

Secondary Metabolites from the Root of *Aralia echinocaulis* Hand. -Mazz.

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Abstract: The root of *Aralia echinocaulis* Hand. -Mazz. (Araliaceae), are used as traditional Chinese medicine for the treatment of rheumatoid arthritis in China. A phytochemical investigation was carried out to this herb, and obtained twelve secondary metabolites, i.e., syringin (**1**), adenosine (**2**), saccharose (**3**), araliasaponin VII (**4**), araliasaponin VI (**5**), araliasaponin XI V (**6**), araliasaponin XVI (**7**), syringaresinol (**8**), 3,4-dihydroxybenzoic acid (**9**), coniferaldehyde (**10**), isovanillin (**11**) and β -sitosterol (**12**). Their structures were determined mainly by comprehensive analyses of ¹H and ¹³C NMR spectrum and comparison with available literature data or the authentic compounds. To the best of our knowledge, it is the first report that all of compounds have been isolated from the titled plant, and syringin should be one of the major active constituents of *A. echinocaulis* for the treatment of rheumatoid arthritis.

Keywords: *Aralia echinocaulis*; Constituents; Rheumatoid arthritis; Bioactivity. © 2016 ACG Publications. All rights reserved.

1. Plant Source

Aralia echinocaulis Hand. -Mazz. (Araliaceae), mainly distributed in the middle eastern areas of China, such as Hunan, Hubai, Anhui and Zhejiang province, which is used as a traditional Chinese medicine for the treatment of rheumatoid arthritis (RA) [1]. Various compounds including saponin, flavonoids and coumarin have been isolated from the genus *Aralia* [2-3]. Some compounds show significant antitumor activity [3]. However, to date, little is known about the constituents of *A. echinocaulis* and their bioactivity. As a part of our studies on Chinese medicinal plant for screening active ingredient from herbs [4-6], we investigated the constituents of the titled plants, and isolated twelve compounds, i.e., syringin (**1**), adenosine (**2**), saccharose (**3**), araliasaponin VII (**4**), araliasaponin VI (**5**), araliasaponin XI V (**6**), araliasaponin XVI (**7**), syringaresinol (**8**), 3,4-dihydroxybenzoic acid (**9**), coniferaldehyde (**10**), isovanillin (**11**) and β -sitosterol (**12**) from the root of

A. echinocaulis (Figure 1). Herein, we report the isolation of these compounds in this paper.

The root of *Aralia echinocaulis* Hand.-Mazz. was collected at Anhui province, China, June, 2012, identified by Prof. Shoujin Liu (plant taxonomist). A voucher specimen (No. Iyz005) has been deposited in School of Pharmacy, Anhui University of Chinese Medicine, P. R. China.

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2. Previous Studies

Six compounds, deglucosylaraloside A, araloside A, butanedioic acid, β -sitosterol and stigmasterol have been isolated from *A. echinocaulis* [7]. Pharmacological studies demonstrated that *A. echinocaulis* was effective in the treatment of adjuvant arthritis in rats by inhibiting the inflammatory cytokines [8, 9].

