

## ***In vitro* antitumor activity of chemical constituents of EtOAc extract from *Artemisa gmelinii*.**

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### **Abstract**

**Objective:** To investigate the chemical composition of EtOAc extract from *Artemisa gmelinii* Web. ex Stechm. (*A. gmelinii*) and evaluate their *in vitro* antitumor activity.

**Methods:** The EtOAc crude extract from the aerial parts of *A. gmelinii* was isolated by chromatography and the structures of the isolated compounds were elucidated based on spectral analysis. All the isolated compounds were investigated for their *in vitro* activity against four human cancer cell lines by SRB (Sulforhodamine B) assay.

**Main findings:** Eight compounds, namely amelarioside (1), annphenone (2), 6, 8-dimethoxycoumarin-7-O- $\beta$ -D-glucuronide (3) 6-methoxycoumarin-7-O- $\beta$ -D-glucuronide (4), sacroflavone A (5), sacroflavone B (6), sacric acid A (7) and sacric acid B (8) were isolated from the EtOAc extract from *A. gmelinii*. Compounds 3-6 have certain activity against these tested human cancer cell lines. Among of them, compound 5 (IC<sub>50</sub>: 5.03-6.78  $\mu$ mol/L) was found more potent than those of the reference Etoposide (IC<sub>50</sub>: >50  $\mu$ mol/L) against Hela and MKN-45.

**Conclusion:** Compounds 1-8 are isolated from this plant for the first time. Compound 5 (IC<sub>50</sub>: 5.03-6.78 $\mu$ mol/L) was found more potent than those of the reference Etoposide (IC<sub>50</sub>: >50  $\mu$ mol/L) against Hela and MKN-45. Compound 5 have good antitumor effect which may be used as potential antitumor agent.

**Keywords:** *Artemisa gmelinii*, Chemical compositions, sacroflavone A, Antitumor activity. **Abbreviations:** SRB: Sulforhodamine B; HPLC: High-Performance Liquid Chromatography; HMBC: <sup>1</sup>H Detected Heteronuclear Multiple Bond Correlation.

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### **Introduction**

Tumor is the most common and frequently occurring diseases, of which malignant carcinoma is one of the most serious diseases that endanger human health [1-3]. It is estimated that annual tumor cases will rise from 14 million in 2012 to 22 million within the next two decades [4-6]. Today, although the synthetic antitumor drugs are dominating the market, their negative side-effects and the risk of resistance remain a pressing matter in their clinical use. These issues drive the research and development of herbal medicines, which have made a comeback to improve our basic health needs. Many medicinal plants such as *Radix ophiopogonis*, *Panax quinquefolium*, *Ganoderma lucidum* Karst and *Angelica sinensis*, have been shown to exhibit potent antitumor effects [7-10].

*Artemisa gmelinii*, family composite, is widely distributed in Inner Mongolia of China [11]. *Artemisa gmelinii* (aerial parts) are used as a characteristic medicine in Mongolian folk to treat cancer and its related diseases. It is widely used in Mongolian as a substitute of the material medica, *Artemisa sacrorum*

Ledeb., which is used in treatment of infantile convulsion, hepatitis, appendicitis and trauma. The secondary metabolites including flavonoids [12] and sesquiterpenes [13] have been isolated from the aerial parts of *Artemisa gmelinii*. However, there is few reported scientific study to support these claimed therapeutic and medicinal effects. In our previous pharmacological studies [14] on this plant showed that the EtOAc extracts of *Artemisa gmelinii* had anti-tumour activity, which urges us to study the EtOAc extracts from *Artemisa gmelinii*. In our phytochemical investigation, eight compounds were isolated, such as amelarioside (1), annphenone (2), 6, 8-dimethoxycoumarin-7-O- $\beta$ -D-glucuronide (3) 6-methoxycoumarin-7-O- $\beta$ -D-glucuronide (4), sacroflavone A (5), sacroflavone B (6), sacric acid A (7) and sacric acid B (8), whose structures are shown in Figure 1.

For searching more potent antitumor agents, we centered our attention on the isolated compounds from the EtOAc extracts of *Artemisa gmelinii*. All the isolated compounds were investigated for their *in vitro* activity against four human cancer cell lines, including HepG2 (liver carcinoma), Hela (cervical cancer), MCF-7 (breast cancer) and MKN-45 (gastric

cancer) by SRB. This paper describes the antitumor activity and the chemical composition from the EtOAc extract of *Artemisa gmelinii*.

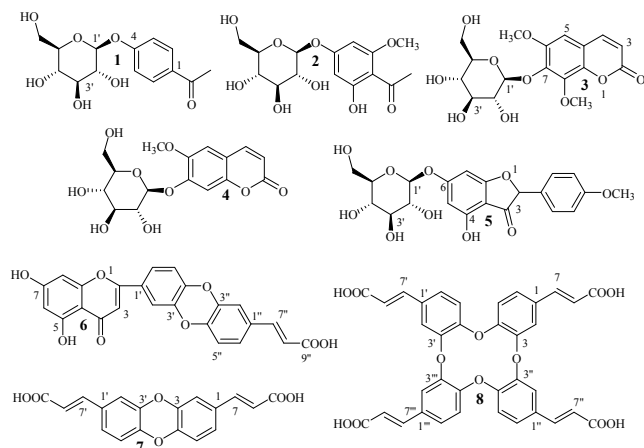


Figure 1. Structures of compounds 1-8.

