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Research Article

Chemical Constituents from the Roots of *Polygala arillata* and Their Anti-Inflammatory Activities

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A new compound, named arillatanoside E, which was elucidated as 3-O-β-D-glucopyranosyl presenegenin 28-O-β-D-xylopyranosyl-(1 ⟶ 3)-β-D-xylopyranosyl-(1 ⟶ 4)-α-L-rhamnopyranosyl-(1 ⟶ 2)-(4-O-acetyl)-β-D-fucopyranosyl ester, along with 11 known compounds was isolated from the ethanolic extract of the roots of Polygala arillata. The 11 known compounds were identified as oleanolic acid (2), 3′-E-3,4,5-trimethoxy cinnamoyl-6-benzoyl sucrose (3), trans-ferulic acid (4), trans-feruloyl-glucoside (5), feruloyl-glucoside (6), 2,4,6-trimethoxy-1-O-β-D-glycoside (7), 3-methoxy-4-hydroxybenzoic acid (8), monopentadecanoin (9), sinapic acid (10), p-hydroxybenzaldehyde (11), and palmitic acid (12). Among them, seven isolated compounds 1, 2, 4, 5, 7, 8, and 10 exhibited little cytotoxic activity on macrophage RAW 264.7 cells. Then, the inhibitory effects of 7 isolates on nitric oxide (NO) production in lipopolysaccharide-activated macrophages were evaluated. As a result, 3 compounds have significant anti-inflammatory activity, and they were arillatanoside E (1), oleanolic acid (2), and 2,4,6-trimethoxy-1-O-β-D-glycoside (7).

1. Introduction

Polygala arillata Buch.-Ham. ex D. Don belongs to the genus of *Polygala* (Polygalaceae), which is mainly distributed in Nepal, India (Sikkim), Myanmar, and the north of Vietnam. In China, it is mainly distributed in several provinces in the south of the Yangtze River; among them, Yunnan Province has the largest production. The roots and stems of this plant are commonly used to treat irregular menstruation, hepatitis, pneumonia, and so on [1].

Up to now, few compounds have been isolated from the plant of *P. arillata*. It is reported that it mainly contains saponins, oligosaccharides, and ketones [2], which has various biological activities, such as immunoenhancement, sedation and hypnotism, antibacterial activity, and dispelling cold and relieving pain. [2]. To further study the chemical constituents of the roots of *P. arillata*, several kinds of chromatographic methods such as silica gel column chromatographic technology, octadecylsilyl (ODS) column chromatographic technique, Sephadex LH-20 column, and preparative high performance liquid chromatography (Pr-HPLC) were used. As a result, a new compound, named arillatanoside E, which was elucidated as 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-xylopyranosyl-(1 ⟶ 3)-β-D-xylopyranosyl-(1 ⟶ 4)-α-L-rhamnopyranosyl-(1 ⟶ 2)-(4-*O*-acetyl)-β-D-fucopyranosyl ester, along with 11 known compounds was isolated from the ethanolic extract of the roots of *P. arillata*. The other 11 known compounds were identified as oleanolic acid (**2**) [3], 3′-E-3,4,5-trimethoxy cinnamoyl-6-benzoyl sucrose (**3**) [4], *trans*-ferulic acid (**4**) [5], *trans*-feruloyl-glucoside (**5**) [6], feruloyl-glucoside (**6**) [6], 2,4,6-trimethoxy-1-*O*-β-D-glycoside (**7**) [7], 3-methoxy-4-hydroxybenzoic acid (**8**) [8], monopentadecanoin (**9**) [9], sinapic acid (**10**) [10], *p*-hydroxybenzaldehyde (**11**) [11], and palmitic acid (**12**) [12] by means of mass spectrometry (MS),

ultraviolet spectrophotometry (UV), and nuclear magnetic resonance (NMR) spectroscopy, including 1D-NMR and 2D-NMR, such as ^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, and ^{1}H-^{1}H COSY.

During inflammation, activation of macrophages leads to the production of many proinflammatory cytokines, prostaglandin E_2, and nitric oxide (NO). NO is a short-lived free radical and is synthesized by NO synthase (NOS). Three different isoforms of NOS exist and are referred to as neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) [13]. Lipopolysaccharides (LPS) are constituents of the cell walls of Gram-negative bacteria and act as the activator of the immune system. LPS-stimulated macrophages induce the expression of iNOS, which stimulates NO production [13].

NO plays a key role in the regulation of cytokines especially related to inflammation and has anti-inflammatory with cytotoxic effects in inflammatory reaction [13, 14]. In this case, by measuring the amount of NO produced by mouse macrophages, we can screen out the compounds with anti-inflammatory activity in *P. arillata* [15]. Here, the concentration of NO secreted by cells was detected by the Griess reagent method, and the potentially anti-inflammatory activity of the compounds was screened [16].

We observed that with higher three compounds (**1**, **2**, **7**) concentration, the levels of nitrite concentration were decreased, which indicated that the three compounds have potential anti-inflammatory activity against the damage induced by LPS, and **1**, **2** and **7** may be therapeutic agents for inflammation. However, among the three compounds, **1** and **7** with 12.5 μg/mL had no significant inhibitory effect for NO, maybe because the drug concentration was too low to reach the effective concentration. Our results will promote further application of the three compounds in pharmacology.

