Chemical Constituents of *Eleutherococcus sessiliflorus* (Rupr. & Maxim.)

Ye Ma¹,², Donghu Zhang¹,², and Mingyan Jiang¹,²

**Abstract**

Ten compounds were separated and purified by chromatographic methods from the 70% ethanol extract of the leaves of *Eleutherococcus sessiliflorus* (Rupr. & Maxim.). Their structures were identified according to their physicochemical properties and the spectral data of dihydromyricetin (1), quercetin (2), taxifolin (3), naringenin (4), liquiritigenin (5), butein (6), syringaresinol (7), dehydrodiconiferyl alcohol (8), gallic acid methyl ester (9), and alphitolic acid (10). Compounds 1, 3, 6, and 10 were isolated from the genus *Eleutherococcus* for the first time. All compounds showed weak cytotoxic activity against the A549 cell line.

**Keywords**

*Eleutherococcus sessiliflorus*, chemical constituent, flavones, phenolic acid, separation and purification

Received: November 18th, 2019; Accepted: December 30th, 2019.

The leaves of *Eleutherococcus sessiliflorus* (Rupr. & Maxim.) S.Y.Hu, commonly known as Ciwujia, have been used in traditional Chinese medicine for the treatment of rheumatism.¹ It has been reported that *E. sessiliflorus* is rich in flavonoids,² terpenoids,³-⁵ and xylogen.⁶ As a continuation of our phytochemical investigations, 6 flavonoids, 3 phenolic acids, and 1 triterpenoid were isolated from the n-butanol solvent fraction of the leaves of *E. sessiliflorus*. Herein, we report the isolation, structural elucidation, and cytotoxic activity of the compounds.

**Results and Discussion**

The 70% ethanol extract of the dried leaves of *E. sessiliflorus* was subjected to multiple chromatographic steps over silica gel, Sephadex LH-20 and recycling preparative high-performance liquid chromatography (HPLC). Ten compounds were obtained: dihydromyricetin (1),⁷ quercetin (2),⁸ taxifolin (3),⁹ naringenin (4),¹⁰ liquiritigenin (5),¹¹ butein (6),¹² syringaresinol (7),¹³ dehydrodiconiferyl alcohol (8),¹⁴ gallic acid methyl ester (9),¹⁵ and alphitolic acid (10).¹⁶ The structures of 1-10 (Figure 1) were identified by physical data analyses, including 1-dimensional and 2-dimensional nuclear magnetic resonance (NMR), and high-resolution electrospray ionisation-mass spectrometry (ESI-MS).

The *Eleutherococcus* Maxim. (*Acanthopanax* [Decne. Et Planch] Witte) is a genus of 38 species growing in Eastern Asia, from the Himalaya to Vietnam, and from Northeastern Russia to North Philippines, with 18 of them coming from China.¹⁷ The main chemical substances are eleutherosides and flavonoids. The roots of *Eleutherococcus senticosus* are a source of flavonoids (hyperin, rutin, afzelin, quercetin, naringenin, and kaempferol), phenylpropiolic acids, triterpenic acids, and anthocyanins.¹⁸ Compounds 1 and 3 were isolated from genus *Eleutherococcus* for the first time. Compound 6, named butein, was first isolated from *Rhus verniciflua*. This structure was responsible for various activities such as inhibiting the activation of protein tyrosine kinase, nuclear factor kappa-B, signal transducers and activators of transcription 3, and epidermal growth factor receptor.¹⁹ Compound 10, a triterpenoid compound, is abundant in *Ziziphus jujuba* Mill. This compound may have potential to be developed as a dietary supplement.²⁰ The in vitro cytotoxic activities of all compounds are shown in Table 1. The 6 flavonoids, the 3 phenolic acids, and the triterpenoid isolated from the leaves of *E. sessiliflorus* showed weak cytotoxicity against the A549 cell line.

