SUPPLEMENTARY MATERIAL

Chemical composition, antioxidant, antibacterial and cytotoxic effects of *Artemisia marschalliana* Sprengel extract

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Abstract

The present study was to investigate the gas chromatography/mass spectrometry (GC/MS), *in vitro* antioxidant, antibacterial and anticancer activity of the ethanolic extract from aerial parts of *Artemisia marschalliana* Sprengel against human gastric carcinoma (AGS) and L929 cell lines. Phytochemical analysis of *A. marschalliana* Sprengel extract showed 22 major components and the most dominant compounds were trans-phytol (29.22%), α -Linolenic acid (13.47%) and n-Hexadecanoic acid (9.28%). In addition, the antioxidant and anti-cancer activity of *A. marschalliana* Sprengel extract was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) methods, respectively. Antibacterial activity against selected pathogenic bacteria was also determined. According to the present obtained results, it seems that this plant has potential uses for pharmaceutical industries and further studies were suggested to be performed for *A. marschalliana* Sprengel pharmaceutical importance.

Key words: GC-MS ,Phytocomponent ,*Artemisia marschalliana* Sprengel, Cytotoxicity

Experimental

Plant material and extract preparation

The aerial parts of *A. marschalliana* Sprengel were collected from Arasbaran, East Azarbaijan province, Iran, in October 2014. The identity of the plants was confirmed by Y. Ajaniat the Herbarium of Iranian Biological Resources Center, Tehran, Iran (Voucher specimens No. IBRCP1000141). The ethanolic extract of *A. marschalliana* Sprengel was prepared by mixing 10 g of dry aerial parts plant sample with 100 ml of 50% ethanol at 60 °C for 10 min. The solution was filtered with the help of filter paper using Whatman No. 1 filter paper (Whatman Plc., Kent, UK) and then the extracts were transferred to a closed container for further use and storage.

GC/MS analysis

The extract of *A. marschalliana* Sprengel was analyzed using an Agilent 6890 gas chromatography-mass spectrometer (GC-MS) fused with a capillary column of silica DB-5 (30 m × 0.25 mm i.d., 0.25 µm film thickness) with ionization potential of 70 ev. The injector temperature and split ratio were adjusted at 250 °C and 1/31, respectively. The flow rate of helium as a carrier gas was 15 ml/min and the oven temperature programming was as follows: temperature was kept at 60 °C for 2 min, then it raised to 250 °C at a rate of 2 °C/min. Afterwards, it was maintained at 250 °C for 2 min.

Identification of phyto-components

The results of GC/MS were compared with the database of National Institute Standard and Technology (NIST) and the mass spectra were analyzed. In addition, the name, molecular weight, and structure of the components of the test materials were determined.

In vitro cytotoxicity assay

Cell line and culture medium

AGS and L929 cells were obtained from the Pasteur Institute cell bank in Tehran (Iran). The cells were grown in Dulbecco's modified eagle (DMEM) medium with 10% fetal bovine serum

(FBS), 100 µg/ml of streptomycin, and 100 U/ml of penicillin (All from Gibco, Scotland) at 37°C in a 5% carbon dioxide.

MTT assay

In vitro cytotoxicity assay of *A. marschalliana* Sprengel extract on AGS and normal L929 cell lines were evaluated using MTT technique. Approximately, 1×10^4 (cells/well) cells in 96 well plates followed by overnight incubation. The cells were then treated with different concentrations of extract (0.2, 2, 20, 40 and 60 mg/ml) for 24 h. Consequently, 25 µl of the MTT solution (5 mg/ml) was added to each well, followed by incubation for 2 h. Finally, for solubilization of formazan crystals, the medium of each well was removed and 100 µl of dimethyl sulfoxide (DMSO) was added. The intensity of the solution was measured using a micro plate spectrophotometer at 570 nm. The 50% inhibition (IC₅₀) of cells was measured by using the following formula:

$$(\%) \text{ inhibition} = \frac{\text{Abs of Control} - \text{Abs of Test}}{\text{Abs of Control}} \cdot 100$$

Antibacterial activity of A. marschalliana Sprengel extract

The antibacterial activity of the extract was evaluated against three types of pathogenic bacteria, including *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 6633), *Klebsiella pneumonia* (ATCC 10031), *Salmonella typhimurium* (ATCC 14028) *Bacillus subtilis* (ATCC 6633), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 6538). The antimicrobial activity was done by determining of minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using micro dilution method as recommended by the clinical and Laboratory Standards Institute (CLSI) (Bazzaz et al., 2005).

DPPH radicals scavenging assay

DPPH radicals scavenging of *A. marschalliana* Sprengel extract was estimated according to the method of Miliauskas (Brand-Williams et al., 1995; Boonchum et al., 2011). DPPH radicals absorbed maximum at 515 nm, which disappears with reduction by an antioxidant compound (s). Three milliliters (3 ml) DPPH solution in methanol (0.1 mM) was mixed with 100 µl of

extracts (0.13, 0.33, and 0.46 mg/ml). In control (blank), 100 μ l methanol (without extracts) was mixed with DPPH solution. The samples were incubated in a water bath for 20 min at 37°C and the decrease in absorbance at 515 nm was measured. Ascorbic acid was used as the standards and all tests were performed in triplicate. Radical scavenging activity was calculated using the following formula:

$$\text{Scavenging (100\%)} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where A is the absorbance.