2. Experimental

2.1. General. HPLC was run on Agilent 1260 HPLC (Agilent, Palo Alto, CA, USA). Semipreparative HPLC was performed on Waters 2489 equipped with a diode array detector and a C_{18} column (250 mm × 10 mm, 5 μm; Waters, Maple St. Milford, MA, USA). NMR spectra were measured on an AV-400 spectrometer (Bruker Corporation, Faellanden, Switzerland) using methanol-d_4 (CD_3OD), chloroform-*d* ($CDCl_3$), and dimethyl sulfoxide-d_6 (DMSO-d_6) (Sigma-Aldrich, St. Louis, MO, USA) as solvent. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed on an electrostatic field orbital trap mass spectrometer (Thermo Scientific, Bremen, Germany) using an ESI source. Thin-layer chromatography (TLC) was performed on glass-precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), detection under UV light or by heating after spraying with 10% sulfuric acid (H_2SO_4) in 90% ethanol (EtOH). Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), and Sephadex LH-20 (Pharmacia Biotech, Switzerland) were used for the chromatography column. Other chemicals and reagents of analytical grade were from Tianjin Concord Technology (Tianjin, China).

2.2. Plant Material. The roots of *P. arillata* were collected from Yunnan province, China. The plant material was identified by Associate Prof. Chun-hua Wang of the School of Pharmaceutical Engineering, Tianjin University of Traditional Chinese Medicine and a voucher specimen (No. 201612CH03) has been deposited in Tianjin International Joint Academy of Biotechnology and Medicine.

2.3. Extraction and Isolation. The extraction process was as follows: 30 kg of air-dried samples were extracted three times with 75% aqueous ethanol solution (v/v) at room temperature by infusion each for a week. The isolation process was as follows: (1) The extracting solution was merged for rotatory evaporation till no alcohol taste. (2) The liquid remaining in the still was suspended in water (H_2O) and then partitioned successively with petroleum ether, ethyl acetate (EtOAc), and *n*-butyl alcohol to give petroleum ether, EtOAc (A, 100.0 g), and *n*-butyl alcohol (B, 300.0 g) fractions, respectively. (3) Fractions A and B were chromatographed over silica gel, eluting with methanol (CH_3OH) in dichloromethane (0–100%, stepwise), yielding ten fractions (Fr.1–Fr.10), respectively. (4) The crude fraction Fr.5 (13.6 g) (fraction A) was further purified by semipreparative HPLC using CH_3OH and 0.1% formic acid solution in water as the mobile phase, to yield compounds **4** (16 mg), **5** (32 mg), **6** (7 mg), **7** (12 mg), **8** (12 mg), **9** (8 mg), **10** (17 mg), **11** (6 mg), and **12** (9 mg). (5) The total fractions Fr.5 (11.3 g) and Fr.8 (17.6 g) (fraction B) were chromatographed over a silica gel column eluting with dichloromethane-methanol (CH_2Cl_2-CH_3OH) (0–100%, stepwise), to afford compound **1** (8 mg), **2** (11 mg), and **3** (4 mg).

*2.4. Arillatanoside E (**1**).* It is white powder; for ^{1}H-NMR, ^{13}C-NMR, and DEPT-135 spectroscopic data, see Table 1 and Figures S1–S3 (Supplementary Materials); ^{1}H-^{1}H COSY, HSQC, and HMBC, see Figures S4–S6 (Supplementary Materials); HR-ESI-MS: *m*/*z* 1277.5839 $[M\text{-}H]^-$ (Calc. for $C_{60}H_{94}O_{29}$: 1277.5803), see Figure S7 (Supplementary Materials).

2.5. Acid Hydrolysis and HPLC Analysis. 4 mg of arillatanoside E was accurately weighed and dissolved in 2 M hydrogen chloride (HCl) (2 mL) for 1 h at 85°C in the reaction bottle and then extracted twice with isovolumic EtOAc. After vacuum evaporation of water layer, the residue was dissolved in 2 mL pyridine containing L-cysteine methyl ester (4 mg) and *O*-tolyl isothiocyanate (4 mg) and reacted at 60°C for 1 h, respectively, and successively [13]. Derivatives were further detected with a steady flow (0.8 mL/min) and column temperature (35°C) by HPLC using 25% acetonitrile-water (0.01% formic acid (HCOOH)) as the mobile phase, and the eluate was monitored at 250 nm. As in the above case, the standard sugar samples (1 mg) were

Table 1: The spectral data of ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) of compound 1 in CD3OD.

