscopic analysis; secondary metabolites; chemotaxonomic significance

1 Introduction

The genus *Viburnum* L., a member of the family Viburnaceae, encompasses 204 species, widely distributed in temperate regions of the Northern Hemisphere and secondarily in subtropical areas of Asia and Latin America [1,2]. *Viburnum awabuki* K. Koch (*V. awabuki*) is a *Viburnum* L. of the family Viburnaceae, also known as the Japanese coral tree, French Holly or early grass, distributed in southern Korea, Japan, Zhejiang and Taiwan Province of China [3]. *V. awabuki* grows as an evergreen shrub or small tree characterized by an obovate crown, perennial verdure, well-developed root system, and tolerance to pruning. The fruit, shaped like coral, is beautiful [4]. Plants within this genus have been traditionally used to treat various diseases such as rheumatism, tumefaction, bone fractures and other injuries in ethnic medicine [5,6]. Several species have demonstrated a range of pharmacological properties including antitumor, hypoglycemic, and antioxidant activities [7-9]. Motivated by the extensive medicinal applications of the genus *Viburnum* L., we investigated the phytochemical composition of *V. awabuki.*

Previous phytochemical investigations of *V. awabuki* have revealed a diverse array of

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bioactive compounds. These compounds mainly include vibsane-type diterpenes [10-12], such as vibsanin O, vibsanin P, furanovibsanin A, and neovibsanin A [13-15]. Triterpenoids (3*β*,28-dihydroxy-12-oleanene-l-one and 6*β*-hydroxyl-3, 20-dioxo-30 norlupane-28-oic acid) have also been isolated from *V. awabuki* [7,16]. In addition, lignans (dihydrodehydrodiconiferyl alcohol, 9'-*O*-methylvibsanol, and vibsanol) [17], lignan glycosides (7*R*,8*S*-dihydrodehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside and 7*S*,8*R*-dihydrodehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside) [18] and coumarin glycosides (2',6'-di-*O*-acetylscopolin and 6'-*O*-acetylscopolin) have been found in the plant [4]. Moreover, three sesquiterpenoids (awabukinol, 4-hydroperoxyawabukinol, and 3-hydroperoxyawabukinol) have been identified from *V. awabuki* [19].

2 Materials and methods

2.1 General experimental procedure

The NMR experiments were performed on Bruker AV-600 spectrometers (Bruker Corporation, Bremen, Germany) with tetramethylsilane as an internal standard and the chemical shifts were indicated as *δ* values (ppm). Chromatographic silica gel (200-300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) was employed for column chromatography (CC). HP-20 macroporous resin (Mitsubishi Chemical Co., Japan) and ODS gel (60-80 *μ*m, Merck, Germany) were used. All solvents for extraction and chromatography were commercially purchased. Semi-preparative HPLC was performed on a Shimadzu LC-20A equipped with an SPD-20A UV/vis detector (Shimadzu, Kyoto, Japan) using a YMC Pack ODS-A column (250 mm× 10 mm, 5 *μ*m, YMC Company, Kyoto, Japan).

2.2 Plant material

The plant material *Viburnum awabuki* was collected from Chengdu in Sichuan province (E 103.47°, N 30.13°) in November 2020 and authenticated by Anrui Lou (Bozhou Zhiyao Agricultural Technology Co., Ltd). A voucher sample with the serial number 20201123 has been deposited in the herbarium of Shenyang Pharmaceutical University.