**Experimental**

**General Experimental Procedures**

The NMR spectra were measured in methanol-*d₄* on a Bruker ARX-400 or AV600 instrument with tetramethylsilane as an internal standard. The 1H and 13C chemical shifts were measured relative to tetramethylsilane. The 1H-1H COSY and HSQC experiments were used to determine the connectivity of protons and carbons. The HSQC-TOCSY experiments were used to determine the spin systems of the molecules. The 2D NMR spectra were recorded on a Bruker ARX-400 or AV600 instrument with tetramethylsilane as an internal standard.

1Department of Pharmacy, The First Hospital of China Medical University, Shenyang, Liaoning Province, China
2School of Pharmacy, China Medical University, Shenyang, Liaoning Province, China

Corresponding Author:
Mingyan Jiang, The First Hospital of China Medical University, Nanjingbei Street 155, Shenyang, Liaoning Province 110001, China.
Email: syjmy@126.com
internal standard. The circular dichroism spectrum was tested using the JASCO pu-2080 spectrometer. Infrared spectra were taken on a Bruker IFS-55 infrared spectrophotometer with a KBr disk. Optical rotations were measured on a Peking-Elmer 241 MC Spectropolarimeter at 20°C. ESI-MS spectra were recorded on Waters Quattro micro API LC/MS/MS spectrometer (Waters) with C18 reversed-phase column (HS12S05-2546WT 5 µm; 250 × 4.6 mm [YMC, Japan]; 25°C). HPLC was performed on JAI LC9103 recycling preparative HPLC (Japan Analytical Industries) equipped with JAIGEL-ODS-AP-P column (YMC-Pack ODS-AQ 15 µm; 500 × 20 mm [YMC, Japan]; 25°C) using a JAI refractive index detector and a JAI UV-3702 detector with MultiChro 2000 workstation. Thin-layer chromatography (TLC) was performed on precoated GF254 plates (Merck) and detected by spraying with 10% sulfuric acid followed by heating. The mobile phase for TLC was eluted with dichloromethane (CH2Cl2)–methanol (MeOH) (100:5).

**Plant Material**

The leaves of *E. sessiliflorus* were collected in October 2017 at Dandong, Liaoning, China, and authenticated by Professor Mingyan Jiang (The School of Pharmacy, China Medical University). A voucher specimen has been deposited in our laboratory (voucher No. MY-2017-012).

**Extraction and Isolation**

The leaves of *E. sessiliflorus* (7 kg) were extracted 3 times (3 × 30 L) with 70% (v/v) aqueous ethanol under reflux at 80°C to give 500 g of crude extract. The crude extract was suspended in 2 L of water. The suspension was successively partitioned with ethylacetate (3 × 2 L) and n-butanol (BuOH) (3 × 2 L). The n-BuOH soluble fraction (80 g) was subjected to silica gel column chromatography (CC), eluting with gradients of CH2Cl2–MeOH (100:1 2500 mL; 100:3 2500 mL; 100:7 3000 mL; 100:15 2500 mL; 100:30 2500 mL; 100:70 1500 mL) on silica gel column (column dimension: 6.0 × 80 cm) to give 6

| Table 1. Cytotoxicity Data of Isolated Compounds 1-10. |
|------------------------------------------------------|
| **Compound** | **IC50** | **A549** |
| 1            | 98.1     |          |
| 2            | 66.7     |          |
| 3            | >100     |          |
| 4            | >100     |          |
| 5            | >100     |          |
| 6            | 88.2     |          |
| 7            | >100     |          |
| 8            | 69.1     |          |
| 9            | >100     |          |
| 10           | 55.7     |          |
| Etoposideb   |          | 5.1      |

IC50, half-maximal inhibitory concentration. A549: human lung cancer cell line. 
Data expressed as IC50 values (µM).

[1] Data expressed as IC50 values (µM).