3. Present Study

Dried root of *A. echinocaulis* (5 Kg) was reflux-extracted with 95% EtOH three times (3×10 L, 2h each), yielded about 373 g of residue after evaporating the solvent in vacuo. Then, the crude extract was suspended in H₂O, partitioned with petroleum ether (3×1.5 L), EtOAc (3×1.5 L), and *n*-BuOH (3×1.5 L), affording 57 g of petroleum ether extract, 32 g of AcOEt extract and 87 g of *n*-BuOH extract, respectively. The *n*-BuOH extract was subjected to column chromatography (silica gel) and eluted with CH₂Cl₂ : MeOH (100 : 0 – 0 : 100) to provide 9 fractions (Fr.1-Fr.9). Fr.3 (17 g) was further subjected to silica gel column chromatography on the gradient elution with CH₂Cl₂ : MeOH : H₂O (8 : 1 : 0.1 - 4 : 1 : 0.2) and purified by Sephadex LH-20 column with MeOH to yield syringin (**1**, 1.5 g) [10], adenosine (**2**, 9 mg) [11]. A bulk crystal was formed in Fr.5, which was identified as saccharose (**3**, 1.2 g). Fr.7 (15 g) was subjected to silica gel column chromatography gradient eluted with CH₂Cl₂ : MeOH : H₂O (12 : 3 : 0.8, 7 : 3 : 0.5), yielded 11 sub-fractions (SF1 - SF11). SF-5 was further purified by column chromatography (SiO₂) with CHCl₃ : MeOH : H₂O (7 : 3 : 0.5) to obtain araliasaponin VII (**4**, 230 mg) [12]. SF-7 (0.89 g) was purified by using the same proportional eluent in SiO₂ column chromatography and obtain three saponins, araliasaponin VI (**5**, 50 mg) [11], araliasaponin XI V (**6**, 17 mg) [13] and araliasaponin XVI (**7**, 10 mg) [13], respectively. EtOAc extract was chromatographed over silica gel column eluting with a gradient of CH₂Cl₂-MeOH (100 : 0 – 0 : 100) to yield ten fractions (Fr.1 - Fr.10). Fr.3 (2.8 g) was subjected to LH-20 to provide 16 sub-fractions (SF1 - SF16). SF 6 was further purified by silica gel column chromatography eluted with petroleum ether and acetone (4 : 1) to obtain (+)-syringaresinol (**8**, 2 mg) [14]. SF-9 was purified by Sephadex LH-20 with MeOH, to yield 3, 4-dihydroxybenzoic acid (**9**, 10 mg) and coniferaldehyde (**10**, 5 mg) [14]. Fr.5 was purified by silica gel column chromatography eluting with a gradient of CH₂Cl₂ : MeOH (25 : 1, 12 : 1) to obtained isovanillin (**11**, 2 mg) [15]. The petroleum ether extract was subjected to silica gel column chromatography using a mixture of petroleum ether and EtOAc (100 : 0 – 0 : 100) as eluent to afford 4 fractions (F₁-F₄). F₄ (13 g) was further subjected to silica gel column chromatography eluted with petroleum ether-acetone (5 : 1) to yield β -sitosterol (**12**, 150 mg) [16].

The structures of isolated compounds were elucidated by means of spectroscopic experiments mainly ¹H-NMR, ¹³C-NMR and mass spectrometry and comparison with literature data or the authentic compounds. As far as we know, all of compounds have been reported for the first time from the titled plant.

In this experiment, there is a total weight of 1.5 g for syringin (**1**) obtained from the extracts of *A. echinocaulis*, which was a relatively large content as to phytochemistry investigation. It has been proved that syringin had a good bio-activity to treat rheumatoid arthritis [17]. Therefore, syringin is perhaps one of the major active constituents of *A. echinocaulis* for the treatment of RA. In this study, four saponins (**4**, **5**, **6**, **7**) were also isolated from the titled plant. These saponins have the same aglycone, i.e., oleanane acid. The difference among them is that aglycone of compounds **4** is linked with five monosaccharides, while the others are linked with four monosaccharides. The previous investigation demonstrated that saponins are the important constituents existed in the plants of the genus *Aralia*. Our results are in accord with this conclusion, and these four saponins can serve as potential chemotaxonomic markers for *A. echinocaulis*.

In chemical structure, compounds **4-7** belong to oleanane-type saponins, which is a kind of important natural products possessing various bioactivity, such as antitumor [18], antidiabetics [19], anti-rheumatoid arthritis[20], immunocompetence [21], and hepatoprotective effect [22], etc. To date, it is unclear whether these four saponins contribute to effect against the rheumatoid arthritis. We plan

to evaluate their bioactivity and structure-activity relation in the future experiment.

