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# CHEMICAL CONSTITUENTS FROM *Acanthopanax senticosus* AND THEIR CYTOTOXIC ACTIVITIES

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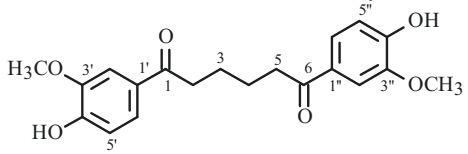
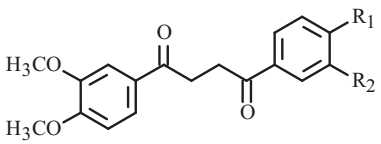
*A new lignan, named 1,6-bis-(3-methoxy-4-hydroxyphenyl)hexane-1,6-dione (**1**), together with seven known compounds (**2–8**) were isolated from the EtOAc-soluble extract of* Acanthopanax senticosus*. Their structures were elucidated based on extensive spectroscopic analyses. All isolates were evaluated for* in vitro *cytotoxic activity against human cancer cells of HepG2, A549, HeLa, and MCF-7. Among them, compound **8** showed significant cytotoxic activity on HepG2 and MCF-7 cells with $IC_{50}$ values of 10.9 ± 1.7 and 9.2 ± 1.5 μM, respectively.*

**Keywords**: *Acanthopanax senticosus*, Araliaceae, lignan, cytotoxicity, A549.

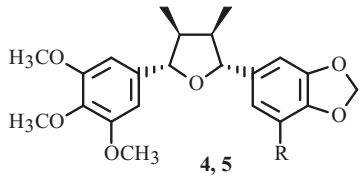
*Acanthopanax senticosus* (Rupr. & Maxim.) Harms, belonging to the family of Araliaceae, is a shrub which widely distributes in China, Korea, Japan, and Russia [1]. As a Chinese traditional medicine, *A. senticosus* was developed into different clinical drugs and used for treating ischemic heart diseases, hydroncus, hypertension, and arthritis [2, 3]. Published articles have confirmed that *A. senticosus* possesses a large range of bioactivities, such as antitumor [4], anti-inflammatory [5], antioxidative [6], and hypoglycaemic effects [7], thus attracting much interest. Further phytochemical investigations on *A. senticosus* resulted in various chemical constituents including flavonoids [8], lignans [9], coumarins [10], triterpenoids [11], sesquiterpenoids [4], as well as triterpenoid saponins [12]. Some compounds displayed significant antitumor, hepatoprotective [13], anti-inflammatory [10], antioxidant and other biological activities [14]. In order to search novel bioactive compounds from plants, a series of phytochemical studies on *A. senticosus* were carried out. As a result, a new lignan, named 1,6-bis-(3-methoxy-4-hydroxyphenyl)hexane-1,6-dione (**1**), along with seven known compounds (**2**–**8**), were isolated from the EtOAc-soluble extract of *A. senticosus*. In addition, their cytotoxic activities on HepG2, A549, HeLa, and MCF-7 were also evaluated.