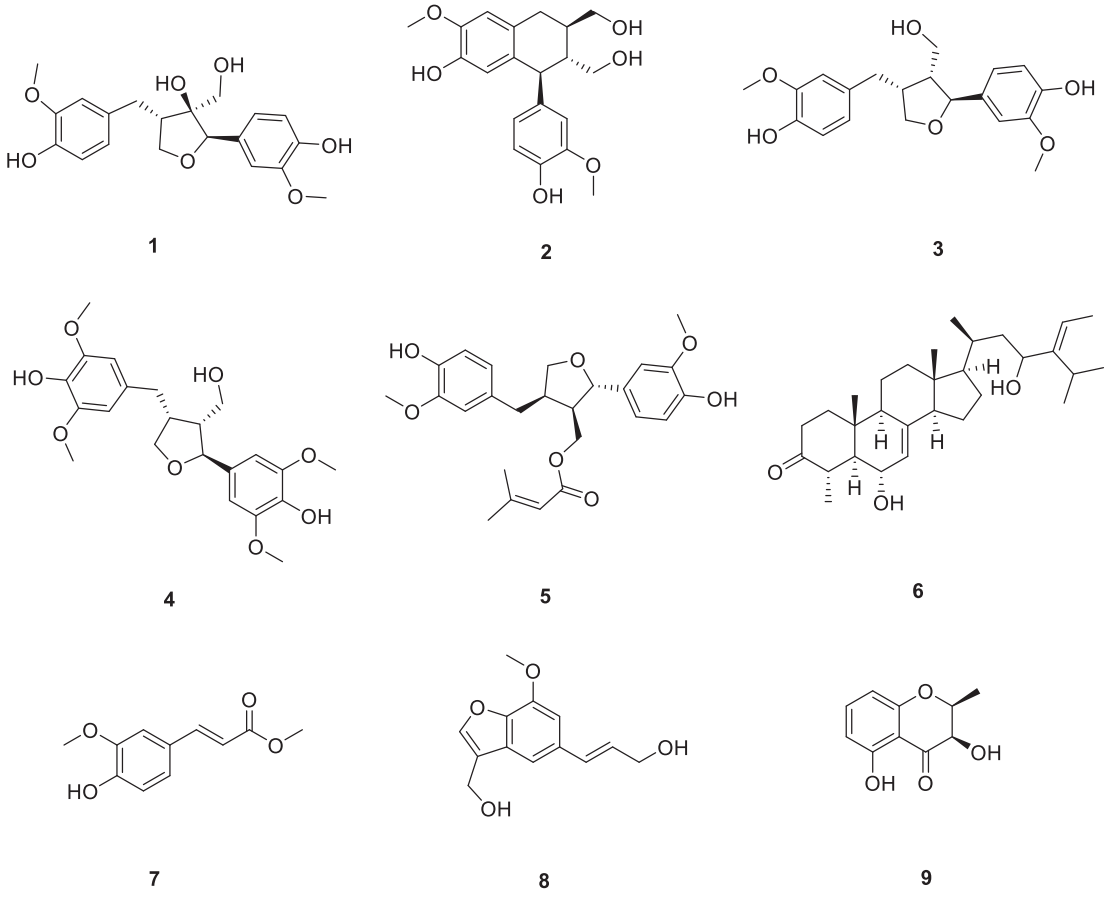
2.3 Extraction and isolation

Dried branches of *Viburnum awabuki* (40 kg) were refluxed three times with 75% industrial EtOH (3 × 75 L). Then, the ethanol extract was concentrated and extracted with *n*-BuOH. The *n*-BuOH extract (1.8 kg) was subjected to silica gel column chromatography and eluted with CH_2Cl_2-MeOH (50:1 to 5:1, *v*/*v*) of increasing polarity to produce three fractions (Fr. A-C) on the basis of silica gel TLC analysis. Subsequently, Fr. A (90 g) was purified by polyamide column chromatography using a stepwise ethanol-water gradient (10%-80%, *v*/*v*) to obtain two fractions (Fr. A1-2). Fr. A2 (44 g) was then subjected to ODS column chromatography with a stepwise ethanol-water gradient (10%-80%, *v*/*v*), yielding three subfractions Fr. A2.1 (10 g), Fr. A2.2 (6 g), and Fr. A2.3 (12 g). Then, Fr. A2.1 was purified by preparative HPLC eluted with MeOH-H_2O (40:60, *v*/*v*) to yield the compounds **10** (6 mg, t_R = 37.6 min); Fr. A2.2 was purified by preparative HPLC eluted with MeOH-H_2O (45:55, *v*/*v*) to yield the compounds **6** (6 mg, t_R = 24.6 min), **9** (6 mg, t_R = 19.7 min), and **13** (3 mg, t_R = 39.8 min); Fr. A2.3 was purified by preparative HPLC eluted with MeOH-H_2O (50:50, *v*/*v*) to give A2.3.1 (768 mg), Fr. A2.3.2 (454 mg), and Fr. A2.3.3 (923 mg). Compound **1** (13 mg, t_R = 23.9 min) and compound **2** (7 mg, t_R = 29.1 min) were obtained from Fr. A2.3.1 by semi preparative HPLC eluted with

