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## Chemical Composition and Antioxidant Activity Achillea millefolium L. Essential Oils

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### Abstract

Aromatic plants are potential natural sources of their essential oils as main sources of terpenoids, flavonoids and phenolics. *Achillea millefolium* was characterized by means of its antioxidant properties. Results clearly show

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## Chemical Composition and Antioxidant Activity *Achillea millefolium* L. Essential Oils

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**Abstract:** Aromatic plants are potential natural sources of novel antibiotics and particular interest has focused on their essential oils as main sources of potent antimicrobial and antifungal compounds classified as terpenoids, flavonoids and phenolics. EO extracted by hydrodistillation from Iranian *Achillea millefolium* was characterized by means of GC-MS. The EO was also subjected to evaluation for antioxidant properties. Results clearly show the antioxidant effects of the plant essential oil.

**Key words:** GC-MS analysis, *Achillea millefolium*, Chemical composition, Antioxidant activity.

### Introduction

Aromatic plants are potential natural sources of novel antibiotics and particular interest has focused on their essential oils as main sources of potent antimicrobial and antifungal compounds classified as terpenoids, flavonoids and phenolics. In the search for sources of natural products, in the last years some medicinal plants have been extensively studied for their biological properties <sup>1-3</sup>. Among plant extracts, essential oils (EOs) are gaining increasing interest in the food, cosmetic and pharmaceutical industries because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use <sup>4</sup>.

Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth <sup>5</sup>. Therefore, our primary objective was to characterize the EOs of *Achillea millefolium*, and compare their antioxidant activity.

### Materials and methods

#### *Plant material and extraction of essential oils*

The plant was identified by Dr. Esmaili, and the voucher specimen was deposited at private herbarium of Dr F. Esmaili (Voucher no. 22). Aerial parts of *A. millefolium* was collected during 2014-2015 (June-July). Aerial parts were harvested and air dried at ambient temperature in the shade. The *A. millefolium* aerial parts were ground and the resulting powder was subjected to hydrodistillation for 3 hrs in an all glass Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia <sup>6</sup>.

#### **Essential oil analysis**

The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored at +4°C until tested and analysed. GC-MS analyses were executed on a Hewlett-Packard 5973N gas chromatograph equipped with a column (HP-5MS; 30 m length × 0.25 mm i.d., film thickness 0.25 µm) coupled with a Hewlett-Packard 5973N

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mass spectrometer. The column temperature was programmed at 50°C as an initial temperature, holding for 6 min with 3°C increases per minute to 240°C, followed by a temperature enhancement of 15°C per minute up to 300°C, then holding at the mentioned temperature for 3 min. Injector port temperature was 290°C and helium was used as carrier gas at a flow rate 1.5 ml/min. Ionization voltage of mass spectrometer in the EI-mode was equal to 70 eV and ionization source temperature was 250°C. Linear retention indices for all components were determined by coinjection of the samples with a solution containing homologous series of C8-C22 *n*-alkanes and comparing their mass spectra with those of authentic samples or with available library data of the GC-MS system (WILEY 2001 data software) and Adams libraries spectra <sup>7</sup>.

### Antioxidant activities

#### *Free radical-scavenging activity*

For the isolation and identification of the active compounds in the essential oil, PTLC was performed using the conditions previously described according to Hanato *et al.*,<sup>8</sup>. The regions showing DPPH scavenging activity were scrapped off then, they were eluted with chloroform. All resulting constituents were analyzed by GC-MS and also tested for their antioxidant activities. The scavenging activity was estimated using the following equation:

$$\text{Scavenging effect (\%)} = [100 \times (\text{Ac-AS}/\text{Ac})]$$

where Ac is the absorbance of the control reaction (containing all reagents except the test sample) and AS is the absorbance of the tested sample.

#### $\beta$ -Carotene bleaching assay

The  $\beta$ -carotene method was carried out according to Koleva *et al.*,<sup>9</sup>. The antioxidant capacity (AA%) of the solutions tested was calculated as:

$$\text{AA (\%)} = [(A_0 - A_1)/A_0] \times 100$$

All samples were prepared and analyzed in triplicate.

#### Rapid screening for antioxidants

For screening of antioxidant compounds in

*A. millefolium* essential oil, the TLC-bioautography method was carried out <sup>10,11</sup>. The diluted oil (1:20 in methanol) was spotted on silica gel sheets (silica gel 60 F254 TLC plates) and developed in *n*-hexane-ethyl acetate (9:1). Plates were sprayed with the methanolic solution of DPPH (0.2 %). The active constituents were detected as yellow spots on a violet background. Only zones where their color turned from violet to yellow within the first 30 min (after spraying) were taken as positive results.