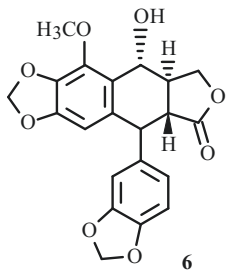

**1**



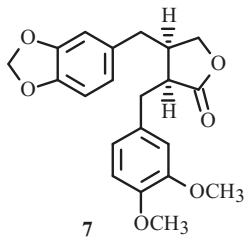

**2, 3**



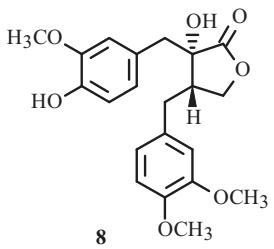

**4, 5**




**6**




**7**




**8**

**2:** $R_1 = R_2 = OCH_3$; **3:** $R_1 = OH$, $R_2 = H$; **4:** R = H; **5:** $R = OCH_3$

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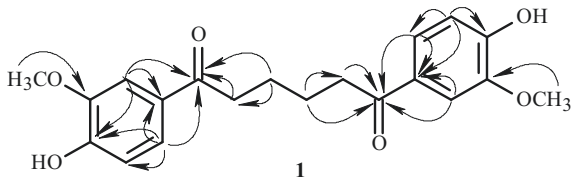
TABLE 1. $^{1}H$ (500 MHz) and $^{13}C$ (125 MHz) NMR Data of Compound **1** ($CD_3OD$, δ, ppm, J/Hz)

| C atom | $\delta_H$ | $\delta_C$ | C atom | $\delta_H$ | $\delta_C$ |
|---|---|---|---|---|---|
| 1 | – | 199.7 | 5′ | 6.72 (d, J = 8.0) | 115.5 |
| 2 | 3.96 (t, J = 6.0) | 59.0 | 6′ | 6.76 (dd, J = 8.0, 2.0) | 122.3 |
| 3 | 3.20 (t, J = 6.0) | 41.7 | 1″ | – | 129.8 |
| 4 | 3.20 (t, J = 6.0) | 41.7 | 2″ | 6.89 (d, J = 2.0) | 107.2 |
| 5 | 3.96 (t, J = 6.0) | 59.0 | 3″ | – | 146.2 |
| 6 | – | 199.7 | 4″ | – | 148.6 |
| 1′ | – | 129.8 | 5″ | 6.72 (d, J = 8.0) | 115.5 |
| 2′ | 6.89 (d, J = 2.0) | 107.2 | 6″ | 6.76 (dd, J = 8.0, 2.0) | 122.3 |
| 3′ | – | 146.2 | $OCH_3$ | 3.90 (s) | 56.9 |
| 4′ | – | 148.6 | | | |

TABLE 2. Cytotoxic Activities of Compounds **1–8** ($IC_{50}$, μM)

| Compound | HepG2 | A549 | HeLa | MCF-7 |
|---|---|---|---|---|
| **1** | 34.2 ± 1.9 | 43.2 ± 2.4 | 31.2 ± 2.1 | 40.2 ± 2.5 |
| **2** | 47.1 ± 1.7 | > 50 | > 50 | > 50 |
| **3** | 48.4 ± 2.7 | > 50 | 45.8 ± 2.4 | > 50 |
| **8** | 10.9 ± 1.7 | 14.0 ± 1.4 | 17.0 ± 2.0 | 9.2 ± 1.5 |
| Doxorubicin* | 1.9 ± 0.7 | 2.1 ± 1.1 | 3.0 ± 1.2 | 1.5 ± 1.7 |

$IC_{50}$ values represent the means ± SD of three parallel measurements. $IC_{50}$ values for compounds **4–7** are > 50 μM.
* Positive control.




**1**

Fig. 1. Key HMBC correlations of compound **1**.