CH_3CN-H_2O (41:59, *v/v*). Compound **3** (82 mg, t_R = 37.6 min) and compound **4** (5 mg, t_R = 24.8 min) were isolated from Fr. A2.3.2 by semi preparative (CH_3CN-H_2O, 45:55, *v/v*). Compound **7** (15 mg, t_R = 22.3 min), compound **8** (5 mg, t_R = 26.6 min) and compound **12** (70 mg, t_R = 38.5 min) were obtained from Fr. A2.3.3 by semi preparative HPLC eluted with CH_3CN-H_2O (48:52, *v/v*). Furthermore, Fr. B (76 g) was purified by HP-20 macroporous resin column chromatography using a stepwise ethanol-water gradient (10-90%, *v/v*) to obtain three fractions (Fr. B1-3). Fr. B1 (44 g) was then subjected to ODS column chromatography employing a stepwise ethanol-water gradient (10%-90%, *v/v*), and yielded three distinct fractions (Fr. B1.1-3). Then, Fr. B1.1 (76 g) was purified by preparative HPLC eluted with MeOH-H_2O (50:50, *v/v*) to yield the compounds **5** (3 mg, t_R = 33.4 min) and **11** (13 mg, t_R = 40.2 min).

3 Results

The structures of these compounds were elucidated through extensive spectroscopic analyses and experimental data with literature data. These compounds were identified as (−)-berchemol (**1**) [20], (+)-isolariciresinol (**2**) [21], (+)-lariciresinol (**3**) [22], (+)-5,5′-dimethoxylariciresinol (**4**) [23], (+)-9′-*O*-senecioyllariciresinol (**5**) [24], viburodorol A (**6**) [25], methyl ferulate (**7**) [26], fructusol A (**8**) [27], (2*S*,3*R*)-2,3-dihydro-3,5-dihydroxy-2-methyl-4H-1-benzopyran-4-one (**9**) [28], ilelatifol A (**10**) [29], 11,12-dehydroursolic acid lactone (**11**) [30], 6*α*-hydroxylup-20(29)-en-3-on-28-oic acid (**12**) [31], and 3-linoleoyl-sn-glycerol (**13**) [32] (Fig. 1).



