Secondary metabolites from *Ajania salicifolia* and their chemotaxonomic significance

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**Abstract**

Forty-seven secondary metabolites were isolated from *Ajania salicifolia* (Mattf.) Poljak, including eight sesquiterpenoids, two diterpenoids, three triterpenoids, four steroids, three flavonoids, five coumarins, five lignans, nine phenylpropanoids, five other phenolic compounds, and three acetylenes. Their chemotaxonomic significance within the genus *Ajania* (the tribe Anthemideae) of the family Asteraceae is discussed.

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1. **Subject and source**

The genus *Ajania* (Asteraceae) contains approximately 30 species and is mainly distributed in Central and South Asia, China, and Japan (Editorial Committee for Flora of the Chinese Academy of Science, 1983). Several species of the *Ajania* have long been used as Chinese traditional folk medicines for treatment of bronchitis, lung diseases, emphysema, tuberculosis, intestinal ulcers, appendicitis, rheumatism, spasmodylic, vasodilatory, and diuretic (Editorial Committee for Flora of the Chinese Academy of Science, 1983; Adekenov et al., 1998; Li et al., 1999). *Ajania salicifolia* (Mattf.) Poljak is mainly distributed in high plateau of the northwest of China (Editorial Committee for Flora of the Chinese Academy of Science, 1983). The whole plants were collected from Huzhu county, in Qinghai province, China in August 2006. A voucher specimen (No. Asa-20060802) was deposited at College of Chemistry and Chemical Engineering, Lanzhou University, P. R. China.

2. **Previous work**

Previous phytochemical investigations on *A. salicifolia* have reported the presence of four compounds, including quinines and coumarins (Wu et al., 2015).

