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Original article

Chemical Composition and Some Biological Activities of *Artemisia marschalliana* Essential Oil

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Abstract

Background and objectives: The aerial parts of *Artemisia marschalliana* Sprengel as an indigenous species of genus *Artemisia* in the East Azerbaijan province of Iran, was subjected to phytochemical analysis, as well as anti-proliferative, free-radical-scavenging and anti-malarial activities. **Methods:** The chemical composition of the essential oil obtained from the aerial parts of *A. marschalliana* was analyzed by GC/MS (gas chromatography/mass spectrometry) and GC/FID (gas chromatography/ flame ionization detector). The anti-proliferative, anti-oxidant, and anti-malarial activities of the essential oil were assessed by MTT, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and cell-free β-hematin formation assays, respectively. **Results:** A total of 38 constituents were identified, which represented 95.55% of the oil. The essential oil was characterized by a high content of oxygenated sesquiterpenes. The major components of the oil were spathulenol (38.25%), isoaromadendrene epoxide (8.5%), and caryophyllene oxide (7.31%). The oil exhibited cytotoxic activity against the human breast adenocarcinoma (MCF-7) cell line. The half maximal inhibitory concentration (IC_{50}) of anti-malarial assay was 0.38±0.04 mg/mL; the oil, however, displayed low anti-oxidant activity. **Conclusion:** These findings will be beneficial for the further development of new chemotherapeutic or anti-malarial agents.

Keywords: *Artemisia*; DPPH; GCMS; heme; MTT

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Introduction

The genus *Artemisia*, a fairly large genus within the Asteraceae family, is mostly distributed all over Asia (Caucasus, Siberia, Turkmenistan, Afghanistan, Pakistan, Central Asia, Armenia, Anatolia, Iraq, Himalayas and Tibet), Europe, North America, and South Africa; 34 annual and perennial species of this genus have been documented in the flora of Iran [1-3].

The plants have long been used in folk medicine of Asian countries as a remedy for various diseases; for example, for the treatment of intestinal worms, cough, cold, toothache, joint pain, fever, throat infection, bronchitis, jaundice, and dermatitis. In addition, different species of *Artemisia* have been used for antibiotic, anti-mycotic, anti-septic, tonic, stimulant, aphrodisiac,

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anti-malarial, anti-hepatitis, anti-cancer, and anti-inflammatory properties [4-6].

The phytochemical analysis of different species of genus *Artemisia* revealed the presence of various natural constituents, with monoterpene and sesquiterpene structures in volatile oil [6,7]. Moreover, sesquiterpene lactones, sterols, flavonoids, lignans, coumarins, phenylpropanoids, and acetylenes were the main constituents of different extracts of the *Artemisia* species [8,9].

Artemisia marschalliana Sprengel or *Artemisia campestris* subsp. *inodora* Nyman., commonly known as "Dermane arasbarani", is an Iranian evergreen or semi-evergreen sub-shrub of the genus *Artemisia*, which grows in the Ahar, Arasbaran region, East Azerbaijan Province, Iran [3,10]. There are limited reports about the phytochemical and biological effects of *A. marschalliana*. According to a previous study, the ethanol extract of *A. marschalliana* has shown anti-bacterial, anti-oxidant, and anti-cancer activities. In addition, the most dominant compounds of the essential oil of this plant consisted of diterpene and sesquiterpene derivatives as well as certain saturated and unsaturated fatty acids [11,12].

The objectives of this study were to evaluate the chemical compositions of the essential oil of *A. marschalliana* and assessment of the cytotoxic, free-radical-scavenging, and anti-malarial activities of the essential oil.

Material and Methods

Ethical considerations

Ethical approval for this study was granted by the Ethical Committee of Tabriz University of Medical Sciences, Tabriz, Iran (ethics committee reference number: IR.TBZMED.VCR.REC.1397.012, 2018).

Plant material

The aerial parts of *Artemisia marschalliana* were collected in June 2017 from Ahar, Arasbaran area, East Azerbaijan Province, Iran. The voucher specimen of this collection (Tbz-fph-4037) was deposited at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Tabriz University of Medical Sciences.

Essential oil extraction

One hundred g of air-dried powdered aerial parts of *A. marschalliana* was subjected to hydrodistillation for three hours using a Clevenger-type apparatus. The resulting oil was dehydrated over anhydrous sodium sulfate and stored in a sealed glass vial at 4-5 °C for subsequent analyses [13].

