Chemical Composition and Antimicrobial Activity of *Artemisia annua* L. Essential Oil from Iran

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Abstract

The composition of the essential oil obtained from the dried flowering aerial parts of *Artemisia annua* L. (Compositae) was analysed by GC and GC/MS. Thirty-two components were identified in the essential oil of *A. annua* L. with campher (48.00%), 1,8-cineole (9.39%), camphene (6.98%) and spathulenol (4.89%) as major components. The essential oil was evaluated for antibacterial and antifungal activities. The activity was more pronounced against fungal organisms than against Gram-positive and Gram-negative bacteria.

Keywords: ***Artemisia annua* L., Essential oil, GC/MS, Antimicrobial activity**

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Introduction

The genus *Artemisia* (Family Compositae) belongs to a useful group of aromatic and medicinal plants comprising about 300 species found in the northern hemisphere [1]. There are approximately 34 native *Artemisia* spp. in Iran [2]. This large genus has been the subject of numerous chemical studies [3]. *Artemisia* spp. have been reported to contain number of coumarins, flavones and terpenes [4].

Artemisia annua L. is an annual herb native of Asia and has been used for many centuries for the treatment of fever and malaria. Many secondary metabolites of terpene peroxides are isolated from *Artemisia annua* L., such as artemisia ketone, artemisinic alcohol, arteannuin B and myrcene hydroperoxide [5, 6]. Some of them also can be found in essential oil [7]. With the increasing tendency for the use of volatile oils in both the food and the pharmaceutical industries, a systematic examination of plant extracts for antimicrobial activity is very important.

As a part of our studies on the chemical composition of the essential oils and screening programe for bioactive compounds from plants that grow in Iran, this paper describes the flowering aerial parts essential oil composition of *A annua* L. and its antimicrobial activity (Table 1).

Table 1. The chemical omposition of the essential oil from *A. annua* L.

No.	Components	RI	Percentage
1	Tricylene	914	0.63
2	α – Thujene	919	0.28
3	α – Pinene	926	3.13
4	Camphene	941	6.98
5	Sabinene	967	0.16
6	Dehydro – 1,8- cineole	981	0.25
7	Delta-3- carene	997	0.65
8	α - Terpinene	1008	0.21
9	Cymol	1017	1.31
10	1,8-Cineole	1024	9.39
11	γ-Terpinene	1049	0.39
12	Artemisia ketone	1055	2.68
13	*cis*- Sabinene hydrate	1058	0.29
14	Terpinolene	1078	1.27
15	*trans*-Sabinene hydrate	1094	0.47
16	Camphor	1148	48.00
17	Borneol	1162	2.53
18	Terpinne-4-ol	1170	1.40
19	Myrtenal	1184	0.78
20	Myrtenol	1187	0.93
21	*trans*-Carveol	1209	0.79
22	*cis*-Carveol	1219	0.42
23	Pregeijerene	1282	0.53
24	Eugenol	1342	0.18
25	Benzyl 2-methyl butyrate	1372	0.52
26	*trans*-Caryophyllene	1481	0.41
27	α – Neoclovene	1442	0.12
28	Farnesene	1459	1.25
29	β – Selinene	1468	1.01
30	Spathulenol	1564	4.89
31	Ledenoxid	1611	3.07
32	*epi*-Cubenol	1615	1.84

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Material and Methods

Plant material

The flowering aerial parts of *A. annua* L. were collected in July 2006, from the Gorgan province, north of Iran. A voucher specimen was deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

Isolation of the Essential Oil

The flowering aerial parts (100 g) were dried at 25 °C in the shade and subjected to hydrodistillation, using a Clevenger-type apparatus for 4 h. The oil was dried with anhydrous sodium sulphate, weighed and stored at 4 – 6 °C until use.