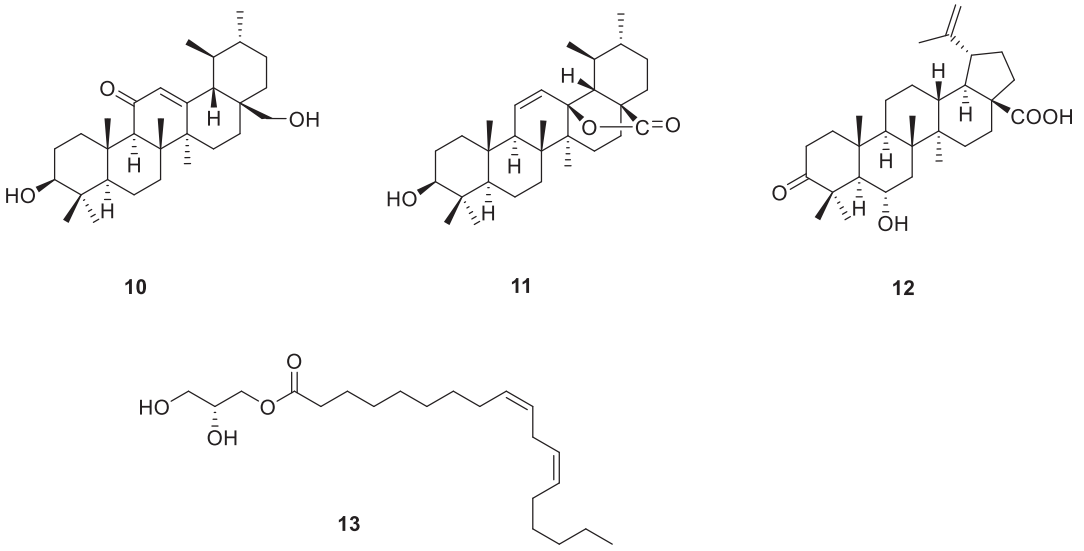


Fig. 1 Chemical structures of compounds **1-13** from the branches of *Viburnum awabuki* K. Koch

4 Discussion

The Viburnaceae family comprises two subtribes known as Subfam. Opuloideae Raf. and Oleinae. The Subfam. Opuloideae Raf. contains only one genus, *Viburnum* L., which includes 204 species of shrubs or small trees [33]. There are about 74 species of *Viburnum* L. in China, which are widely distributed in all provinces. These species are particularly recognized worldwide for their ecological and economic value, as well as for their medicinal uses [34,35]. *Viburnum awabuki* (syn. Japanese coral tree), the focus of this research, commonly known as the French Holly or early grass is widely distributed from southern Korea and Japan regions of China, including Zhejiang Province and Taiwan Province [36].

Dammarane-type, ursane-type, and oleanane-type are the predominant types of triterpenes found within this genus [37,38]. Vibsane-type diterpenoids are unique natural products, restricted to only a few species of the genus *Viburnum* L. based on the research so far [39]. Although iridoid compounds are also found within this genus, their distribution is relatively limited compared to these primary constituents [5]. Furthermore, small quantities of monoterpenes, sesquiterpenes, flavonoids, phenols, lignans, coumarins, lactones, and alkaloids have been identified in the genus *Viburnum* L. [6].

The present study of *V. awabuki* resulted in the characterization of five lignans (**1-5**), one phytosterol (**6**), two phenylpropanoids (**7-8**), one chromone derivative (**9**), three pentacyclic triterpenoids (**10-12**), and one glyceride (**13**). This work significantly expanded the chemical profile of *V. awabuki* and provided proof for chemotaxonomic analysis of the Viburnaceae family. Notably, five compounds (**7-10**, **13**) represent novel reports for the family Viburnaceae and were isolated from *Viburnum awabuki* for the first time. The distribution network of compounds **1-13** from *V. awabuki* was shown in Fig. 2, and the distribution of the compounds was shown as well (Table 1). The networks were constructed by Cytoscape (version 3.10.0).