GC-MS and GC-FID analyses

The GC/MS analysis of the essential oil of *A. marschalliana* was carried out on a Shimadzu GC/MS-QP5050A gas chromatograph-mass spectrometer (GC/MS) fitted with a fused methyl silicon DB-1 column (60 m × 0.25 mm i.d. and 0.25 μm film thickness). The carrier gas was helium with a flow rate of 1.3 mL/min. The column temperature was programmed at 50 °C for 3 min and then increased to 260 °C at the rate of 3 °C/min and finally kept constant at 260 °C for 9 min. The injector temperature was 230 °C and the split ratio was set up at 1:33. The injection volume was 1 μL. The mass operating parameters were obtained under the following conditions: ionization potential = 70 eV; ion source temperature = 260 °C; solvent delay 2.0 min; resolution = 2000 amu/second; scan range = 30-600 amu.

The constituents of the essential oil were identified by direct comparison of their mass spectra and retention times with standard alkanes (C_8–C_20) from Sigma-Aldrich (USA), computer matching with the NIST 107, NIST 21, and Wiley 229 mass spectral database as well as by the comparison of the fragmentation patterns of the mass spectra with those reported in the literature [14-16].

For quantitation (area %), the GC analysis was also performed by using a Shimadzu GC-17A gas chromatograph, which was equipped with an FID detector. The FID detector temperature was 300 ºC. To obtain the same elution with GC/MS, the duplicate of the same column and operational conditions was applied.

Cytotoxicity assay

The MCF-7 cell line (human breast adenocarcinoma cell line) was purchased from Pasture Institute, Tehran, Iran, and was cultured in a humidified atmosphere at 5% CO_2 in RPMI-1640 (Biosera, United Kingdom) supplemented with 10% fetal bovine serum (Gibco, United Kingdom). The cells were cultivated in 96-well plates at a density of 1.5×10^4 cells per well in 100 μL of the culture medium for 24 hs. Different concentrations of the essential oil were applied to the wells of a 96-well plate that

contained the confluent cell monolayer in duplicate and doxorubicin was used as the reference standard. After 24 h of incubation, cell viability was assessed by MTT assay and the IC_{50} values were calculated as the concentration of the sample decreased by 50% of the viable cells in comparison to that of the control by using the optical density (OD) values of the viable cells [17,18].

Free-radical-scavenging activity: 2, 2-diphenyl-1-picryhydrazyl (DPPH) assay

The free-radical-scavenging property of essential oil was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, Germany) assay, as reported in the literature [19,20]. The essential oil was dissolved in $CHCl_3$ (Duksan, Korea) to obtain the stock concentration of 1 mg/mL. Serial dilutions were prepared to derive concentrations of 5×10^{-1}, 2.5×10^{-1}, 1.25×10^{-1}, 6.25×10^{-2}, 3.13×10^{-2}, and 1.56×10^{-2} mg/mL. Diluted solutions (2 mL each) were mixed with DPPH (0.08 mg/mL, 2 mL) and allowed to stand for 30 min for the reaction to occur. The absorbance was read against a blank at 517 nm, with a Spectronic Genesys 5 UV/Visible Spectrophotometer (USA). The experiment was performed in triplicate and the average absorption was measured at each concentration. The experiment was performed in the same manner for the positive control quercetin. The data was processed by using MS Excel and the concentration that caused a 50% reduction in absorbance (RC_{50}) was computed.

Cell-free β-hematin formation assay

The anti-malarial activity of the essential oil was evaluated by the in vitro cell-free β-hematin formation assay, which was described by Afshar et al. [21], with modifications. Initially, different concentrations (0.4-4 mg/mL in DMSO) of the essential oil were produced and then the samples were incubated with 100 µL of hematin (3 mM, Sigma-Aldrich, Switzerland), 10 µL of oleic acid (10 mM, Fluka, India), and 10 µL of HCl (1 M, Merck, Germany). The final volume was adjusted to 1 mL by using sodium acetate buffer (pH value = 5, Merck, Germany), overnight, at 37 °C, with regular shaking. The incubation was terminated by centrifugation (12000 RPM for 10 min at 21 °C) to collect the β-hematin pellets. The pellets were repeatedly washed with incubation (for 10 min at 37 °C and constant shaking) in 2.5% (w/v) SDS (Merck, Germany) in phosphate buffer saline, which was followed by a final wash in 0.1 M sodium bicarbonate (Merck, Germany) until the supernatant was clear (usually 3-8 washes). To determine the amount of heme crystallized into β-hematin, the pellets were dissolved into 0.1 M NaOH (Merck, Germany) before recording the absorbance at 400 nm (Spectronic Genesys spectrophotometer, USA). The results were computed as percentage of heme crystallization inhibition (I %) in comparison to negative control (DMSO) by using the following equation:

$$I\% = [(An–As)/An] \times 100$$

An: absorbance of negative control and As: absorbance of test samples.

In this method, chloroquine diphosphate (Sigma-Aldrichwas, United Kingdom) was used as the positive control.