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3. Present study

3.1. Extraction and isolation

The dried and powdered whole plants of *A. salicifolia* (5.0 kg) were extracted successively with petroleum ether (PE, 60–90°C)/Et2O/MeOH (1:1:1, v/v/v) and then MeOH, three times (each 38 L) at room temperature. The combined extracts were reduced to dryness in vacuo to give dried residue (873 g). The residue was suspended in distilled water (2.5 L) and then partitioned sequentially with PE (5 × 2.5 L), chloroform (CHCl3) (4 × 2.5 L), ethyl acetate (EtOAc) (5 × 2.5 L), and n-butyl-lactoh (n-BuOH) (4 × 2.5 L), yielding PE (125 g), CHCl3 (115 g), EtOAc (132 g), and n-BuOH (138 g), and aqueous fractions, respectively. Based on their TLC profile, the PE fraction merged with CHCl3 fraction and yielded dried residues (220 g). The residues (215 g) was subjected to silica gel column chromatography (CC) (200–300 mesh which was as same as following silica gel CC) using a gradient of PE/acetone (Me2CO) (1:0, 100:1, 50:1, 30:1, 20:1, 10:1, 8:1, 5:1, 3:1, 2:1, 1:1, 1:0, 1:v) increasing polarity as the eluent, and finally washed with MeOH, to give 13 fractions (F1–F13) based on TLC analysis. F2 (PE/Me2CO, 5:1, 6.5 g) was subjected to silica gel CC with PE/EtOAc (100:1 to 30:1, v/v), to afford two fractions (F2–1–F2–2). F2–1 was subjected to silica gel CC with PE/Me2CO (100:1, v/v), to yield 11 (29.0 mg). The rest of F2–1 was subjected to silica gel CC with PE/EtOAc (80:1, v/v), and further purified by a Sephadex LH-20 column (2.0 × 120 cm) using CHCl3/MeOH (1:1, v/v) as the eluent, to afford 45 (6.0 mg) and 13 (28.0 mg). F3 (PE/Me2CO, 30:1, 4.0 g) was subjected to silica gel CC with PE/Me2CO (30:1, v/v) to obtain 2 (30.0 mg), F4–1 was subjected to silica gel CC with PE/Me2CO (30:1, v/v), to afford three fractions (F4–1–F4–3). F4–1 was subjected to silica gel CC with PE/Me2CO (30:1, v/v), to yield 2 (30.0 mg). F4–2 was subjected to silica gel CC with PE/EtOAc (10:1, v/v), to afford 44 (8.1 mg). F5 (PE/Me2CO, 10:1, 4.0 g) was subjected to silica gel CC with PE/EtOAc (30:1 to 5:1, v/v), to afford four fractions (F5–1–F5–4). F5–2 was subjected to silica gel CC with PE/EtOAc (10:1 to 0:1, v/v), to afford four fractions (F6–1–F6–4). F6–1 was subjected to silica gel CC with PE/EtOAc (15:1 to 5:1, v/v), to yield 34 (12.0 mg) and 39 (8.0 mg). The rest of F6–1 was subjected to a Sephadex LH-20 column (2.0 × 120 cm) using CHCl3/MeOH (1:1, v/v) as the eluent, to afford 40 (9.0 mg). F6–2 was subjected to further silica gel CC with PE/EtOAc (10:1 to 0:1, v/v), then three fractions (F7–1–F7–3). F7–1 was subjected to silica gel CC with PE/EtOAc (8:1, v/v), then purified by a silica gel CC with CHCl3/MeOH (15:1, v/v), to obtain 3 (2.8 mg), 5 (11.0 mg), and 6 (3.1 mg). F7–2 was subjected to a Sephadex LH-20 column (2.0 × 120 cm) with CHCl3/MeOH (1:1, v/v), then purified by silica gel CC with CHCl3/MeOH (15:1, v/v), to obtain 20 (2.3 mg), 27 (3.6 mg), 43 (5.6 mg), and 46 (1.7 mg). The rest section of F7–2 was subjected to silica gel CC with PE/Me2CO (20:1, v/v), then subjected to a Sephadex LH-20 column (2.0 × 120 cm) with CHCl3/MeOH (1:1, v/v), and further purified by silica gel CC with CHCl3/MeOH (8:1, v/v), to afford 8 (0.6 mg), 1 (3.3 mg), and 7 (13.0 mg). F7–3 was subjected to further MCI gel CC (75–150 μm, 3.0 × 60 cm) with H2O/MeOH (7:3, 1:1, 3:7, 1:9, 0:1, v/v), then purified by silica gel CC with CHCl3/MeOH (8:1, v/v), to give 32 (8.0 mg), 42 (8.0 mg) and 14 (12.0 mg). F8 (PE/Me2CO, 2:1, 11.2 g) was subjected to silica gel CC with CHCl3/MeOH (5:1 to 0:1, v/v), to give 4 fractions (F8–1–F8–4). F8–1 and F8–3 were further subjected to a Sephadex LH-20 column (2.0 × 120 cm) with CHCl3/MeOH (1:1, v/v), respectively, then purified by silica gel CC with CHCl3/MeOH (6:1, v/v), to obtain 18 (12.0 mg), 19 (6.0 mg), 24 (17.3 mg), and 33 (6.5 mg). F8–4 was subjected to a Sephadex LH-20 column (2.0 × 120 cm) with CHCl3/MeOH (1:1, v/v), and finally purified by silica gel CC with CHCl3/MeOH (8:1, v/v) to yield 16 (1.6 mg), and 30 (6.3 mg). F9 (PE/Me2CO, 1:1, 18.0 g) was subjected to MCI gel CC with H2O/MeOH (7:3, 1:1, 3:7, 1:9, 0:1) to give 5 fractions (F9–1–F9–5). F9–1 was subjected to silica gel CC with CHCl3/MeOH (5:1 to 0:1, v/v), respectively, then subjected to a Sephadex LH-20 column (2.0 × 120 cm) with CHCl3/MeOH (1:1, v/v), finally purified by silica gel CC with CHCl3/MeOH (8:1, v/v), to obtain 24 (12.3 mg), 26 (11.9 mg), and 28 (11.6 mg). F9–3 was subjected to reverse phase silica gel CC with H2O/MeOH (7:3, 1:1, 3:7, 1:9, v/v), then CHCl3/MeOH (3:1, v/v), to give 37 (5.7 mg). F10 (Me2CO/MeOH, 1:1, 18.0 g) was subjected to MCI gel CC eluted with H2O/MeOH (7:3, 1:1, 3:7, 1:9, 0:1), to afford three fractions (F10–1–F10–3). F10–1 was subjected to a Sephadex LH-20 column (2.0 × 120 cm) with CHCl3/MeOH (1:1, v/v), then purified by silica gel CC with CHCl3/MeOH (20:1, v/v), to give 17 (12.0 mg) and 4 (6.7 mg). Compound 22 (8.0 mg) was given with the same method to F10–3.

The EtOAc extract (130 g) was subjected to CC on a macroporous resin (D–101) eluted with H2O/MeOH (1:0 to 1:1, v/v, gradient system), to give eight fractions (F-ea–1–F-ea–8). F-ea–3 (H2O/MeOH, 7:3, 4.0 g) was subjected to silica gel CC with CHCl3/MeOH (20:1, v/v), then subjected to a Sephadex LH-20 column (2.0 × 120 cm) with CHCl3/MeOH (1:1, v/v), finally purified by silica gel CC with CHCl3/MeOH (20:1, v/v), to give 35 (13.6 mg). With the same method to F-ea–4 (H2O/MeOH, 4:6, 3.4 g), compound 23 (6.8 mg) was obtained. F-ea–6 (H2O/MeOH, 3:7, 5.6 g) was subjected to a Sephadex LH-20 column (2.0 × 120 cm) with CHCl3/MeOH (1:1, v/v), then followed by silica gel CC with CHCl3/MeOH (30:1, v/v), to obtain 21 (8.3 mg) and 38 (3.0 mg).