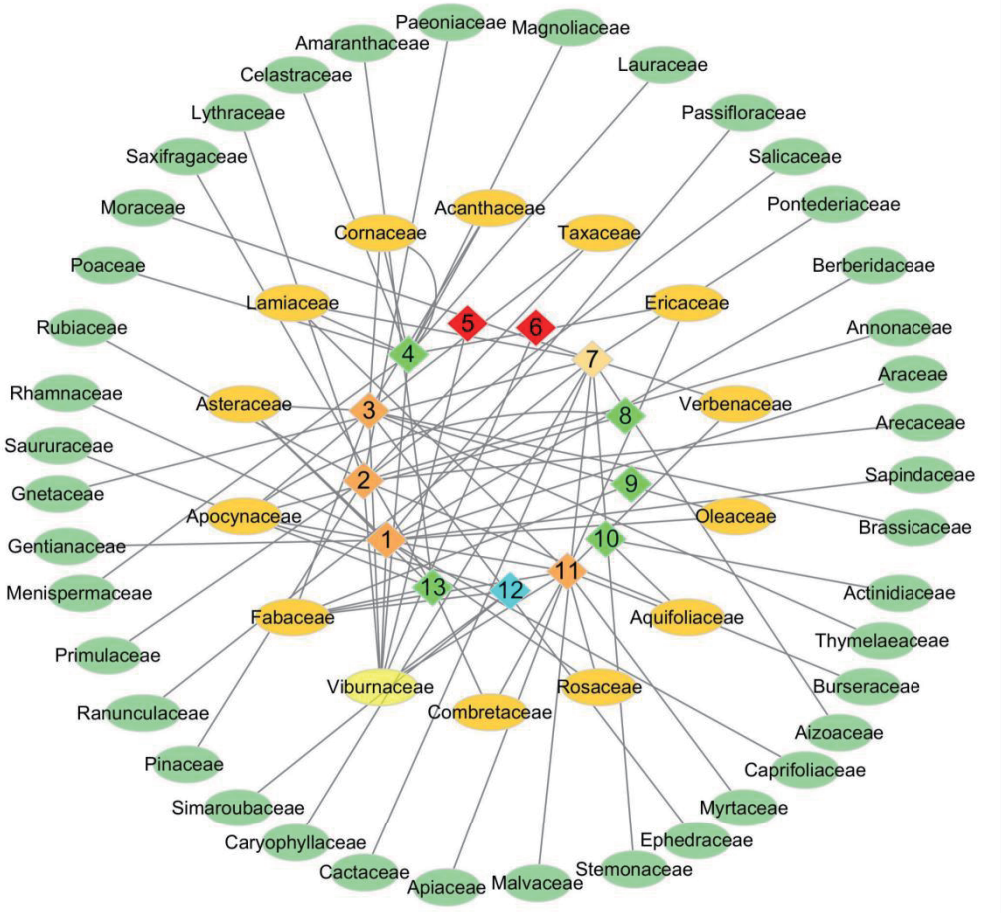


Fig. 2 The compound-family network. Each connecting line represents the families of sources of the compounds. The colorized diamonds represent the compounds from *Viburnum awabuki* and the colorized circles represent the families of sources of the compounds

Table 1 Distribution of compounds **1-13**

Compound	Species	Reference
1 (−)-berchemol	*Sambucus williamsii* (Viburnaceae)	[52]
	Viburnum odoratissimum (Viburnaceae)	[53]
	Gentiana macrophylla (Gentianaceae)	[20]
	Berchemia floribunda (Rhamnaceae)	[59]
	Vladimiria souliei (Asteraceae)	[60]
	Syringa pinnatifolia (Oleaceae)	[61]
	Passiflora foetida (Passifloraceae)	[62]
	Arisaema heterophyllum (Araceae)	[63]
	Acer truncatum (Sapindaceae)	[64]
	Spiraea prunifolia var. *simpliciflora* (Rosaceae)	[65]
	Valeriana jatamansi (Caprifoliaceae)	[66]

(to be continued)

Continued Table 1

Compound	Species	Reference
2 (+)-isolariciresinol	*Sambucus williamsii* (Viburnaceae)	[54]
	Viburnum fordiae (Viburnaceae)	[55]
	Viburnum chingii (Viburnaceae)	[51]
	Areca catechu (Arecaceae)	[67]
	Taxus baccata (Taxaceae)	[68]
	Cathaya argyrophylla (Pinaceae)	[69]
	Rubia yunnanensis (Rubiaceae)	[70]
	Punica granatum (Lythraceae)	[71]
	Populus nigra (Salicaceae)	[72]
	Annona squamosa (Annonaceae)	[73]
	Paeonia lactiflora (Paeoniaceae)	[74]
3 (+)-lariciresinol	*Sambucus williamsii* (Viburnaceae)	[52,56]
	Sambucus nigra (Viburnaceae)	[75]
	Arabidopsis thaliana (Brassicaceae)	[76]
	Daphne genkwa (Thymelaeaceae)	[77]
	Taxus baccata (Taxaceae)	[78]
	Cornus kousa (Cornaceae)	[79]
	Arctium lappa (Asteraceae)	[80]
	Ephedra viridis (Ephedraceae)	[81]
	Parsonsia alboflavescens (Apocynaceae)	[82]
	Osmanthus fragrans 'Latifolius' (Oleaceae)	[83]
	Justicia glauca (Acanthaceae)	[84]
4 (+)-5,5′-dimethoxylariciresinol	*Viburnum chingii* (Viburnaceae)	[51]
	Alangium premnifolium (Cornaceae)	[85]
	Diploclisia glaucescens (Menispermaceae)	[86]
	Lyonia ovalifolia (Ericaceae)	[87]
	Cinnamomum cassia (Lamiaceae)	[88]
	Manglietia insignis (Magnoliaceae)	[89]
	Lindera glauca (Lauraceae)	[90]
5 (+)-9′-*O*-senecioyllariciresinol	*Viburnum odoratissimum* (Viburnaceae)	[24,25]
6 viburodorol A	*Viburnum odoratissimum* (Viburnaceae)	[25]
7 methyl ferulate	*Stemona tuberosa* (Stemonaceae)	[48]
	Hibiscus cannabinus (Malvaceae)	[49]
	Sesuvium sesuvioides (Aizoaceae)	[50]
	Pseudostellaria heterophylla (Caryophyllaceae)	[91]
8 fructusol A	*Gymnema inodorum* (Apocynaceae)	[40]
9 (2*S*,3*R*)-2,3-dihydro-3,5-dihydroxy-2-methyl-4H-1-benzopyran-4-one	*Archidendron clypearia* (Fabaceae)	[28]
	Glycyrrhiza uralensis (Fabaceae)	[42]
10 ilelatifol A	*Actinidia arguta* (Actinidiaceae)	[29]
	Ilex kudmcha (Aquifoliaceae)	[43]