Statistical analysis

All measurements were performed in triplicate and the one-way analysis of variance (ANOVA) was done for expressing the significance of the present work. In cell cytotoxicity assay, P values less than 0.05 were considered as significant.

Figure S1. GC-MS chromatogram of *A. marschalliana* extracts.

Figure. S2. Cytotoxicity of *A. marschilliana* against AGS and L929 cell lines at 24 h. the data is expressed as mean $\pm$ SD of 3 experiments. Cell viability is expressed relative to untreated controls. (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

Table S1. Phytocomponents identified in the extract of *A. marschillianaby* GC-MS.

No.	Compound identified	Molecular formula	MW	RT(min)	Peak area (%)	Structure
1	n-Tetradecane	C ₁₄ H ₃₀	198	10.969	1.219
2	Cycloheptasiloxane, tetradecamethyl	C ₁₄ H ₄₂ O ₇ Si ₇	518	12.113	1.196
3	Phenol, 2,4-bis(1,1-dimethylethyl)	C ₁₄ H ₂₂ O	206	12.410	0.794
4	2,6,10-Trimethyltetradecane	C ₁₇ H ₃₆	240	12.760	0.742
5	Spathulenol	C ₁₅ H ₂₄ O	220	13.370	1.761
6	n-Hexadecane	C ₁₆ H ₃₄	226	13.440	1.243
7	Cyclooctasiloxane, hexadecamethyl	C ₁₆ H ₄₈ O ₈ Si ₈	592	14.212	2.110
8	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl	C ₁₅ H ₂₄ O	220	14.611	0.953
9	Geranyl isovalerate	C ₁₅ H ₂₆ O ₂	238	15.183	0.865
10	1-Heptatriacotanol	C ₃₇ H ₇₆ O	536	16.084	0.804
11	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	17.320	9.285
12	Ethyl hexadecanoate	C ₁₈ H ₃₆ O ₂	284	17.627	3.300
13	Falcarinol	C ₁₇ H ₂₄ O	244	17.983	7.045
14	trans-Phytol	C ₂₀ H ₄₀ O	296	18.690	29.223

15	9,12-Linoleic acid	$C_{18}H_{32}O_2$	280	18.836	6.892
16	α -Linolenic acid	$C_{18}H_{30}O_2$	278	18.884	13.476
17	9,12-Octadecadienoic acid	$C_{20}H_{36}O_2$	308	19.008	3.217
18	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	306	19.052	4.237
19	Oleic acid amide	$C_{18}H_{35}NO$	281	19.133	0.760
20	5,8,11-Heptadecatriynoic acid	$C_{18}H_{24}O_2$	272	19.181	1.569
21	Oleic acid amide	$C_{18}H_{35}NO$	281	20.298	1.211
22	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	21.760	8.071

Table S2. Bioactivity of phytocomponents identified in the ethanolic extracts of *A. marschilliana* by GC-MS.

No.	RT	Name of the compound	Nature of compound	Activity	Reference
1	12.410	Phenol, 2,4-di-tert-butyl	Phenolic compounds	Antibacterial	Bazzaz et al, 2005.
2	17.983	Falcarinol	Alcoholic compounds	No Activity	Loy et al, 2001.
3	18.690	trans-Phytol (Phytol)	Diterpenoids	Anticancer-Antibacterial	Seca et al, 2008.
4	18.836	9,12-Linoleic acid (Linoleic acid)	unsaturated omega-6 fatty acid	Anticancer and Antioxidant	Albishri et al, 2013.
5	18.884	α -Linolenic acid	unsaturated omega-3 fatty acid	Anticancer and Antioxidant	Albishri et al, 2013.

Table S3. MIC and MBC of the *A. marschalliana* extract against pathogenic bacteria.

MIC and MBC concentration (mg/ml) against human pathogenic bacteria		
	MIC	MBC
<i>P. aeruginosa</i>	0.6	1.6
<i>E. coli</i>	0.9	1.8
<i>K. pneumonia</i>	1.6	2.6
<i>S. typhimurium</i>	2.2	2.9
<i>S. aureus</i>	2.6	3.2
<i>S. epidermidis</i>	3.4	4
<i>B. subtilis</i>	4.2	5.2

Table.S4. Free radicals scavenging activity of *A. marschalliana* extract in comparison to standard antioxidant – ascorbic acid.

Sample	SC50 (µg/ml) ^a
<i>A. marschalliana</i> Sprengel extract	375 ± 0.08
Ascorbic acid	11.6± 0.06

^aValues are presented as means ± standard deviation (n=3)

SC₅₀ = 50% scavenging concentration

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