The n-BuOH extract (132 g) was subjected to CC on a macroporous resin (D–101) eluted with H2O/MeOH (1:0 to 1:1, v/v, gradient system), to give eight fractions (F-bu–1–F-bu–8). F-bu–3 (H2O/MeOH, 3:7, 2.5 g) was subjected to MCI gel CC with H2O/
MeOH (7:3; 1:1, 3:7; 0:1), then subjected to a Sephadex LH-20 column (2.0 × 120 cm) with CHCl₃/MeOH (1:1, v/v), and finally followed by silica gel CC with CHCl₃/MeOH (10:1, v/v), to give 36 (10.3 mg).

As a systematic approach to A. salicifolia, 47 constituents were isolated from the plant. By comparing their physical and spectroscopic data with the values reported in the literature, they were identified as 3,4-dihydroxyguaia-10(14),11(13)-dien-6,12-olide (1) (Sigstad et al., 1991), costunolide (2) (El-Feryal and Chan, 1978), 14-oxomelampolide (3) (Czerson et al., 1979), artemorin (4) (Fischdock et al., 2012), 11-hydr-oxy-2-one-1(10)-en-4α,7β-epimerophane (5) (Savona et al., 1987), magnolialide (6) (Küsel and Zielińska, 2001), santamarine (7) (Fang et al., 2005), 5-hydroxy-5,6-seco-caryophyllen-6-one (8) (Ahmed et al., 2003), betulin (9) (Pistelli et al., 2005), 3β-friedelilolin (10) (Salazar et al., 2000), friedelin (11) (Ageta et al., 1995), sitostenone (12) (Seca et al., 2000), β-sitosterol (13) (Edliu et al., 2015), ergosterol peroxide (14) (Hybelbauerová et al., 2008), ergosta-4,6,8,22-tetraene-3-one (15) (Chobot et al., 1997), luteolin-3,4′-dimethylether (16) (Stevens et al., 1999), centaureidin (17) (Glasi et al., 2002), evofolin-B (18) (Wu et al., 1995), isocoumopoletin (19) (Jerezano et al., 2011), scoparone (20) (Zhu et al., 2010), fraxetin (21) (Liu et al., 2005), 6,8-dimethoxy-7-β-0-glucopropargyl-coumarin (22) (Zhu et al., 2010), 5,7-dimethoxy-6-hydroxycoumarin (23) (Hammad et al., 2008), (7R,8R)-three-3′-dimethoxy-8′-oxo-neoligna-4, 7,9′-tetraol (24) (Huang et al., 2013), cedrusin (25) (Kuang et al., 2009), larciresinol (26) (Eklund et al., 2002), sesamin (27) (Jayasinghe et al., 2003), syringaresinol (28) (Leong et al., 1999), ferulic acid (29) (Xing et al., 2003), coniferaldehyde (30) (Sy and Brown, 1999), methyl ferulate (31) (Guo et al., 2015), methyl-p-coumarate (32) (Kwon and Kim, 2003), E-p-coumaric acid tetracosylester (33) (Achenbach et al., 1986), methyl caffeate (34) (Zhu et al., 2010), abietin (35) (Greca et al., 1998), syringoside (36) (Greca et al., 1998), 1-O-methyl-guaiacylglycerol (37) (Kim et al., 2013), 3,4-dihydroxybenzaldehyde (38) (Chiji et al., 1980), vanillin (39) (Pelter et al., 1976), 4-hydroxybenzoic acid methyl ester (40) (Zhang et al., 2014), cannabichromeorcin (41) (Quaghebeur et al., 1994), 5-heneicosy-1,3-benzenediol (42) (Feresin et al., 2003), phytene-1,2-di-ol (43) (Rodríguez and Acosta, 1997), phytol (44) (Brown et al., 2003), 2β-(2′,4′-hexadiynoyl)-1, 6-dioxaspiro[4,5]deca-3-ene (45) (Zhu et al., 2010), icthyothereol acetate (46) (Cascon et al., 1965), icthyothereol (47) (Cascon et al., 1965). Their structures were shown as Fig. 1.