Compound **1** was obtained as a yellow amorphous powder, and its molecular formula was found to be $C_{20}H_{22}O_6$ by HR-ESI-MS at *m/z* 359.1297 for $[M + H]^+$. The IR spectrum displayed typical absorptions attributable to hydroxyl (3432 $cm^{-1}$) and carbonyl (1684 $cm^{-1}$) groups. The $^{1}H$ NMR spectrum (Table 1) of **1** showed a set of ABX-type aromatic protons at δ 6.89 (1H, d, J = 2.0 Hz), 6.72 (1H, d, J = 8.0 Hz), and 6.76 (1H, dd, J = 8.0, 2.0 Hz), two methylene groups at $\delta_H$ 3.96 (2H, t, J = 6.0 Hz) and 3.20 (2H, t, J = 6.0 Hz), and methoxy protons at δ 3.90 (3H, s). The $^{13}C$ NMR spectrum showed 20 carbons corresponding to $^{1}H$ NMR data including a ketone carbonyl carbon at δ 199.7, six aromatic carbons at δ 148.6, 146.2, 129.8, 122.3, 115.5, and 107.2, a methoxy carbon at δ 56.9, and two methylene carbons at δ 59.0 and 41.7. Combined with the preceding 1D NMR and HR-ESI-MS data, a conclusion could be deduced that **1** possesses a symmetrical structure including two aromatic rings, two ketone carbonyl units, and a butane moiety. In addition, the key HMBC correlations (Fig. 1) of $\delta_H$ 6.89 (H-2′/2″), 6.76 (H-6′, 6″), 3.96 (H-2, 5), 3.20 (H-3, 4) with $\delta_C$ 199.7 (C-1, 6), $\delta_H$ 3.96 (H-2, 5) with $\delta_C$ 129.8 (H-1′, 1″), and $\delta_H$ 3.90 ($OCH_3$) with $\delta_C$ 146.2 (C-3′, 3″), further confirmed the structure of **1**. Thus, the structure of **1** was defined as 1,6-bis-(3-methoxy-4-hydroxyphenyl)hexane-1,6-dione.

The known compounds were identified as 1,4-bis-(3,4-dimethoxyphenyl)butane-1,4-dione (**2**) [15], 1-(3,4-dimethoxyphenyl)-4-(4-hydroxyphenyl)butane-1,4-dione (**3**) [15], 7′-epi-henricine (**4**) [16], saurucinol I (**5**) [16], cleistantoxin (**6**) [17], (–)-kusunokinin (**7**) [15], trachelogenin (**8**) [18] by the comparison of their spectroscopic data with those reported in the literature.

All compounds were evaluated for their cytotoxic activities against human cancer cells including HepG2, A549, HeLa, and MCF-7, and doxorubicin was used as a positive control. As shown in Table 2, compounds **1–3**, and **8** showed different levels of cytotoxic activities on four cancer cells with $IC_{50}$ values from 9.2 ± 1.5 to 48.4 ± 2.7 μM, and compounds **4–7** did not exhibit any inhibitory effect on cell proliferation. Among these isolates, compound **8** showed significant cytotoxic activities on HepG2 and MCF-7 cells with $IC_{50}$ values of 10.9 ± 1.7 and 9.2 ± 1.5 μM, respectively. In addition, compound **1** also exhibited moderate cytotoxic activity toward HeLa cells with $IC_{50}$ value of 31.2 ± 2.1 μM.

In the past 20 years, more than 200 articles have been published to report the chemical constituents of *A. senticosus* and their biological activities. Flavonoids, lignans, triterpenoids, and phenylpropanoids were identified to be the main material basis to exert the various biological activities of *A. senticosus*. Regarding the aspect of the antitumor effect, it was found that triterpenoid saponins and lignans play a key role based on the literature research, which is consistent with our current research. Although some investigations have reported that *A. senticosus* possesses inhibitory effects on lung and liver cancer, the study regarding the chemical constituents of *A. senticosus* with antitumor activity is still not in-depth. Thus, effort should be intensified to search for the leading compounds with cytotoxic activity from *A. senticosus*.

## EXPERIMENTAL

**General Experimental Procedures**. UV spectra were measured on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan). IR spectra were made by Nicolet 5700 spectrometer with KBr disc (Thermo, Waltham, MA, USA). NMR spectra were obtained from a Bruker DRX-500 spectrometer (Bruker, Rheinstetten, Germany). Mass spectra were obtained on a QTOF2 high-resolution mass spectrometer (Micromass, Wythenshawe, UK). Column chromatography was performed using silica gel (200–300 μm particle size, Yantai Xinde Chemical Co., Ltd., Yantai, China) and RP-18 (150–63 μm particle size, Merck, Darmstadt, Germany). TLC was performed with precoated silica gel GF254 glass plates (Qingdao Marine Chemical Co., Ltd.). HPLC was carried out using a Shimadzu System LC-20AT pump equipped with an SPD-10Avp UV detector (Shimadzu, Tokyo, Japan), and a YMC ODS-A column (250 mm × 4.6 mm, 5 μm).

