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

Yu Hua, Qinghu Wang*, Biligetu Pa, Wenqiang Bao, Junsheng Hao, Jingjing Han

College of Traditional Mongolian Medicine, Inner Mongolia University for Nationalities, No. 996 Xilamulun District, Tongliao, PR China

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 ameliaroside (1), annphenone (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 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_{50}: 5.03-6.78 μmol/L) was found more potent than those of the reference Etoposide (IC_{50}: >50 μmol/L) against Hela and MKN-45.

Conclusion: Compounds 1-8 are isolated from this plant for the first time. Compound 5 (IC_{50}: 5.03-6.78μmol/L) was found more potent than those of the reference Etoposide (IC_{50}: >50 μmol/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: ^{1}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 ameliaroside (1), annphenone (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), 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*.

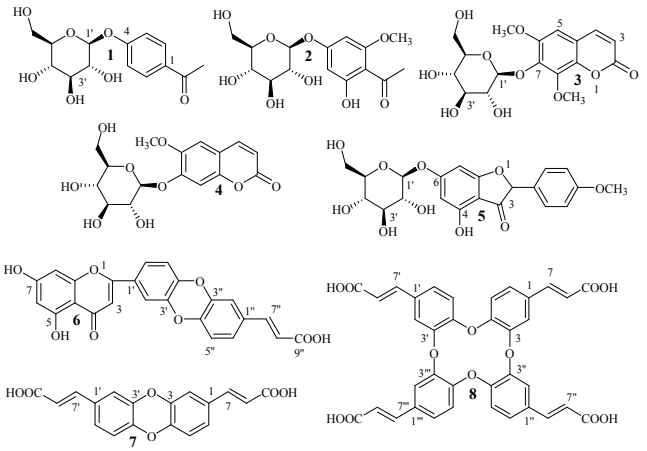

Figure 1. *Structures of compounds 1-8.*

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 CHCl3 (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 gradiently eluted with $CHCl_3$-MeOH (50:1 to 10:1) to give 3 fractions (Fr. C_{1-3}). Fr. C1 ((15 g, $CHCl_3$-MeOH (30:1)) eluate was further chromatographed on Sephadex LH-20 column eluting with MeOH, and then separated by semipreparative HPLC (CH_3CN-H_2O, 59:41) yielding 1 (51 mg), 2 (75 mg), 3 (67 mg), 4 (48 mg) and 5 (92 mg). Fr. C_3 (21 g, $CHCl_3$-MeOH (10:1) eluate) was further separated by semipreparative HPLC (CH_3CN-H_2O, 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 ameliaroside (1), annphenone (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_{50}: 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_{50}: 5.03-35.56 μmol/L) are more active than Etoposide (IC50: >50 m mol/L) against Hela and MKN-45. Moreover, compound 5 was found to have potent activity (IC_{50}: 5.03-13.66 μmol/L) against all of the tested cell lines and was IC_{50} (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_{50}[a] (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

[a]IC_{50} 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_6-C_2-C_6 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_2) 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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*Correspondence to

Qinghu Wang

College of Traditional Mongolian Medicine

Inner Mongolia University for Nationalities

PR China