Position	δ_H	δ_C	Position	δ_H	δ_C
1	2.12 (1H, m), 1.21 (1H, m)	44.6	3-*O*-Glc-1	4.55 (1H, d, J = 7.8 Hz)	105.6
2	3.55 (1H, m)	70.9	Glc-2	3.95 (m)	75.1
3	3.50 (1H, m)	87.7	Glc-3	3.26 (m)	77.7
4	—	53.2	Glc-4	3.92 (m)	71.7
5	1.70 (1H, m)	52.9	Glc-5	3.26 (m)	77.7
6	1.67 (1H, m), 1.21 (1H, m)	21.6	Glc-6	3.82 (m), 3.72 (1H, dd, J = 6.6, 4.8 Hz)	62.3
7	1.67 (1H, m), 1.27 (1H, m)	34.3	28-*O*-Fuc-1	5.44 (1H, d, J = 8.4 Hz)	95.0
8	—	41.7	Fuc-2	3.85 (m)	72.3
9	1.89 (1H, m)	50.1	Fuc-3	3.38 (m)	75.5
10	—	37.5	Fuc-4	5.10 (1H, d, J = 3.6 Hz)	75.3
11	1.99 (1H, m), 1.64 (1H, m)	23.9	Fuc-5	4.32 (m)	71.2
12	5.67 (1H, t)	128.8	Fuc-6	1.10 (3H, d, J = 6.6 Hz)	16.5
13	—	139.3	Ac at 4	2.19 (3H, s)	20.8
14	—	48.6	—	—	172.8
15	1.64 (1H, m), 1.44 (1H, m)	24.9	Rha-1	5.46 (1H, brs)	101.1
16	1.99 (1H, m), 1.91 (1H, m)	24.7	Rha-2	3.55 (m)	71.1
17	—	47.9	Rha-3	3.84 (m)	73.7
18	2.92 (1H, dd, J = 10.2, 3.6 Hz)	42.8	Rha-4	3.54 (m)	85.0
19	1.62 (1H, m), 1.23(1H, m)	46.2	Rha-5	3.87 (m)	68.5
20	—	31.6	Rha-6	1.32 (3H, d, J = 6.6 Hz)	18.2
21	1.39 (1H, s), 1.27 (1H, m)	34.7	Xyl-1 (inner)	4.51 (1H, d, J = 7.8 Hz)	106.8
22	1.75 (1H, m), 1.61 (1H, m)	33.0	Xyl-2	3.95 (m)	75.1
23	—	181.9	Xyl-3	4.13 (1H, d, J = 3.6 Hz)	85.9
24	1.40 (3H, s)	13.7	Xyl-4	3.54 (m)	69.4
25	1.29 (3H, s)	17.7	Xyl-5	3.77 (m), 3.56 (m)	66.5
26	0.76 (3H, s)	18.9	Xyl-1 (outer)	4.36 (1H, d, J = 7.8 Hz)	104.9
27	3.77 (1H, m), 3.55(1H, m)	64.7	Xyl-2	3.95 (m)	75.0
28	—	177.8	Xyl-3	3.37 (m)	77.6
29	0.94 (3H, s)	33.4	Xyl-4	3.27 (m)	71.1
30	0.97 (3H, s)	24.1	Xyl-5	3.92 (m), 3.24 (m)	67.2

subjected to the same reaction and HPLC conditions. Then, the retention times of the derivatized sugars in HPLC were 17.464 (for D-glucose), 20.496 (for D-xylose), 24.340 (for D-fucose), and 30.146 min (for D-rhamnose), respectively.

2.6. Biological Activity Assays

2.6.1. Cell Culture. RAW 264.7 mouse macrophages were obtained from the Binhai lab (Bio-Swamp, MD, USA) and cultured in Dulbecco's modified eagle medium (DMEM) (high glucose), complete medium, which was refreshed every other day supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in an incubator (STIK (Shanghai) Co., Ltd., Shanghai, China) which is a 5% carbon dioxide-(CO_2-) humidified atmosphere. The subculture was carried out at 2 to 3 day intervals. When the cells were approximately 80% confluent, cells were kept in the logarithmic phase by cell scraper and subcultured.

2.6.2. CCK-8 Cell Viability Assay. The cells in the logarithmic phase were seeded in 96-well culture plates at 1×10^4 cells per well (100 μL) and incubated for 2 h for adhesion at 37°C in a humidified atmosphere with 5% CO_2. Meanwhile, blank control wells were added to 100 μL of culture medium and maintained under the same conditions. Next, 100 μL of the above culture medium containing three concentrations of 7 compounds (50, 25, and 12.5 μg/mL), dexamethasone sodium phosphate (5 μg/mL), and normal culture medium (untreated control group) were added, respectively, and incubated for 1 h. Moreover, in the dark environment, 10 μL of CCK-8 was added to each well and incubated for 1 h to 4 h. Last, the absorbance at 450 nm was measured with an enzyme-linked immunosorbent reader (FlexStation 3, Molecular Devices, San Francisco, CA, USA). The survival rate of cell was calculated as survival rate (%) = $(A_{sample} - A_{blank})/(A_{control} - A_{blank}) \times 100\%$.