Statistical Analysis

All the experiments were performed in triplicate and presented as Mean ± SD. RC_{50} and IC_{50} values were calculated using Excel 2012 and GraphPad prism 8.0.1 software (GraphPad Software Inc., USA).

Results and Discussion

The yield of the essential oil obtained by 3 h hydrodistillation of the dried aerial parts of *A. marschalliana* was 0.06%v/w. The GC/MS analysis of essential oil led to the identification and quantification of 38 compounds, which have been listed in table 1.

These compounds represent 95.55% of the total components. The essential oil predominantly included hydrocarbon sesquiterpenes (78.19%). The most prominent components were spathulenol (38.25%), isoaromadendrene epoxide (8.5%), and caryophyllene oxide (7.31%). Spathulenol accounted for one-third of the total constituents. No study has ever been performed on the oil of *A. marschalliana*, except for the study that was performed by Ahmadi et al. in 2002 [12]. Qualitative and quantitative comparison between the present study and the data reported by Ahmadi et al. revealed that the samples were collected from the same season and different geographical regions. According to the aforementioned study, 20 components, equal to 99% of the total compositions of the essential oil were identified and the major components of the oil were hydrocarbon sesquiterpene, which

contributed to 47.5% of the total oil. Germacrene-D (23.7%) and bicyclogermacrene (14.9%) were the main components. The differences in the GC/MS profiles of the essential oils of *A. marschalliana*, collected from different locations, might be a result of the differences in local climates and seasonal factors.

Table 1. Volatile compounds identified in the aerial part of *Artemisia marschalliana*

No.	Compounds	Percentage	Calculated KI	Reported KI
1	Alpha pinene	2	931	939
2	Beta pinene	0.62	970	979
3	D-limonene	0.76	1022	1029
4	Trans-beta-Ocimene	0.31	1038	1050
5	Cis-6-Nonenal	0.26	1072	1101
6	Alpha-campholene aldehyde	0.87	1105	1119
7	Trans-pinocarveol	0.73	1124	1139
8	(S)-cis-verbenol	1.09	1129	1141
9	Terpinen-4-ol	0.36	1163	1177
10	Myrtenol	0.34	1180	1196
11	Octyl acetate	0.55	1194	1211
12	Trans-(+)-carveol	0.44	1199	1217
13	Citronellol	3.3	1211	1226
14	Z-2-decenal	0.36	1239	1250
15	Citronellyl acetate	2.58	1335	1353
16	Geranyl acetate	0.39	1361	1372
17	Alpha Copaene	0.79	1378	1387
18	Beta caryophyllene	1.37	1420	1433
19	Neryl acetone	0.54	1428	1436
20	Cis-.beta.-farnesene	0.56	1445	1448
21	Alpha.-humulene	0.58	1451	1455
22	Alloaromadendrene	1.04	1458	1462
23	Alpha-curcumene	2.21	1468	1481
24	Gamma-muurolene	0.99	1470	1483
25	Germacrene D	5.31	1475	1485
26	1-pentadecene	1.11	1484	1492
27	Bicyclogermacrene	5.16	1490	1500
28	Gamma.-cadinene	0.56	1510	1514
29	Delta.-cadinene	1.23	1518	1523
30	Nerolidol	0.68	1551	1563
31	**Spathulenol**	**38.25**	1573	1578
32	**Caryophyllene oxide**	**7.31**	1577	1583
33	Globulol	0.39	1581	1585
34	Salvial-4(14)-en-1-one	1.62	1585	1595
35	**Isoaromadendrene epoxide**	**8.5**	1589	1612
36	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	0.89	1637	1690
37	Hexahydrofarnesyl acetone	0.75	1832	1846
38	N-Hexadecanoic acid	0.75	1943	1963
Total			95.55	
Non-terpenoids			3.03	
Terpenoids			92.52	
Monoterpenes			14.33	
Sesquiterpenes			78.19	

Earlier studies on the essential oils extracted from *A. spicigera* and *A. splendens* exhibited that oxygenated monoterpenes were the major constituents, and cis-chrysanthenyl acetate (24.0%) and 1,8-cineol (4.7%), were the main compounds of the essential oils; these two components were not detected in the present study [22].

The colorimetric MTT test is a sensitive and reliable approach that is used for the screening of anti-proliferative agents. The present study showed that the essential oil of *A. marschalliana* possessed significant anti-proliferative activity against MCF-7 cells with an IC_{50} value of 21.5±2.0 µg/mL (table 2).