**Plant Material**. The stems of *A. senticosus* were collected in Changbaishan, Jilin, China, and authenticated by Prof. Gang Liu (Tongji Medical College, Huazhong University of Science & Technology). A voucher specimen of the plant (No. 20190845) was deposited at Tongji Medical College, Huazhong University of Science & Technology, Wuhan, China.

**Extraction and Isolation**. The dried stems of *A. senticosus* (15.0 kg) were extracted 3 times with 95% EtOH under reflux and concentrated under vacuum to obtain a crude extract (1.2 kg). This extract was suspended in $H_2O$ and partitioned successively with petroleum ether (PE), $CH_2Cl_2$, EtOAc, and *n*-BuOH. Next, the EtOAc-soluble fraction (153.5 g) was subjected to silica gel column chromatography using a gradient of $CH_2Cl_2$–MeOH, and was separated into 13 fractions (Frs. 1–13). Fraction 5 (22.7 g) was chromatographed over silica gel, eluted with a gradient of PE–EtOAc, and separated into 15 subfractions (Subfrs. 5.1–5.15). Subfraction 5.6 (2.3 g) was subjected to the ODS column and eluted with MeOH–$H_2O$ (3:7–10:0) to afford 10 subfractions (Subfrs. 5.6.1–5.6.10). Further purification of Subfr. 5.6.3 (151.1 mg) by HPLC, using an isocratic solvent system of 45% MeOH in $H_2O$ for over 60 min, yielded compounds **1** (6.1 mg, $t_R$ = 34.4 min), **2** (5.8 mg, $t_R$ = 47.1 min), and **3** (8.2 mg, $t_R$ = 53.1 min). Subfraction 5.6.5 (158.1 mg) was purified by HPLC, using a gradient solvent system 45–60% MeOH in $H_2O$ for over 80 min, and yielded compounds **4** (5.5 mg, $t_R$ = 28.4 min), **5** (8.1 mg, $t_R$ = 37.1 min), **8** (6.6 mg, $t_R$ = 53.3 min), **7** (4.7 mg, $t_R$ = 59.2 min), and **6** (6.5 mg, $t_R$ = 66.9 min).

**1,6-Bis-(3-methoxy-4-hydroxyphenyl)hexane-1,6-dione (1),** yellow amorphous powder. UV (MeOH, $\lambda_{max}$, nm) (log ε): 211 (3.04), 235 (4.32), 282 (3.37). IR (KBr, $\nu_{max}$, $cm^{-1}$): 3432, 2847, 1684, 1607, 1502, 1248. $^1H$ (500 MHz, $CD_3OD$) and $^{13}C$ NMR (125 MHz, $CD_3OD$) spectral data, see Table 1. HR-ESI-MS *m/z* 359.1297 $[M + H]^+$ (calcd for $C_{20}H_{23}O_6$, 359.1416).

**Cytotoxicity Assay**. The MTT assay was used to evaluate the cytotoxic activities of compounds **1–8** on four cancer cells according to the published method [19]. Doxorubicin was used as a positive control. Briefly, cells were cultured in a DMEM medium supplemented with 10% foetal bovine serum, 1 × penicillin–streptomycin in a humidified 37 °C incubator supplied with 5% $CO_2$. Then cells were seeded into 96-well flat bottom plate at 5 × 104 cells/mL per well and incubated for 12 h at 37 °C. Next, the test samples were dissolved in DMSO, added to each well. After cells were incubated for another 48 h, MTT (5 mg/mL) was added to each well. Next, the formazan crystal was dissolved with DMSO (150 μL), and the absorbance of each well was measured at 570 nm with a microplate reader (Thermo Scientific Multiskan MK3, Shanghai, China).