2.6.3. Anti-Inflammatory Activity Assay. 500 μL of the above cells in the logarithmic phase were seeded in 24-well culture plates at 5×10^5 cells each well and incubated for 2 h for adhesion at 37°C in a humidified atmosphere with 5% CO_2. After the culture medium was replaced by the medium with different concentrations of 7 compounds (50, 25, 12.5 μg/mL), dexamethasone sodium phosphate (5 μg/mL), and normal culture medium (untreated control group), the volume of them were 400 μL, respectively, and incubated for 1 h, and all of them except the blank control group were exposed to 0.1 μg/mL LPS (100 μL) for 16 h. After that, all of the supernatant was collected which were divided into three wells (50 μL), added to 96-well culture plates, and mixed with an equal volume (50 μL) of Griess reagents

I (Sulfanilamide Solution) and II (NED). Absorbance values were measured at 548 nm using a microplate reader. Here, sodium nitrite was used to generate a standard reference curve (Figure S8), and the curve is obtained as follows: $y = 0.0072x + 0.0557$ ($R^2 = 0.9995$).

2.7. Statistical Analysis. The *t*-test was used to analyze the differences between groups of data. Testing the significance of difference among groups by the statistical method with SPSS version 20.0 (International Business Machines Corporation, Armonk, NY, USA) and making statistical diagram by using GraphPad Prism version 5.0 (GraphPad Software Inc., La Jolla, CA, USA) were further made. p values less than 0.05 ($p < 0.05$) were considered statistically significant. p values less than 0.01 ($p < 0.01$) were considered of notable statistically significance.

3. Results and Discussion

3.1. Structure Elucidation. The known compounds (**2**–**12**) (Figure 1) were identified by comparison of their experimental spectral data with literature data [3–12].

The structure of the new compound **1** was elucidated on the basis of extensive NMR spectroscopic analysis, including a series of 2D-NMR experiments (Figure 2) (heteronuclear singular quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC)), and MS data.

Compound **1** was obtained as a white powder with the molecular formula of $C_{60}H_{94}O_{29}$ based on the negative ion peak at m/z 1277.5839 $[M\text{-}H]^-$ (calculated 1277.5803), implying 14 degrees of unsaturation. The ^{1}H-NMR spectrum revealed the presence of two tertiary methyl protons at δ_H 1.32 (3H, d, J = 6.6 Hz) and 1.10 (3H, d, J = 6.6 Hz), five quaternary methyl protons at δ_H 1.40 (3H, s), 1.28 (3H, s), 0.97 (3H, s), 0.94 (3H, s), and 0.76 (3H, s), an olefinic proton at δ_H 5.67 (1H, t), and five anomeric protons at δ_H 5.46 (1H, brs), 5.44 (1H, d, J = 8.4 Hz), 4.55 (1H, d, J = 7.8 Hz), 4.51 (1H, d, J = 7.8 Hz), and 4.36 (1H, d, J = 7.8 Hz). The ^{13}C-NMR spectrum displayed the presence of six quaternary carbons at δ_C 31.6, 37.5, 41.7, 47.9, 48.6, and 53.2, an oxygen-bearing methylene carbon at δ_C 64.7, two oxygen-bearing methine carbon at δ_C 70.9 and 87.7, five anomeric carbons at δ_C 95.0, 101.1, 104.9, 105.6, and 106.8, a set of olefinic carbons at δ_C 128.8 and 139.3, an ester carbonyl carbon at δ_C 177.8, and a carboxyl carbon at δ_C 182.9. All the above spectral information showed that the aglycone moiety of **1** was presenegenin [17] which belonged to oleanane triterpenoidal saponin, meanwhile combined with the chemical shift of the deshielded carbon C-3 (δ_C 87.7) and the carbonyl C-28 (δ_C 177.8) of presenegenin indicated that compound **1** was the 3,28-bisdesmoside of presenegenin, having five monosaccharide units.

On acid hydrolysis, compound **1** afforded an aglycone which was identified as presenegenin on the basis of the ^{1}H-NMR and ^{13}C-NMR data [17]. The sugars obtained from the saponin hydrolysate were identified as D-glucose, D-fucose, D-xylose, and L-rhamnose based on HPLC analysis (Figure 3). The NMR (Table 1) data of compound **1**, along with the HSQC spectrum, showed the five anomeric proton signals at δ_H 5.46 (1H, brs), 5.44 (1H, d, J = 8.4 Hz), 4.55 (1H, d, J = 7.8 Hz), 4.51 (1H, d, J = 7.8 Hz), and 4.36 (1H, d, J = 7.8 Hz) and were correlated with anomeric carbon signals at δ_C 101.1, 95.0, 105.6, 106.8, and 104.9, respectively. Combined ^{13}C-NMR with distortionless enhancement by polarization transfer (DEPT) data, the carbon signals of five monosaccharide units had been listed as follows: Glc's carbon signals at δ_C 105.6 (Glc-1), 75.1 (Glc-2), 77.7(Glc-3), 71.7 (Glc-4), 77.7 (Glc-5), and 62.3 (Glc-6); Fuc's carbon signals at δ_C 95.0 (Fuc-1), 72.3 (Fuc-2), 75.4 (Fuc-3), 75.3 (Fuc-4), 71.2 (Fuc-5), and 16.5(Fuc-6); Rha's carbon signals at δ_C 101.1 (Rha-1), 71.1 (Rha-2), 73.7 (Rha-3), 85.0 (Rha-4), 68.5 (Rha-5), and 18.2 (Rha-6); Xyl's (inner) carbon signals at δ_C 106.8 (Xyl-1), 75.1 (Xyl-2), 85.9 (Xyl-3), 69.4 (Xyl-4), 65.5 and (Xyl-5); Xyl's (outer) carbon signals at δ_C 104.9 (Xyl-1), 75.0 (Xyl-2), 78.1 (Xyl-3), 71.1 (Xyl-4), and 67.2 (Xyl-5). With the analyzing results of HSQC, HMBC, DEPT, and NMR, five monosaccharide units of **1** were identified as one β-D-glucopyranosyl (Glc), one β-D-fucopyranosyl (Fuc), one α-L-rhamnopyranosyl (Rha) and two β-D-xylopyranosyls (Xyl). The positions of the sugar components in **1** were clarified by an HMBC experiment (Figure 2), which showed a correlation between δ_H 4.55 (Glc-1) and δ_C 87.7 (C-3) revealing that Glc was located at the position of C-3 of presenegenin, δ_H 5.44 (Fuc-1) and δ_C 177.8 (C-28) revealing that Fuc was located at the position of C-28 of presenegenin, δ_H 4.51 (Xyl-1, inner) and δ_C 85.0 (Rha-4) revealing that Xyl (inner) was located at the position of C-4 of Rha, δ_H 4.36 (Xyl-1, outer) and δ_C 85.9 (Xyl-3, inner) revealing that Xyl (outer) was located at the position of C-3 of Xyl (inner), and δ_H 5.48 (Rha-1) and δ_C 72.3 (Fuc-2) revealing that Rha was located at the position of C-2 of Fuc.