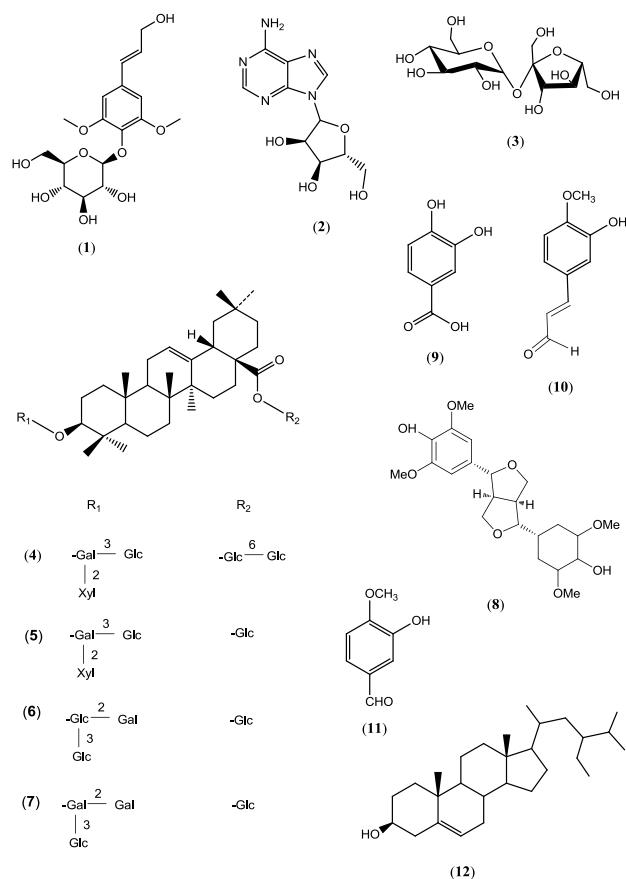


Figure 1. Structure of compounds 1 – 12 from *A. echinocaulis*.

NMR data (δ , ppm):

Syringin (1): $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ : 6.72 (2 H, m, H-3, 5), 6.46 (1H, d, $J = 16$ Hz, H-7), 6.36 (1 H, d, $J = 16$ Hz, H-8), 3.76 (6 H, s, $2 \times -\text{OCH}_3$), 4.92 (1H, d, $J = 7.6$ Hz, H'-1), 4.10 (2 H, t, H-9) ; $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6) δ : 133.9 (C-1), 152.7 (C-2), 104.5 (C-3), 132.6 (C-4), 104.5 (C-5), 152.7 (C-6), 128.4 (C-7), 130.2 (C-8), 61.4 (C-9), 56.3 ($2 \times -\text{OCH}_3$), 102.6 (C-1), 74.2 (C-2), 77.2 (C-3), 69.9 (C-4), 76.5 (C-5), 60.9 (C-6).

Adenosine (2): $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ : 8.34 (1H, s, H-8), 8.13 (1H, s, H-2), 7.32 (2H, br s, H-NH $_2$), 5.88 (1H, d, $J = 6.0$ Hz, H-1'), 5.42 (2H, br s, OH-2', 3'), 5.15 (1H, br s, OH-5'), 4.61 (1H, dd, $J = 6.0, 11.2$ Hz, H-2'), 4.14 (1H, dd, $J = 3.2, 7.8$ Hz, H-3'), 3.96 (1H, dd, $J = 3.2, 6.8$ Hz, H-4'), 3.69 (1H, m, H-5'a), 3.57 (1H, m, H-5'b); $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6) δ : 156.1 (C-6), 152.2 (C-2), 149.0 (C-4), 139.8 (C-8), 119.3 (C-5), 87.8 (C-1'), 85.8 (C-4'), 73.3 (C-2'), 70.5 (C-3'), 61.6 (C-5').