[2] Positive control.
fractions, Fr. A–F (2.3 g, 5.1 g, 10.9 g, 5.1 g, 3.3 g, and 0.2 g), which were detected by TLC. Fraction A was purified by silica gel CC (column dimension: 2.5 × 40 cm) with CH₂Cl₂–MeOH (100:800 mL; 100:200 mL) to afford 230 mg of Fr.A-1. Fr. A-1 was then subjected to HPLC with MeOH–water (H₂O) (60:40) as eluent and each subfraction was further purified on preparative HPLC with MeOH–H₂O (60:40) to yield 1 (12.7 mg), 2 (19.2 mg), 3 (5.9 mg), and 5 (31.0 mg). Fr. B was purified by silica gel CC (column dimension: 2.5 × 40 cm) with CH₂Cl₂–MeOH (100:1800 mL; 100:21000 mL) to afford 370 mg of Fr.B-1. Fr. B-1 was subjected to preparative HPLC with MeOH–H₂O (45:55) as eluent to obtain compounds 4 (12.8 mg), 6 (5.7 mg), and 10 (21.3 mg). Fr. D was purified by silica gel CC (column dimension: 2.5 × 40 cm) with CH₂Cl₂–MeOH (100:3800 mL; 100:41500 mL; 100:51500 mL) to afford 190 mg of Fr.D-1. Fr. D-1 was further purified on preparative HPLC with MeOH–H₂O (300:1) to yield 6 (5.7 mg), and 10 (5.7 mg), and 10 (19.2 mg). Fr. D was further purified by preparative HPLC with MeOH–H₂O (35:65) to yield 7 (21.1 mg), 8 (3.7 mg), and 9 (7.2 mg).

Cytotoxicity

Human lung cancer cell lines (A549) were provided by the American Type Culture Collection (ATCC). The cells were cultured in medium (RPMI1640 for A549) supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (100 units/mL penicillin G sodium, 100 µg/mL streptomycin, and 250 µg/mL amphotericin B). The cells were incubated at 37°C and 5% carbon dioxide in a humidified atmosphere. Etoposide (Sigma, purity >98%) was used as a positive control. Cell viability was determined by the sulforhodamine B protein staining method. Cells were seeded in 96-well plates and incubated for 24 and were fixed (for zero day controls) or treated with test compounds for 72 hours. All compounds were dissolved in dimethyl sulfoxide (final concentration of 0.1% [v/v]), stored at −20°C and diluted to the desired concentration (0.01, 0.1, 1, 10, 100 µM) in normal saline immediately prior to each experiment. Each concentration was tested thrice. At least 3 experiments were performed. After incubation, cells were fixed with 10% trichloroacetic acid, dried and stained in 0.4% sulforhodamine B in 1% acetic acid solution. Unbound dye was washed and stained cells were dried and dissolved in 10 mM Tris (pH 10.0). Absorbance was measured at 515 nm and cell proliferation was determined as follows: cell proliferation (%) = (average absorbancecompound – average absorbancezero day) / (average absorbancecontrol – average absorbancezero day) ×100%. GI₅₀ values were calculated by nonlinear regression analysis using the Table Curve 2D software (Version 5.01, Systat Software Inc., CA, USA).

Conclusions

Ten compounds were separated and purified by chromatographic methods from the 70% ethanol extract of the leaves of Eleutherococcus sessiliflorus. Four compounds were isolated from the genus Eleutherococcus for the first time. All compounds exhibited weak cytotoxicity against the A549 cell line. Our findings can enrich the phytochemical content of genus Eleutherococcus.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID ID

Ye Ma https://orcid.org/0000-0002-1213-2639

Supplemental Material

Supplemental material for this article is available online.