What is noteworthy is that, in the HMBC spectrum, a correlation between the methyl proton at δ_H 2.18 (CH_3CO-) and δ_C 172.8 (CH_3CO-) and between δ_H 5.10 (Fuc-4) and 172.8 (CH_3CO-) indicated acetyl existed and methyl was attached by an ester linkage to the position of C-4 of Fuc. In addition, compared with arillatanoside A [17], the stereochemical structure of compound **1** had not changed. Synthesizing the above analysis of all the proton and carbon signals, we established the structure of compound **1**, which was named as arillatanoside E, as 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-xylopyranosyl-(1 ⟶ 3)-β-D-xylopyranosyl-(1 ⟶ 4)-α-L-rhamnopyranosyl-(1 ⟶ 2)-(4-*O*-acetyl)-β-D-fucopyranosyl ester.

3.2. Biological Activity Assays. The roots of *P. arillata* have anti-inflammatory effect, but its active components are not clear. Here, by measuring the amount of inhibition of LPS-induced NO production in RAW 264.7 mouse macrophages, 7 out of 12 compounds were selected for primary screening of anti-inflammatory components [15, 18].

3.2.1. Cytotoxic Activity Assay. NO participates in the regulation of cytokines which is especially associated with inflammation and has anti-inflammatory protective effect and cytotoxicity in inflammatory reaction [15]. Meanwhile,