Saccharose (3): $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ : 5.18 (1H, d, $J = 3.6$ Hz, H-1), 4.38 (1H, d, $J = 7.2$ Hz, H-3'), 3.25~3.90 (12H, m, sugar-H); $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6) δ : 104.0 (C-1'), 91.8 (C-1), 82.5 (C-3'), 77.0 (C-3), 74.3 (C-2), 72.9 (C-4'), 72.8 (C-2'), 71.6 (C-5), 69.8 (C-4), 62.1 (C-6'), 62.0 (C-6), 60.5 (C-5')

Araliasaponin VII (4): $^1\text{H-NMR}$ (400 MHz, pyridine- d_5) δ : 0.86, 0.88, 0.89, 1.08, 1.09, 1.26, 1.27 ($3\text{H} \times 7$), 3.32 (1H, dd, $J = 9.6, 3.2$ Hz, H-3), 5.41 (t, H-12), 4.80 (1H, d, $J = 6$ Hz, H-Gal-1), 5.33 (1H, d, $J = 6$ Hz, H-Xyl-1), 5.01 (1H, d, $J = 6$ Hz, H-Glc-1), 5.43 (1H, d, $J = 6$ Hz, H-Glc'-1), 6.23 (1H, d, $J = 6$ Hz, H-Glc''-1); $^{13}\text{C-NMR}$ (100 MHz, pyridine- d_5) δ : 38.7 (C-1), 26.7 (C-2), 89.5 (C-3), 39.7 (C-4), 56.0 (C-5), 18.5 (C-6), 33.1 (C-7), 39.9 (C-8), 48.1 (C-9), 37.0 (C-10), 23.4 (C-11), 122.7 (C-12),

144.2 (C-13), 42.2 (C-14), 28.2 (C-15), 23.7 (C-16), 47.0 (C-17), 41.7 (C-18), 46.3 (C-19), 30.7 (C-20), 34.0 (C-21), 32.5 (C-22), 27.8 (C-23), 16.5 (C-24), 15.6 (C-25), 17.5 (C-26), 26.1 (C-27), 176.5 (C-28), 33.1 (C-29), 23.6 (C-30); Sugar moiety: 105.4 (C-1'), 77.6 (C-2'), 84.8 (C-3'), 69.7 (C-4'), 76.1 (C-5'), 62.3 (C-6'); 105.3 (C-1''), 75.8 (C-2''), 79.1 (C-3''), 72.1 (C-4''), 79.1 (C-5''), 62.6 (C-6''); 105.2 (C-1'''), 75.8 (C-2'''), 79.8 (C-3'''), 71.9 (C-4'''), 67.7 (C-5'''); 95.7 (C-1''''), 74.2 (C-2'''), 78.9 (C-3'''), 71.3 (C-4'''), 79.3 (C-5'''), 62.2 (C-6'''); 104.9 (C-1''''), 76.1 (C-2''''), 79.1 (C-3''''), 71.5 (C-4''''), 67.0 (C-5'''').

Araliasaponin VI (5): $^1\text{H-NMR}$ (400 MHz, pyridine- d_5) δ : 0.84, 0.88, 0.91, 1.07, 1.08, 1.27, 1.28 (3H \times 7), 3.24 (1H, dd, $J = 9.6, 3.2$ Hz, H-3), 5.43 (t, H-12), 4.83 (1H, d, $J = 6$ Hz, H-Gal-1), 5.30 (1H, d, $J = 6$ Hz, H-Glc-1), 5.58 (1H, d, $J = 6$ Hz, H-Xyl-1), 6.28 (1H, d, $J = 6$ Hz, H-Glc'-1); $^{13}\text{C-NMR}$ (100 MHz, pyridine- d_5) δ : 38.8 (C-1), 26.6 (C-2), 89.4 (C-3), 39.7 (C-4), 56.0 (C-5), 18.5 (C-6), 33.1 (C-7), 39.9 (C-8), 48.0 (C-9), 37.0 (C-10), 23.4 (C-11), 122.4 (C-12), 144.2 (C-13), 42.1 (C-14), 28.2 (C-15), 23.7 (C-16), 47.0 (C-17), 41.7 (C-18), 46.2 (C-19), 30.8 (C-20), 34.0 (C-21), 32.5 (C-22), 27.8 (C-23), 16.5 (C-24), 15.5 (C-25), 17.4 (C-26), 26.1 (C-27), 176.6 (C-28), 33.1 (C-29), 23.6 (C-30); Sugar moiety: 105.3 (C-1'), 77.3 (C-2'), 84.8 (C-3'), 69.6 (C-4'), 76.1 (C-5'), 62.4 (C-6'); 105.1 (C-1''), 75.3 (C-2''), 78.7 (C-3''), 71.4 (C-4''), 78.9 (C-5''), 63.1 (C-6''); 104.8 (C-1'''), 76.0 (C-2'''), 79.8 (C-3'''), 71.3 (C-4'''), 66.9 (C-5'''); 95.7 (C-1''''), 74.1 (C-2''''), 78.2 (C-3''''), 71.1 (C-4''''), 79.2 (C-5''''), 62.2 (C-6'''').