4. Chemotaxonomic significance

The present phytochemical study reports the isolation and identification of 47 secondary metabolites from the aerial part of A. salicifolia, including eight sesquiterpenoids in five skeletal types (1–8): guaiane lactone (1), germacrane lactones (2–4), eudesmane (5), epimeroplane lactones (6–7), and seco-caryophyllene (8); three triterpenoids (9–11) including lupinane-type (9) and friedelan-ene-type (10 and 11); four steroids (12–15) including stigmasteryl-type (12–13) and ergosterol-type (14–15); three flavonoids (16–18); five coumarins (19–23); five lignans with different skeletons (24–28); nine phenylpropanoids (29–37); five other phenolic compounds (38–42); two phytol diterpenoids (43–44), and three acetylenes (45–47). Among them, 34 compounds (2–6, 8, 9, 12, 14–16, 18, 21, 23–26, 28–33, 35–38, 40–44, 46, and 47) were reported for the first time in the genus Ajania.

The genus Ajania belongs to the tribe Anthemideae of the family Asteraceae (or Compositae). The family Asteraceae is characterized by the occurrence of sesquiterpenoids. In the tribe Anthemideae, sesquiterpenoids were isolated from species in the genera Achillea (Saeidnia et al., 2011), Anthemis (Staneva et al., 2008), Artemisia (Ivanescu et al., 2015), Chrysanthemum (Kumar et al., 2005), and Tanacetum (Ivanescu et al., 2015). The most common sesquiterpenoids are guaiane-type, eudesmane-type, epimeroplane-type and germacrane-type sesquiterpenoids, which are used as chemotaxonomic markers in the tribe Anthemideae (Kumar et al., 2005; Staneva et al., 2008; Sanedia et al., 2011; Ivanescu et al., 2015).

Up to now, nine species in the genus Ajania, Ajania achilloides (Turcz.) Poljak. Ex Grubov. (Belenovskaya and Markova, 1979; Zdero et al., 1990), Ajania fastigiata (Ch.Wendl.) Poljak. (Chumbalov et al., 1973; Yusupov et al., 1982), Ajania fruticulosa (Lcdeb.) Poljak. (Wang et al., 1994; Adekonov et al., 1998; Li et al., 1999; Meng et al., 2001; Tikhonova et al., 2006), Ajania nematoloba (Hand.-Mazz.) Y. Ling et C. Shih (Lin et al., 2015), Ajania nubigena (Wall.) Shih (Wangchuk et al., 2013), Ajania przewalskii Poljak. (Zhang, 2006; Zhang et al., 2006; Zhu et al., 2010), Ajania salicifolia (Mattf.) Poljak. (Wu et al., 2015), Ajania semnanensis Sonboli (Salehi et al., 2015), and Ajania tenuifolia (Jacq.) Tzvel. (Zhang, 2008), have been studied on phytochemical investigations. In the present study, two sesquiterpenoids (1, 7) were obtained previously from two Ajania species, guaiane lactone (1) from the aerial parts of A. fruticulosa, collected in Gansu of China (Meng et al., 2001), epimeroplane lactone (7) from the aerial parts of A. achilloides, growing in Mongolia (Zdero et al., 1990). Although sesquiterpenoids 2–6 and 8 were first isolated from A. salicifolia, four skeletal types of these sesquiterpenoids (1–7), guaianolides, eudesmanolides, germacranoles, and bisabolene have been reported in Ajania: guaiane-type and germacrane-type sesquiterpenoids from six Ajania species, A. fastigiata (Yusupov et al., 1982), A. achilloides (Zdero et al., 1990), A. fruticulosa (Wang et al., 1994; Adekonov et al., 1998; Li et al., 1999; Meng et al., 2001; Tikhonova et al., 2006), A. tenuifolia (Zhang, 2008), A. przewalskii (Zhu et al., 2010), and A. nubigena (Wangchuk et al., 2013); eudesmane-type sesquiterpenoids from A. achilloides (Zdero et al., 1990) and A. przewalskii (Zhu et al., 2010), and a bisabolene from A. przewalskii (Zhu et al., 2010). These evidences confirmed the close chemotaxonomic relationship between A. salicifolia and the above species. Therefore, the study provided powerful evidences to support the taxonomic location of A. salicifolia in the genus Ajania of the tribe Anthemideae of the Asteraceae family. On the other hand, seco-caryophyllene (8) was obtained from the genus Ajania for the first time, suggesting that it may be potential chemotaxonomic markers for A. salicifolia to distinguish it from other Ajania species.