(to be continued)

Continued Table 1

Compound	Species	Reference
11 11,12-dehydroursolic acid lactone	*Sambucus adnata* (Viburnaceae)	[58]
	Eucalyptus tereticornis (Myrtaceae)	[92]
	Centella asiatica (Apiaceae)	[93]
	Lavandula spica (Lamiaceae)	[94]
	Carissa carandas (Apocynaceae)	[95]
	Ilex pernyi (Aquifoliaceae)	[96]
	Eriobotrya japonica (Rosaceae)	[97]
	Terminalia chebula (Combretaceae)	[98]
12 6*α*-hydroxylup-20(29)-en-3-on-28-oic acid	*Viburnum awabuki* (Viburnaceae)	[4]
	Viburnum chingii (Viburnaceae)	[24]
	Viburnum erosum (Viburnaceae)	[47]
	Viburnum odoratissimum (Viburnaceae)	[99]
	Lupinus polyphyllus (Fabaceae)	[31]
13 3-linoleoyl-sn-glycerol	*Bergenia* (Saxifragaceae)	[44]
	Terminalia chebula (Combretaceae)	[45]
	Chenopodium formosanum (Amaranthaceae)	[46]
	Clitoria ternatea (Fabaceae)	[79]

To the best of our knowledge, compounds **5** and **6** were both isolated from *Viburnum* L. genus. (+)-9′-*O*-senecioyllariciresinol (**5**) was identified in *Viburnum odoratissimum* Ker Gawl., as was viburodorol A (**6**) [25]. Therefore, (+)-9′-*O*-senecioyllariciresinol and viburodorol A could serve as chemotaxonomic markers for the identification and classification of *Viburnum* L. plants.

In addition, this research reported the first isolation of compounds **8**-**10**, **13** from the Viburnaceae family. Their isolation demonstrates the existence of phenylpropanoids, pentacyclic triterpenoids, and glycerides in *V. awabuki*, enriching the chemical composition of Viburnaceae plants. Compounds **8**-**10** are used as chemotaxonomic markers. However, compounds **13** cannot be served as chemotaxonomic marker. At the same time, these compounds have already been identified in a small number of other families plants. Fructusol A (**8**) has been reported in Apocynaceae [40] and Asteraceae [27], and (2*S*,3*R*)-2,3-dihydro-3,5-dihydroxy-2-methyl-4H-1-benzopyran-4-one (**9**) has been isolated from Fabaceae [41,42]. Whereas, the pentacyclic triterpenoid, ilelatifol A (**10**), has been reported previously in Aquifoliaceae and Actinidiaceae [29,43]. Moreover, 3-linoleoyl-sn-glycerol (**13**) was found in Saururaceae, Saxifragaceae, Combretaceae, Amaranthaceae, Fabaceae, and Asteraceae [44-46].