Araliasaponin XI V (6): $^1\text{H-NMR}$ (400 MHz, pyridine- d_5) δ : 0.81, 0.88, 0.91, 1.08, 1.09, 1.27, 1.27 (3H \times 7), 3.25 (1H, dd, $J = 9.6, 3.2$ Hz, H-3), 5.42 (t, H-12), 4.81 (1H, d, $J = 6$ Hz, H-Glc-1), 5.60 (1H, d, $J = 6$ Hz, H-Gal-1), 5.34 (1H, d, $J = 6$ Hz, H-Glc'-1), 6.28 (1H, d, $J = 6$ Hz, H-Glc''-1); $^{13}\text{C-NMR}$ (100 MHz, pyridine- d_5) δ : 38.8 (C-1), 26.6 (C-2), 89.3 (C-3), 39.7 (C-4), 55.8 (C-5), 18.5 (C-6), 33.1 (C-7), 39.9 (C-8), 48.0 (C-9), 37.0 (C-10), 23.4 (C-11), 122.7 (C-12), 144.1 (C-13), 42.1 (C-14), 28.2 (C-15), 23.7 (C-16), 47.0 (C-17), 41.8 (C-18), 46.3 (C-19), 30.8 (C-20), 34.0 (C-21), 32.6 (C-22), 28.0 (C-23), 16.6 (C-24), 15.5 (C-25), 17.5 (C-26), 26.1 (C-27), 176.4 (C-28), 33.2 (C-29), 23.8 (C-30); Sugar moiety: 105.2 (C-1'), 79.7 (C-2'), 75.4 (C-3'), 70.2 (C-4'), 77.7 (C-5'), 62.5 (C-6'); 104.0 (C-1''), 73.9 (C-2''), 75.4 (C-3''), 69.7 (C-4''), 76.5 (C-5''), 62.2 (C-6''); 105.0 (C-1'''), 75.3 (C-2'''), 78.6 (C-3'''), 71.5 (C-4'''), 78.4 (C-5'''), 63.4 (C-6'''); 95.8 (C-1''''), 74.2 (C-2''''), 78.9 (C-3''''), 71.1 (C-4''''), 79.3 (C-5''''), 62.3 (C-6'''').

Araliasaponin XVI (7): $^1\text{H-NMR}$ (400 MHz, pyridine- d_5) δ : 0.83, 0.90, 0.92, 1.10, 1.15, 1.27, 1.35 (3H \times 7), 3.26 (1H, dd, $J = 9.6, 3.2$ Hz, H-3), 5.43 (t-like, H-12), 4.81 (1H, d, H-Gal-1), 5.54 (1H, d, $J = 6$ Hz, H-Gal'-1), 5.30 (1H, d, $J = 6$ Hz, H-Glc-1), 6.33 (1H, d, $J = 6$ Hz, H-Glc'-1). $^{13}\text{C-NMR}$ (100 MHz, pyridine- d_5) δ : 38.8 (C-1), 26.7 (C-2), 89.5 (C-3), 39.8 (C-4), 56.0 (C-5), 18.5 (C-6), 33.2 (C-7), 39.9 (C-8), 48.1 (C-9), 37.0 (C-10), 23.4 (C-11), 122.7 (C-12), 144.2 (C-13), 42.2 (C-14), 28.3 (C-15), 23.7 (C-16), 47.0 (C-17), 41.8 (C-18), 46.2 (C-19), 30.8 (C-20), 34.1 (C-21), 32.6 (C-22), 28.1 (C-23), 16.7 (C-24), 15.5 (C-25), 17.5 (C-26), 26.1 (C-27), 176.3 (C-28), 33.2 (C-29), 23.7 (C-30); Sugar moiety: 105.4 (C-1), 77.6 (C-2), 84.7 (C-3), 69.6 (C-4), 76.1 (C-5), 61.5 (C-6); 104.7 (C-1'), 73.7 (C-2'), 75.4 (C-3'), 69.7 (C-4'), 76.5 (C-5'), 62.2 (C-6'); 105.0 (C-1''), 75.3 (C-2''), 78.6 (C-3''), 71.5 (C-4''), 78.4 (C-5''), 62.4 (C-6''); 95.7 (C-1'''), 74.2 (C-2'''), 78.9 (C-3'''), 71.2 (C-4'''), 79.3 (C-5'''), 62.3 (C-6''').