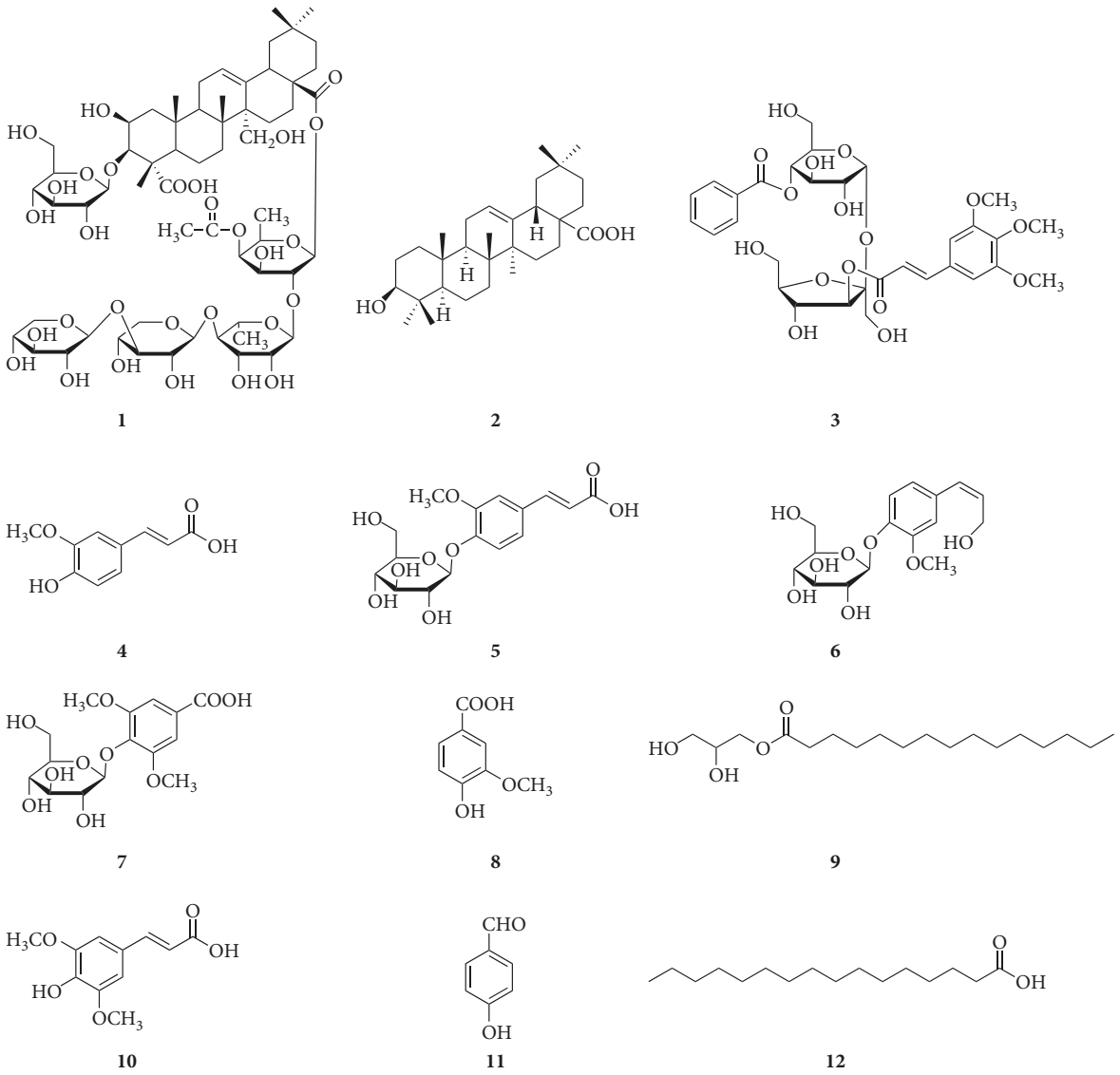


Figure 1: Chemical structures of compounds **1**–**12**.

NO is an effector in cytotoxicity of activated macrophages [15]. To further confirm, cytotoxicity screenings on the isolated compounds were measured by cell counting kit-8 (CCK-8) assay in LPS-induced RAW 264.7 macrophage cells. 7 compounds including arillatanoside E (**1**), oleanolic acid (**2**), *trans*-ferulic acid (**4**), *trans*-feruloyl-glucoside (**5**), 2,4,6-trimethoxy-1-*O*-*β*-D-glycoside (**7**), 3-methoxy-4-hydroxybenzoic acid (**8**), and sinapic acid (**10**) were tested against RAW 264.7 macrophage cells using cell counting kit-8 (CCK-8) (Dojindo, Japan). The results (Figure 4) showed that there was no significant cytotoxicity for the compounds at the corresponding concentrations, which also means its cytotoxicity had no impact on anti-inflammatory activity.

3.2.2. Anti-Inflammatory Activity Assay. As an important physiological messenger and effector molecule, NO can regulate various physiological and pathological responses. However, it was rapidly metabolized into nitrite, which was relatively stable and could be detected by the Griess reagent kit (Promega, USA). For this, the Griess reagent kit was used to determine the level of NO which was secreted by macrophage cells, and 7 isolated compounds (**1**, **2**, **4**, **5**, **7**, **8**, and **10**) which did not exhibit cytotoxic activity on LPS-induced RAW 264.7 mouse macrophages were screened for anti-inflammatory activity with the LPS group as control. Meanwhile, dexamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$) was taken as the positive control group [18]. Compared with the positive control group and negative control group, the results (Figure 5) indicated that three compounds (**1**, **2**, and **7**) had significant anti-inflammatory activity and **5** had anti-inflammatory activity at the highest concentration, while the other three compounds (**4**, **8**, and **10**) without anti-inflammatory activity. With higher three compounds (**1**, **2**, and **7**) concentration, the levels of nitrite concentration were decreased, which indicated that the three compounds have potential anti-inflammatory activity

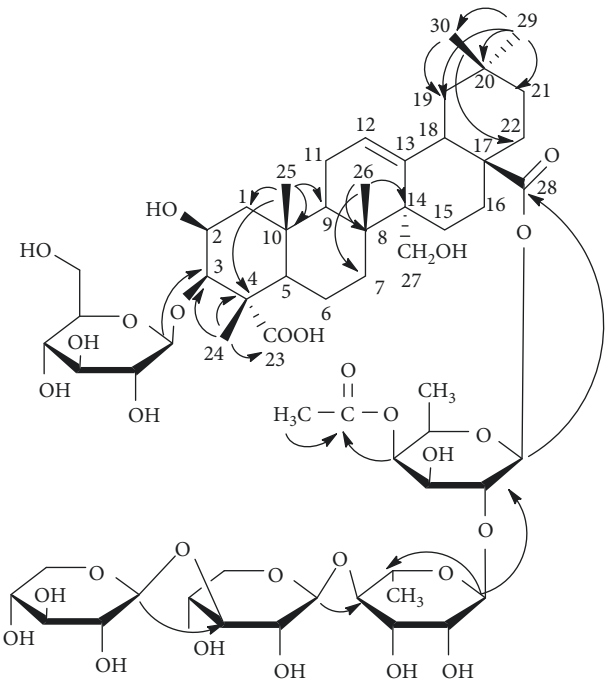


Figure 2: Key HMBC correlations of compound **1** (arrows point from proton to carbon).