Additionally, this investigation also revealed the occurrence of pentacyclic triterpenoid 6*α*-hydroxylup-20(29)-en-3-on-28-oic acid (**12**). It has also been discovered in *V. awabuki*, in a previous study [4]. Compound **12** had already been isolated from various *Viburnum* L. species, including *Viburnum odoratissimum*, *Viburnum chingii* P. S. Hsu and *Viburnum erosum* Thunb. [24,47], which belong to the same section as *Viburnum* L. Furthermore, compound **12** had also been reported in a small number of other family plants, such as Fabaceae, Apocynaceae, and Simaroubaceae [31].

On the other hand, methyl ferulate (**7**) was obtained for the first time from the *Viburnum* L. genus. Moreover, it has been reported in over 30 families, such as Stemonaceae, Malvaceae, and Aizoaceae [48-50]. Since compound **7** is wildly distributed in plants, it has no significance for the chemical classification of the genus *Viburnum* L.

Besides, (+)-5,5′-dimethoxylariciresinol (**4**) has been reported previously in *Viburnum chingii* [51]. Since compound **4** had also been reported in a large number of other species from different sections, there is no significance for the chemical classification of lignan **4** in the genus *Viburnum* L.

Finally, lignans (**1-3**) and pentacyclic triterpenoid (**11**) were isolated from *V. awabuki* for the first time, and they have already been isolated from various *Viburnum* L., *Sambucus* L. species in Viburnaceae family and lots of other families. (−)-berchemol (**1**) was found in *Sambucus williamsii* Hance [52], *Viburnum odoratissimum*, and *Viburnum corylifolium* Hook. f. & Thomson [53]; (+)-isolariciresinol (**2**) has been reported in *Sambucus williamsii* [54], *Viburnum chingii*, and *Viburnum fordiae* [55]; (+)-lariciresinol (**3**) has been isolated from *Sambucus williamsii*, *Sambucus adnata* Wall. ex DC., and *Sambucus nigra* L. [56,57]; 11,12-dehydroursolic acid lactone (**11**) was found in *Sambucus adnata* [58]. Accordingly, lignans (**1-3**) and pentacyclic triterpenoid (**11**) revealed the chemotaxonomic relationship between the genera *Viburnum* and *Sambucus* in the Viburnaceae family.

These isolated compounds highlight the diversity of secondary metabolites in *V. awabuki* and the family Viburnaceae, enriching the chemical profile of this plant and providing candidate molecules for further pharmacological investigation. This study establishes a theoretical framework and provides data to support future chemotaxonomic studies within *Viburnum* species and between the Viburnum family and other genera. Although primarily a fundamental chemical investigation, most of the discovered lignans, phenylpropanoids, and triterpenoids exhibit potential pharmacological activities. Notably, the first-time identification of bioactive compounds such as fructusol A in Viburnaceae, which has well-documented biological activities in other plant families [40], provides theoretical support for the development of *V. awabuki* as a novel natural medicine source.

5 Conclusion

In summary, chemotaxonomy is essential for resolving taxonomic ambiguities and clarifying phylogenetic relationships among various plant groups and within closely related sections of the same genus. The investigation underscores the remarkable biochemical diversity of the *Viburnum* L. genus. This study presents the comprehensive phytochemical investigation of *V. awabuki*, leading to the isolation and structural elucidation of thirteen known compounds. Notably, five compounds (**7-10**, **13**) were identified for the first time in the family Viburnaceae, significantly expanding the known chemical diversity of this taxon. Additionally, compounds **7-10** were isolated from *V. awabuki* for the first time, underscoring the species untapped phytochemical potential. The identification of unique chemical profiles provides strong evidence for species delimitation and evolutionary divergence. The shared phytosterols between *V. awabuki* and *Viburnum odoratissimum* support their classification within the same Viburnum section, and the identified lignans in *V. awabuki* strengthens their potential as chemotaxonomic markers.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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