Table 2. Anti-proliferative activity against MCF-7, anti-oxidant, and anti-malarial properties of the essential oil of *Artemisia marschalliana* aerial parts

Sample	Anti-proliferative Assay	Anti-oxidant assay	Anti-malarial assay
	IC_{50} (µg/mL)	RC_{50} (mg/mL)	IC_{50} (mg/mL)
Essential oil	21.5±2.0	2.69±0.12	0.38±0.04
Positive control *	0.35±0.07	0.0039±0.0001	0.014 ± 0.003

*Doxorubicin, quercetin, and chloroquine were the positive controls of the anti-proliferative, anti-oxidant, and anti-malarial tests, respectively.

Previous studies showed that the essential oil of *A. herba-alba* possessed significant anti-proliferative activity against the acute lymphoblastic leukemia (CEM) cell line with an IC_{50} value of 3 µg/mL [23]. On the other hand, the essential oil of *A. iwayomogi* exhibited cytotoxic activity against the human oral epidermoid carcinoma cell line (KB cells) and phenolic compounds are the active components responsible for the induction of the apoptosis of KB cells through a mitochondrial and caspase-dependent mechanism [24]. Moreover, it has been reported in the literature that caryophyllene exhibited high anti-proliferative activity against the human erythroleukemia K562 cells (IC_{50} = 98.7 µM) [25]. Interestingly, trans-caryophyllene exerted cytotoxic activity on colon cancer (HCT-116) cells (IC_{50} = 65.2 µg/mL) and was more active against murine macrophage (RAW 264.7) cell lines (IC_{50} = 35.2 µg/mL) [26]. Another study showed that spathulenol was demonstrated to be quite cytotoxic with an IC_{50} value of 23±2 µM against human gastric adenocarcinoma (AGS) cells [27]. Based on the cytotoxic activity of the aforementioned compounds, it can be concluded that the anti-proliferative activity of the essential oil of *A. marschalliana* could be due to the synergistic effects of terpenoids in the oil. In our

study, the low IC_{50} value of the essential oil confirmed the strong anti-cancer properties of the plant. Hence, the investigation of the molecular mechanisms of the anti-tumor activities seemed rational.

The free-radical-scavenging activity of the corresponding oil was investigated in vitro by DPPH assay. According to table 2, the essential oil of *A. marschalliana* showed weak anti-oxidant activity. However, the essential oil of *A. afra* possessed significant anti-oxidant activity with IC_{50} of 1.1 µL/mL [28]; while, the volatile oils of *A. spicigera* and *A. splendens* exhibited weak free radical-scavenging properties with the RC_{50} values of 55.6 and 106.4 µg/mL, respectively [22].

In the next step, the anti-malarial activity of essential oil was evaluated by the in vitro β-hematin formation assay, which was developed by Afshar et al. Malaria is a life-threatening disease that is caused by the reproduction of the parasite *Plasmodium falciparum* in a host erythrocyte [29]. During the intra-erythrocytic cycle, the parasite utilizes the host's hemoglobin as the main source of nutrition for its development and proliferation [30]. The degradation of hemoglobin is accompanied by the production of brown heme crystals that are harmful for parasites [31]. Subsequently, the *Plasmodium* protects itself by detoxification of free heme through different pathways, predominantly via the biocrystalization of heme into an inert and insoluble crystal that is known as hemozoin or malaria pigment in acidic digestive vacuoles [32]. Thus, the inhibition of hemozoin or β-hematin (the synthetic analogue of hemozoin) formation is an important drug target in anti-malarial drug discovery [33].

As observed in table 2, the *A. marschalliana* essential oil showed significant anti-malarial activity with the IC_{50} value of 0.38 ± 0.041 mg/mL in comparison to the standard anti-malarial compound chloroquine (IC_{50} = 0.014 ± 0.003 mg/mL).

Reviews of the previous literature demonstrated that sesquiterpenes, such as artemisinin and its derivatives, are being developed for a new generation of potent anti-malarial drugs. The chloroquine-resistant strains of *P. falciparum* are still susceptible to artemisinin derivatives [34]; therefore, it seems that the anti-malarial activity of essential oil might be due to high content of sesquiterpenes and the synergistic with other components in the volatile oil.

Overall, our findings demonstrated the cytotoxic and anti-malarial activity of the essential oil. The result from this study will be beneficial for further development of new chemotherapeutic or anti-malarial agents.

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Author contributions

Parina Asgharian and Abbas Delazar conceived and planned the study; Masumeh Zadehkamand and Elham Safarzadeh carried out the experiment and collected available literature; Solmaz Asnaashari prepared the manuscript, analyzed the statistical data and verified the accuracy of the tests.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

GC/MS: gas chromatography/ mass spectrometry; GC/FID: gas chromatography/ flame ionization detector; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DPPH: 2,2-diphenyl-1-picrylhydrazyl; SDS: sodium dodecyl sulfate; IC_{50}: the half maximal inhibitory concentration; RC_{50}: the half maximal reduction concentration; RPMI: Roswell Park Memorial Institute