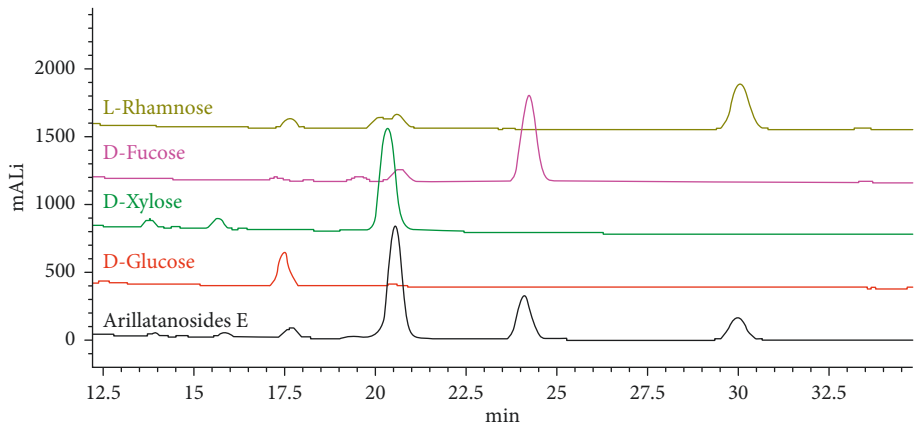


Figure 3: The hydrolyzed sugar's identification chromatogram of compound **1**.

against the damage induced by LPS and **1**, **2**, and **7** may be therapeutic agents for inflammation. However, among the three compounds, **1** and **7** with 12.5 μg/mL had no significant inhibitory effect for NO, maybe the drugs concentration was too low to reach the effective concentration. In addition, the compound **5** was effective at the highest concentration, for the effective concentration was too high, in-depth research on it is unnecessary either in theory or in reality.

4. Conclusion

In this research, a new compound, named as arillatanoside E (**1**), which was established as a derivative of the chemotaxonomic marker 3-*O*-(β-D-glucopyranosyl) presenegenin 28-[*O*-β-D-xylopyranosyl-(1 $\longrightarrow$ 4)-*O*-α-L-rhamnopyranosyl-(1 $\longrightarrow$ 2)-β-D-fucopyranosyl] ester, 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-xylopyranosyl-(1 $\longrightarrow$ 3)-β-D-xylopyranosyl-(1 $\longrightarrow$ 4)-α-L-rhamnopyranosyl-(1 $\longrightarrow$ 2)-(4-*O*-acetyl)-β-D-fucopyranosyl ester, was found in *P. arillata*. During the past ten years' literatures, we have also found that two compounds, 3-*O*-(β-D-glucopyranosyl) presenegenin 28-{*O*-β-D-galactopyranosyl-(1 $\longrightarrow$ 3)-*O*-β-D-xylopyranosyl-(1 $\longrightarrow$ 4)-*O*-α-L-rhamnopyranosyl-(1 $\longrightarrow$ 2)-β-D-fucopyranosyl} ester [19] and arillatanoside A [17], have the same structure character and were found in this genus, whose type may be the chemotaxonomic marker of *Polygala* genus. On the other hand, more than 100 saponins have been isolated from *Polygala* genus, and their aglycones are almost oleanane triterpenoids. Thus, in our opinion, oleanane triterpenoids with diverse biological activities [20] are the chemotaxonomic markers of this genus. Furthermore, seven isolated compounds (**1**, **2**, **4**, **5**, **7**, **8**, and **10**) exhibited little cytotoxic activity on RAW