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**REFERENCES**

1. X. Q. Zhang, Y. C. Lai, L. Wang, F. F. Xu, M. H. Gao, H. Li, Y. L. Li, and W. C. Ye, *Biochem. Syst. Ecol.*, **39**, 861 (2011).
2. A. L. Jia, Y. H. Zhang, H. Gao, Z. Zhang, Y. H. Zhang, Z. Wang, J. M. Zhang, B. Deng, Z. D. Qiu, and C. M. Fu, *J. Ethnopharmacol.*, **268**, 113586 (2021).
3. M. Yu, B. Wang, A. N. Qiao, and Y. B. Ji, *Saudi. J. Biol. Sci.*, **26**, 1932 (2019).
4. Z. F. Li, Q. Wang, G. Chen, S. L. Yang, Y. L. Feng, and Y. H. Pei, *Chem. Nat. Compd.*, **53**, 273 (2017).
5. Y. Jiang and M. H. Wang, *J. Med. Food*, **18**, 468 (2015).
6. Y. H. Kim, M. L. Cho, D. B. Kim, G. H. Shin, J. H. Lee, J. S. Lee, S. O. Park, S. J. Lee, H. M. Shin, and O. H. Lee, *Molecules*, **20**, 13281 (2015).
7. K. Watanabe, K. Kamata, J. Sato, and T. Takahashi, *J. Ethnopharmacol.*, **132**, 193 (2010).
8. C. M. Liu, J. M. Zhao, H. M. Li, and F. R. Song, *Chem. Res. Chinese U.*, **23**, 233 (2008).
9. J. L. Li, N. Li, H. S. Lee, S. S. Xing, S. Z. Qi, Z. D. Tuo, L. Zhang, B. B. Li, J. G. Chen, and L. Cui, *Fitoterapia*, **109**, 185 (2016).
10. Z. F. Li, Z. H. Wu, G. Chen, Q. H. Zhang, and Y. H. Pei, *J. Asian Nat. Prod. Res.*, **11**, 716 (2019).
11. Z. W. Yan, J. P. Liu, D. Lu, P. Groundwater, C. Y. He, and P. Y. Li, *Nat. Prod. Res.*, **24**, 1523 (2010).
12. W. H. Jiang, W. Li, L. K. Han, L. J. Liu, Q. B. Zhang, S. J. Zhang, T. Nikaido, and K. Koike, *J. Nat. Prod.*, **69**, 1577 (2006).
13. L. Z. Huang, H. F. Zhao, and B. K. Huang, *Pharmazie*, **66**, 83 (2011).
14. S. Lee, D. Son, J. Ryu, Y. S. Lee, S. H. Jung, J. Kang, S. Y. Lee, H. S. Kim, and K. H. Shin, *Arch. Pharm. Res.*, **27**, 106 (2004).
15. B. B. Li, J. L. Li, N. Li, H. S. Lee, X. B. Wang, and L. Cui, *Phytochem. Lett*., **20**, 123 (2017).
16. Y. F. Cheng, Z. Y. Yin, F. Q. Jiang, J. Xu, H. Chen, and Q. Gu, *Fitoterapia*, **144**, 147 (2020).
17. T. T. T. Van, C. P. Van, D. T. M. Huong, L. Marc, G. Franɜoise, R. Pascal, H. N. Van, and M. C. Van, *J. Nat. Prod.*, **75**, 1578 (2012).
18. H. Chang, Y. W. Wang, X. Gao, Z. H. Song, S. Awale, N. Han, Z. H. Liu, and J. Yin, *Fitoterapia*, **121**, 31 (2017).
19. D. V. Tarbeeva, S. A. Fedoreyev, M. V. Veselova, A. S. Blagodatski, A. M. Klimenko, A. I. Kalinovskiy, V. P. Grigorchuk, D. V. Berdyshev, and P. G. Gorovoy, *Fitoterapia*, **135**, 64 (2019).