Gas chromatography/ mass spectrometry

FID – GC was carried out using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane, 25 m. 0.25 mm i.d., 0.25 μm film thickness); carrier gas, He; split ratio, 1:25, and flame ionization detector. Temperature programme: 60 °C (2 min) rising to 240 °C at 4 °C/min; injector temperature, 250 °C, detector temperature, 260 °C. GC–MS was performed using a Hewlett-Packard 6859 with a quadrupole detector, on a HP-5 column (see GC), operating at 70 eV ionization energy, using the same temperature programme and carrier gas as above. Retention indices were calculated by using retention times of *n*-alkanes that were injected after the oil at the same chromatographic conditions according to Van Den Dool method.

Identification of components

The linear retention indices for all the compounds were determined by coinjection of the sample with a solution containing the homologous series of C8 – C22 *n*-alkanes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature [8] and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database.

Antimicrobial activity

The antimicrobial and antifungal activities of the essential oil was determined against *Staphylococcus aureus* (ATCC 29737), *Echerichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), Saccharomyces cerevisiae (ATCC 16404) and *Candida albicans* (ATCC 14053). Bacterial and fungal strains were tested on soybean casein digest agar and Sabouraud dextrose agar, respectively. Sterilized paper disks were loaded with different amount of *A. annua* L. essential oil (0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 mg/ml) and applied on the surface of agar plates. All plates were incubated at 37 °C for 24 h for bacteria; at 25 °C for 24 h for *C. albicans*. The MIC was defined as the lowest drug concentration, resulting in a clear zone of growth inhibition around the disk after conventional incubation period. 23 Paper disks containing different concentrations of fluconazole and gentamycin (Sigma Chemical Co.) were applied over the test plates as a comparative positive control.

Results and Discussion

The hydrodistillaton of the dried flowering aerial parts of *A. annua* L. gave a light yellowish oil with yield of 1.2 % (v/w). Thirty – two components were identified in the oil, representing 96.83 % of the total composition (Tab.1). The major components of the essential oil were campher (48.00 %), 1, 8 – cineole (9.39 %), camphene (6.98 %) and spathulenol (4.695 %). The oil consist of 24 monoterpenoids (83.72%) and 7 sesquiterpenoids (12.59 %). The essential oil of the dried flowering aerial parts of *A. annua*

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L. was rich in monoterpenoids.

The results obtained in the antimicrobial assay are shown in table 2. the oil showed antimicrobial activity against all the tested microorganisms, excepted *Pseudomonas aeruginosa*. Maximum activity was observed against fungal microorganisms Saccharomyces cerevisiae (MIC = 2 mg/ml) and *Candida albicans* (MIC = 2 mg/ml). Moderate inhibitory activity of the oil against *Staphylococcus aureus* and *Echerichia coli* were also determined with MIC value of 32 mg/ml and 64 mg/ml respectively. No activity was observed against *Pseudomonas aeruginosa*. In the present study Gram-positive bacteria *Staphylococcus aureus* was more susceptible than Gram-negative bacteria strains. It has frequently been reported that Gram-negative bacteria were resistant to the inhibitory effects of essential oils and their components [9]. This resistance has been attributed to the presence of cell wall lipopolysaccharides, which can screen out the essential oils; the lipids are thus prevented from accumulating on the transporting cell membrane, and from entering the cells.

Table 2. Antimicrobial activity (MIC) of essential oil of *Artemisisa annua* L. (mg/ml).

Strains	MIC (mg/ml)		
	Essential oil	Gentamycin	Fluconazole
Staphylococcus aureus (ATCC 29737)	32	4×10^{-3}	ND
Echerichia coli (ATCC 8739)	64	1×10^{-3}	ND
Pseudomonas aeruginosa (ATCC 9027)	-	8×10^{-3}	ND
Saccharomyces cerevisiae (ATCC 16404)	2	ND	10×10^{-3}
Candida albicans (ATCC 14053)	2	ND	10×10^{-3}

[a] ND, not determined.

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