Syringaresinol (8): $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 6.58 (4 H, s, H-2, 2', 6, 6'), 4.72 (2 H, d, $J = 3.6$ Hz, H-7, 7'), 3.09 (2 H, m, H-8, 8'), 3.90 (2 H, dd, $J = 8.8, 2.8$ Hz, H-9^a, H-9^a'), 4.28 (2 H, dd, $J = 8.8, 6.8$ Hz, H-9^b, 9^b'), 3.90 (12 H, s, $-\text{OCH}_3$, H-3, 3', 5, 5'). $^{13}\text{C-NMR}$ (400 MHz, CDCl_3) δ : 132.2 (C-1, 1'), 102.9 (C-2, 2', 6, 6'), 147.3 (C-3, 3', 5, 5'), 134.5 (C-4, 4'), 86.2 (C-7, 7'), 54.5 (C-8, 8'), 71.9 (C-9, 9'), 56.5 (3, 5, 3', 5'- OCH_3).

3,4-dihydroxybenzoic acid (9): $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 7.33 (1H, d, $J = 2.4$ Hz, H-2), 6.78 (1H, d, $J = 8.0$ Hz, H-5), 7.27 (1H, dd, $J = 8.0, 2.4$ Hz, H-6), 9.26 (1H, brs, -OH), 9.63 (1H, brs, -OH), 12.28 (1H, brs, -COOH).

Coniferaldehyde (10): $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 9.65 (1 H, d, $J = 8.0$ Hz, H-9), 7.40 (1 H, d, $J = 15.6$ Hz, H-7), 7.13 (1H, d, $J = 8.0$ Hz, H-6), 7.07 (1H, d, $J = 1.6$ Hz, H-2), 6.96 (1H, d, $J = 8.0, 1.6$ Hz, H-5), 6.60 (1H, dd, $J = 15.6, 8.0$ Hz, H-8), 3.94 (3H, s, 3- OCH_3). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3)

δ :193.7 (C-9), 153.2 (C-7), 149.1 (C-1), 147.1 (C-4), 126.8 (C-2), 126.5 (C-8), 124.2 (C-5), 115.1(C-6), 109.6 (C-3), 56.1 (3-OCH₃).

Isovanillin (**11**): ¹H-NMR (400 MHz, CDCl₃) δ : 3.96 (3H, s, -OCH₃), 7.05 (1 H, d, *J* = 8.4 Hz, 5-H), 9.82 (1H, s, -CHO), 7.41 (1H, d, *J* = 1.8 Hz, 2-H), 7.42 (1H, dd, *J* = 1.6, 8.4 Hz, 6-H).

β -sitosterol (**12**): ¹H-NMR (400 MHz, CDCl₃) δ : 5.35 (1H, d, H-6), 3.53 (1H, m, H-3), 1.01 (3H, s, *J* = 6.4 Hz, H-19), 0.97 (3H, d, *J* = 6.4 Hz, H-21), 0.87 (3H, d, *J* = 6.0 Hz, H-26), 0.84 (3H, d, *J* = 6.4 Hz, H-27), 0.82 (3H, d, *J* = 6.4 Hz, H-29), 0.69 (3 H, s, H-18).

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Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/RNP>

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