References

1. Lee S, Kim B-K, Cho SH, Shin KH. Phytochemical constituents from the fruits of Acanthopanax sessiliflorus. Arch Pharm Res. 2002;25(3):280-284.
2. Jung IH, Kim SE, Lee Y-G, et al. Antihypertensive effect of ethanolic extract from Acanthopanax sessiliflorus fruits and quality control of active compounds. Oxid Med Cell Longev. 2018;2018(2):1-14.
3. Chen C, Zhang D, Zhao Y, Cai E, Zhu H, Gao Y. A new 3,4-seco-lupane triterpenene glycosyl ester from the leaves of Eleutherococcus sessiliflorus. Nat Prod Res. 2014;1-4.
4. Song Y, Yang C, Wang Z-B, Zhao N, Feng X-S, Meng F-H. Chemical constituents of Eleutherococcus sessiliflorus extract and its sedative-hypnotic effect. Nat Prod Res. 2017;31(17):1995-2000.
5. Yang C, An Q, Xiong Z, Song Y, Yu K, Li F. Triterpenes from Acanthopanax sessiliflorus fruits and their antiplatelet aggregation activities. Planta Med. 2009;75(6):656-659.
6. Ma T, Ma H, Zhao H, Qi H, Zhao J. Identification, characterization, and transcription analysis of xylogen-like arabino-galactan proteins in rice (Oryza sativa L.). BMC Plant Biol. 2014;14(1):299-302.
7. Du Q, Cai W, Xia M, Ito Y. Purification of (+)-dihydromyricetin from leaves extract of Ampelopsis grossedentata using high-speed countercurrent chromatograph with scale-up triple columns. J Chromatogr A. 2002;973(1-2):217-220.
8. Boots AW, Haenen GRMM, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. Eur J Pharmacol. 2008;585(2-3):325-337.
9. Janeiro P, Corduneau O, Oliveira Brett AM. Chrysin and (±)-taxifolin electrochemical oxidation mechanisms. Electroanal. 2010;17(12):1059-1064.
10. Iris E. Review of the flavonoids quercetin, hesperetin, and naringenin. dietary sources, bioactivities, bioavailability, and epidemiology. Nutr Res. 2004;24(10):851-874.
11. Kim YW, Zhao RJ, Park SJ, et al. Anti-Inflammatory effects of liquiritigenin as a consequence of the inhibition of NF-κB-dependent iNOS and proinflammatory cytokines production. Br J Pharmacol. 2008;154(1):165-173.
12. Moon D-O, Kim M-O, Choi YH, Hyun JW, Chang WY, Kim G-Y. Butein induces G2/M phase arrest and apoptosis in human hepatoma cancer cells through ROS generation. Cancer Lett. 2010;288(2):204-213.

13. Park B-Y, Oh S-R, Ahn K-S, Kwon O-K, Lee H-K. (-)-Syringaresinol inhibits proliferation of human promyelocytic HL-60 leukemia cells via G1 arrest and apoptosis. Int Immunopharmacol. 2008;8(7):967-973.

14. Lee J, Kim D, Choi J, et al. Dehydrodiconiferyl alcohol isolated from Cucurbita moschata shows anti-adipogenic and anti-lipogenic effects in 3T3-L1 cells and primary mouse embryonic fibroblasts. J Biol Chem. 2012;287(12):8839-8851.

15. Hynes MJ, Coinceanainn MO. The kinetics and mechanisms of the reaction of iron(III) with gallic acid, gallic acid methyl ester and catechin. J Inorg Biochem. 2001;85(2-3):131-142.

16. Hao J. Efficient access to isomeric 2,3-dihydroxy lupanes: first synthesis of alphitolic acid. Cheminform. 2009;38(38):7975-7984.

17. Tumiłowicz J. Woody species of Araliaeae at the rogów arboretum. Rocznik Dendrologiczny. 2006;54:35-50.

18. Zaluski D, Olech M, Galanty A, et al. Phytochemical content and pharma-nutrition study on Eleutherococcus senticosus fruits intratum. Oxid Med Cell Longev. 2016;2016(11):1-10.

19. Pandey MK, Sandur SK, Sung B, Sethi G, Kunnunakkara AB, Aggarwal BB. Butein, a tetrahydroxychalcone, inhibits nuclear factor (NF)-κB and NF-κB-regulated gene expression through direct inhibition of IκBα Kinase β on cysteine 179 residue. J Biol Chem. 2007;282(24):17340-17350.

20. Li JW. Optimization of the ultrasonically assisted extraction of polysaccharides from Zizyphus jujuba cv. jinsixiaozao. J Food Eng. 2007;80(1):176-183.