## Materials and Methods

### General experimental procedures

NMR experiments were performed on a Bruker Avance III-500 NMR spectrometer (Bruker, Germany). Semipreparative HPLC was performed by using a Japanese liquid chromatograph equipped with an EZ0566 column. Column chromatography was performed by using silica gel (200-300 mesh, Marine Chemical Factory, Qingdao, China). High-Performance Liquid Chromatography (HPLC)-grade acetonitrile was purchased from Merck (Darmstadt, Germany). All other chemicals and reagents were analytical grade.

### Plant material

*Artemisa gmelinii* (aerial parts) were collected in Humeng, Inner Mongolia, China, in July 2016. The plant material was identified by Prof. Wuxiangjie (Inner Mongolia University for Nationalities) and a voucher specimen was stored in the Mongolian Medicine Research Center, Inner Mongolia University for Nationalities.

### Extraction and isolation

The air-dried and powdered aerial parts of *Artemisa gmelinii* (2.0 Kg) were extracted twice with EtOAc (20 L) at 80°C for 4 h after extracting with CHCl<sub>3</sub> (10 L). The combined EtOAc extracts were concentrated to a residue (180 g, yield 9.0%) under reduced pressure. The EtOAc extract (180.0 g) was isolated by column chromatography on silica gel and gradually eluted with CHCl<sub>3</sub>-MeOH (50:1 to 10:1) to give 3 fractions (Fr. C<sub>1-3</sub>). Fr. C<sub>1</sub> (15 g, CHCl<sub>3</sub>-MeOH (30:1)) eluate was further chromatographed on Sephadex LH-20 column eluting with MeOH, and then separated by semipreparative HPLC (CH<sub>3</sub>CN-H<sub>2</sub>O, 59:41) yielding 1 (51 mg), 2 (75 mg), 3 (67 mg), 4 (48 mg) and 5 (92 mg). Fr. C<sub>3</sub> (21 g, CHCl<sub>3</sub>-MeOH (10:1) eluate) was further separated by semipreparative HPLC

(CH<sub>3</sub>CN-H<sub>2</sub>O, 41:59) to yield 6 (32 mg), 7 (29 mg) and 8 (45 mg).

### Anti-tumor activities

All the isolated compounds were investigated for their *in vitro* activity against four human cancer cell lines, including HepG2 (liver carcinoma), Hela (cervical cancer), MCF-7 (breast cancer) and MKN-45 (gastric cancer) by SRB assay [15].

## Results and Discussion

From the EtOAc extract of *Artemisa gmelinii*, eight compounds including amelarioside (1), annaphenone (2), 6, 8-dimethoxycoumarin-7-O-β-D-glucuronide (3), 6-methoxycoumarin-7-O-β-D-glucuronide (4), sacroflavone A (5), sacroflavone B (6), sacric acid A (7) and sacric acid B (8) were obtained from this plant for the first time. Their structures were elucidated on the basis of comparing their NMR data with those reported in the literature [16-22].

According to the biological evaluation results shown in Table 1, the compounds 1-8 (IC<sub>50</sub>: 10.14-45.23 μmol/L) have certain activity against HepG2 and MCF-7. Among of them, compounds 3-6 have certain activity against these tested human cancer cell lines. All of them (IC<sub>50</sub>: 5.03-35.56 μmol/L) are more active than Etoposide (IC<sub>50</sub>: >50 μmol/L) against Hela and MKN-45. Moreover, compound 5 was found to have potent activity (IC<sub>50</sub>: 5.03-13.66 μmol/L) against all of the tested cell lines and was IC<sub>50</sub> (5.03-6.78 μmol/L) found more potent than those of the reference against Hela and MKN-45.

Table 1. *In vitro* activity of compounds 1-8 against four cell lines.

Compounds	IC <sub>50</sub> <sup>a</sup> (μmol/L)			
	HepG2	Hela	MCF-7	MKN-45
1	45.23	38.00	40.12	>50
2	43.15	36.45	38.45	>50
3	23.07	20.12	35.56	31.46
4	20.19	18.99	33.36	29.65
5	13.66	6.78	10.14	5.03
6	16.55	21.54	26.66	32.47
7	19.32	38.22	25.37	>50
8	17.88	>50	23.22	>50
Etoposide	1.99	>50	16.32	>50

<sup>a</sup>IC<sub>50</sub> values were presented as the concentration of drug inhibition 50% cell growth and determined by at least three separate tests and reported.

Compound 5 is a derivative of diphenylethene. Diphenylethene is a group of natural organic compounds with a C<sub>6</sub>-C<sub>2</sub>-C<sub>6</sub> unit in the parent nucleus, which have a variety of biological activities, such as anti-tumor, antihypertensive, ester, anti-platelet aggregation, antibacterial and so on. For example, resveratrol is a widely known natural product, which exhibited

significant pharmacological activities [23-26] and considered a plant antitoxin. The structure of compound 5 can be regarded as the oxidized and cyclization of the vinyl group (C<sub>2</sub>) in diphenylethene. The structural characteristics of compound 5 may be the reason why compound 5 have good antitumor effect. In addition, the type of sugar linkage with the aglycone should be an important factor for the antitumor activity of compound 5.

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### Conflict of Interest

The authors declare no financial or commercial conflicts of interest.

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