#### Activity guided fractionation of the essential oil for antioxidants

For the isolation and identification of the active compounds in the essential oil, PTLC was performed using the conditions previously described <sup>11</sup>. The regions showing DPPH scavenging activity were scrapped off then, they were eluted with chloroform. All resulting constituents were analyzed by GC-MS and also tested for their antioxidant activities.

#### Data analysis

All the experiments were performed in triplicate, being the results expressed as mean  $\pm$  SEM of three independent experiments. The means were statistically compared using two-way ANOVA, with a Dunnett's multiple comparison test. The differences between the means were considered significant for values of  $p < 0.001$ .

### Results and discussion

#### *Yield and chemical composition of the essential oil*

The EOs were obtained in yields of  $0.87 \pm 0.21$  % (v/w) dried mass. This is comparable to the yield of the essential oil obtained from the aerial part and seeds of other species considered economically important, such as *A. millefolium* with 17.4 % (v/w) <sup>12</sup>. The results obtained by GC-MS analysis of the essential oils of *A. millefolium* is demonstrated in Table I. GC-MS analysis resulted in the identification of twenty-two from *Achillea millefolium*. The major constituents of the EO from the aerial parts of *A. millefolium* were thymol (21.14 %), carvacrol (18.56 %). Carvacrol and thymol were the main component of the essential oils. Javidnia *et al.*,<sup>13</sup> reported carvacrol

**Table 1. Chemical composition of *Achillea millefolium* volatile oil constituents**

Compound	%	RI
$\alpha$ -Pinene	5.65	932
Camphene	4.11	950
Sabinene	6.32	975
$\alpha$ -Phellandrene	3.45	1000
Limonene	5.47	1020
<i>trans</i> -limonene oxide	1.21	1140
Borneol	1.08	1160
$\gamma$ -Terpinene	4.25	1195
Carone	1.09	1200
Carvone	1.65	1240
Bornyl acetate	0.78	1281
Thymol	21.14	1300
Carvacrol	18.56	1308
$\beta$ -caryophyllene	0.21	1418
$\gamma$ -Elemene	0.36	1430
$\gamma$ -Muurolene	0.78	1476
$\beta$ -Selinene	0.98	1490
$\beta$ -Guaiene	0.54	1496
$\alpha$ -Selinene	0.75	1490
Calarene	0.65	1500
Calacorene	0.24	1529
Caryophyllene oxide	0.41	1579
Total	79.68	
Yields	0.87 $\pm$ 0.21	

as the main constituent of the *Achillea tenuifolia* essential oil. Our results on chemical profiling of the *Achillea* species EO are in agreement with some other studies <sup>14,15</sup>.

#### Antioxidant activity

The *A. millefolium* EO showed the high radical scavenging activity ( $IC_{50}$ : 23.11  $\pm$  0.04 mg/ml), more than trolox (23.51  $\pm$  0.05 mg/ml). Table 3 also shows the results of  $\beta$ -carotene bleaching inhibition based on the loss of the yellow color of  $\beta$ -carotene due to its reaction with radicals produced during linoleic acid oxidation in an emulsion. The *A. millefolium* oil (2.05  $\pm$  0.01  $\mu$ l/ml) was better than the trolox (2.06  $\pm$  0.0  $\mu$ l/ml). Compared to Trolox the *A. millefolium* exhibited stronger antioxidant activity. Recent reports indicate that the *Achillea* genus displays a relevant anti-

oxidant activity that is associated or correlated well with its flavonoid and total phenolic contents <sup>16</sup>. Components identified and their antioxidant activity relative percentages have been showed in Table 3. The major compound found in the active band were thymol (71 %) and carvacrol (16 %) (Table 2). In conclusion, it is worthwhile to screen the commonly used plants from the local flora for different biological activities because they might present a new alternative source for possible

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**Table 2. Components identified and their antioxidant activity relative percentages constituents**

Compounds	%
Other component	6
Thymol	71
Carvacrol	16

**Table 3. Antioxidant potential of *Achillea millefolium* L. essential oils**

	<i>A. millefolium</i>	Trolox
DPPH scavenging activity (IC <sub>50</sub> mg/ml)	23.11±0.04 <sup>b</sup>	23.51±0.05 <sup>a</sup>
β-Carotene bleaching inhibition (IC <sub>50</sub> (μl/ml)	2.06±0.01 <sup>b</sup>	3.05±0.5 <sup>a</sup>

Values are the mean of three replication ± SD. Mean separation among treatments was done by Duncan test at  $p \leq 0.01$ . Mean values followed by different letters are significantly different

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