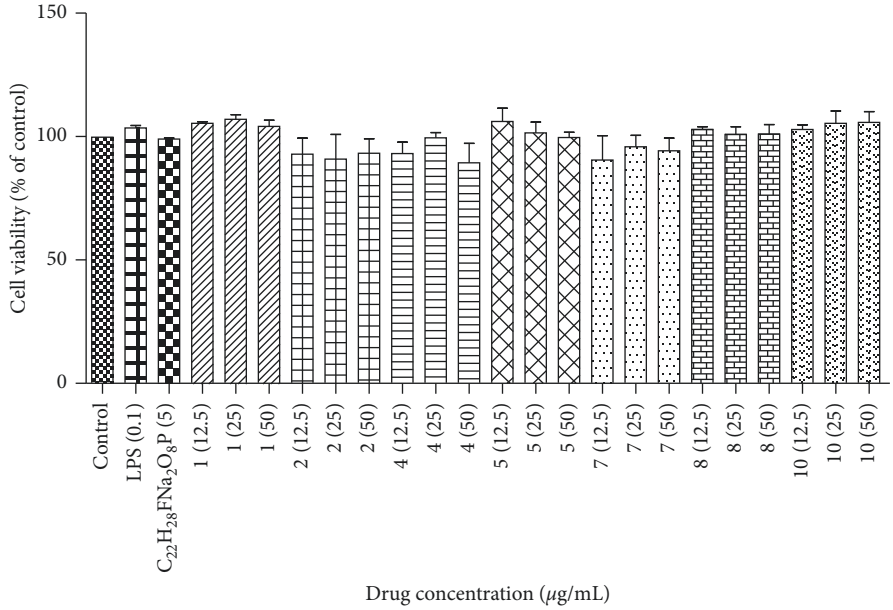


Figure 4: The effect of the isolated compounds on the survival rate of RAW 264.7 macrophage cells. The cells were pretreated with concentrations (12.5, 25, and 50 μg/mL) of compounds (**1**, **2**, **4**, **5**, **7**, **8**, and **10**) or $C_{22}H_{28}FNa_2O_8P$ (5 μg/mL) for 1 h. The data show the mean ± SD of three independent experiments performed in triplicates. Arillatanoside E (**1**), oleanolic acid (**2**), *trans*-ferulic acid (**4**), *trans*-feruloyl-glucoside (**5**), 2,4,6-trimethoxy-1-*O*-β-D-glycoside (**7**), 3-methoxy-4-hydroxybenzoic acid (**8**), and sinapic acid (**10**).

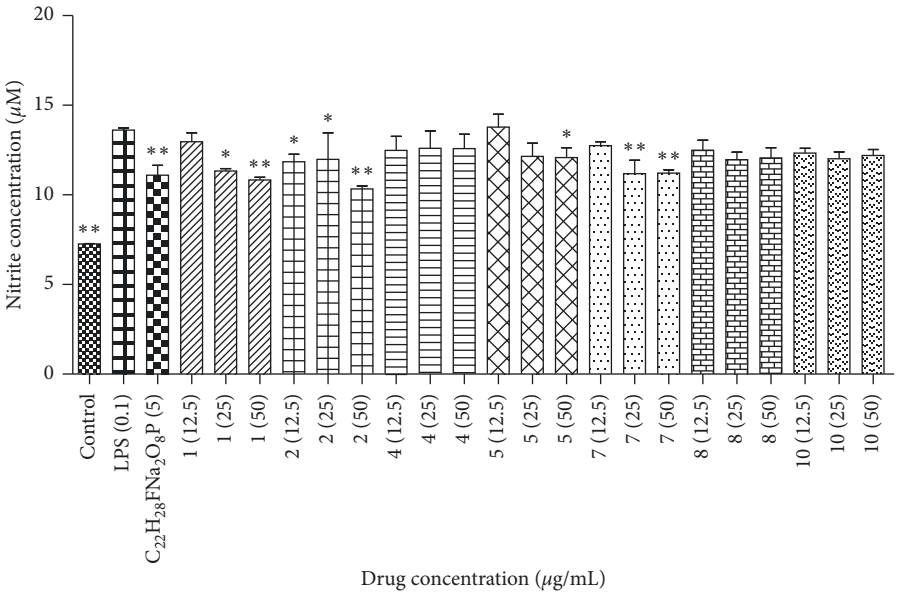


Figure 5: Anti-inflammatory effects of the isolated compounds and inhibition of LPS-induced NO production in RAW 264.7 mouse macrophages. The cells were pretreated with concentrations (12.5, 25, and 50 μg/mL) of compounds (**1**, **2**, **4**, **5**, **7**, **8**, and **10**) or $C_{22}H_{28}FNa_2O_8P$ (5 μg/mL) for 1 h and then stimulated with LPS (0.1 μg/mL) for 16 h. The data show the mean ± SD of three independent experiments performed in triplicates. $^*p < 0.05$ and $^{**}p < 0.01$ compared to the LPS-treated values. Arillatanoside E (**1**), oleanolic acid (**2**), *trans*-ferulic acid (**4**), *trans*-feruloyl-glucoside (**5**), 2,4,6-trimethoxy-1-*O*-β-D-glycoside (**7**), 3-methoxy-4-hydroxybenzoic acid (**8**), and sinapic acid (**10**).

264.7 mouse macrophages; then, the inhibitory effects of 7 isolates on nitric oxide (NO) production in lipopolysaccharide-activated macrophages were evaluated. As a result, arillatanoside E (**1**), oleanolic acid (**2**), and 2,4,6-trimethoxy-1-*O*-β-D-glycoside (**7**) possessed potential anti-inflammatory activity.

Data Availability

The NMR and MS data used to support the findings of this study are included within the supplementary information file. The other data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

In this paper, Chun-hua Wang, Zheng Li, and Fang-Yi Li conceived and designed the experiments; Wei Xiang, Guo-Dong Zhang, and Teng-long Wang performed the experiments; Wei Xiang, Tong-Chuan Suo, and Teng-long Wang analyzed the data; and Wei Xiang, Chun-hua Wang, Zheng Li, and Yan Zhu wrote the paper.

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Supplementary Materials

Figure S1: the ^{1}H-NMR spectrum of compound **1**. Figure S2: the ^{13}C-NMR spectrum of compound **1**. Figure S3: the DEPT-135 spectrum of compound **1**. Figure S4: the HMBC spectrum of compound **1**. Figure S5: the ^{1}H-^{1}H COSY spectrum of compound **1**. Figure S6: the HSQC spectrum of compound **1**. Figure S7: the HR-ESI-MS spectrum of compound **1**. Figure S8: the standard reference curve of sodium nitrite. (*Supplementary Materials*)

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