In previous study, oleane-type, ursane-type, friedelane-type, taraxastane-type, and taraxerane-type triterpenoids were previously reported from Ajania species, A. fruticulosa (Wang et al., 1994; Li et al., 1999; Khan et al., 2011), A. przewalskii (Zhang, 2006), and A. tenuifolia (Zhang, 2008). Steroids including stigmasteryl-type and ergosterol-type were isolated from four...
Ajania species, *A. fruticulosa* (Li et al., 1999), *A. przewalsk* (Zhang, 2006), *A. tenuifolia* (Zhang, 2008), and *A. nematoloba* (Lin et al., 2015). In the present study, 3β-friedelanol 10 was reported previously from *A. przewalsk* (Zhang, 2006), while friedelin 11 and β-sitosterol 13 from the aerial parts of *A. przewalsk*, growing Huzhu county in Qinghai province of China (Zhang, 2006) and whole plants of *A. tenuifolia*, growing Maqu county in Gansu province of China (Zhang, 2008). Hence, combining the known chemical characteristics data in *Ajania* show a very close relationship between *A. salicifolia* and those species *A. przewalsk* and *A. tenuifolia* with similar geographical environment. However, there have not been report on the occurrence of lupinane-type triterpenoids in this genus, suggesting that lupinane-type triterpenoid 9 may be potential chemotaxonomic markers for *A. salicifolia* to distinguish it from other species of *Ajania*.

Fig. 1. Structures of compounds 1–47 from *A. salicifolia*.
Phenolic constituents are widely distributed in Ajania species: flavonoids from A. fastigiata (Chumbalov et al., 1973), A. achilleoides (Belenovsky and Markova, 1979), A. fruticulosa (Li et al., 1999), A. przewalsk (Zhang et al., 2006), A. tenuifolia (Zhang, 2008), A. nubigena (Wangchuk et al., 2013), A. nematoloba (Lin et al., 2015); coumarins from A. achilleoides (Belenovsky and Markova, 1979), A. przewalsk (Zhu et al., 2010), and A. tenuifolia (Zhang, 2008); other phenolics from A. nubigena (Wangchuk et al., 2013) and A. nematoloba (Lin et al., 2015). Furthermore, flavonoids are in the classification of temperate members of the Anthemideae (Williams et al., 2001). Seven phenolic compounds (17, 19, 20, 22, 27, 34, and 39) isolated in our study have been reported from other Ajania species, flavonoid 17 from A. fruticulosa (Meng et al., 2001), A. przewalsk (Zhang et al., 2006), and A. tenuifolia (Zhang, 2008), respectively; coumarins 19, 20, and 22 from the Mongolian species A. achilleoides (Belenovsky and Markova, 1979) and A. przewalsk (Zhu et al., 2010); lignan 27 from A. przewalsk (Zhang et al., 2006); methyl caffeate 34 from A. przewalsk (Zhu et al., 2010); vanillin 39 from A. przewalsk (Zhang et al., 2006). The present study revealed the close chemotaxonomic relationships between A. salicifolia and these species in Ajania. Therefore, phenolic compounds, especially flavonoids, could be considered as important chemotaxonomic markers for the genus Ajania. However, lignans 24–26, phenylpropanoids 29–33 and 35–37, and phytol diterpenoids 43–44 have not been isolated from other species of this genus, suggesting that they could be used to distinguish A. salicifolia from other species of Ajania as designated fingerprints.

Acetylene 45 was previously isolated from A. przewalsk (Zhu et al., 2010). Acetylenes are not common in the Ajania, but have been only reported from A. przewalsk, collected from Qinghai in China (Zhu et al., 2010) and the aerial parts of A. nubigena, collected from Lungzhi in Bhutan (Wangchuk et al., 2013). Interestingly, these three species, A. salicifolia, A. przewalsk, and A. nubigena, are all from Himalaya regions of China and Asia, in the similar geographical environment. The occurrence of acetylenes suggest that these three species could be systematically related and also could be used to differentiate from the other species of Ajania.

To conclude, this study indicated that A. salicifolia has the close chemotaxonomic relationships with some other species in Ajania, especially more close to the four species, A. fruticulosa, A. nubigena, A. przewalsk, and A. tenuifolia, with the similar growth environment and similar chemical constituents. Sesquiterpenoids (mainly guaiane-type and germacrene-type sesquiterpenoids) and phenolic constituents (mainly flavonoids, coumarins, and other phenolic compounds) are systematically important, and serve as suitable taxonomic markers for the genus Ajania. In addition, lupanine-type triterpenoid 9, lignans 24–26, phenylpropanoids 29–33 and 35–37, and phytol diterpenoids 43–44 could be considered chemotaxonomic markers of A. salicifolia since they have not previously been isolated from any species of the genus Ajania.

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References