

## THE BIOACTIVE AND VOLATILE COMPOSITIONS OF *ACHILLEA MOLLIFOLIUM* USING GC/MS AND NANO SCALE INJECTION TECHNIQUE

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The essential oil *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae) were investigated. GC-MS analysis of the essential oil resulted in the identification of 36 compounds constituting 97.21% of the total oil  $\alpha$ -pinene(2.53%), linalool (3.15%), geraniol(33.43%), neryl acetate(17.48%) faranesol(7.61%) and benzyl benzoate(6.08%) were the principal components comprising 70.7% of the oil.

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*Keywords:* *Achillea millefolium*, Essential oil, GC-MS

### 1. Introduction

*Achillea millefolium* is a well-known species amongst the members of *Achillea* (Asteraceae) [1]. It is known as "Bomadaran" and used in folk remedies as an appetizer, wound healer, diuretic, carminative or menstrual regulator [2]. *Achillea* which is native to Europe, Asia, North America and distributed in low temperate zones of the world [3]. It is a commonly used herb both in the ethno-pharmacology and in the up-to-date phytotherapy, they assure a valuable source of natural remedies [4]. It is used as an anti-inflammatory, antispasmodic, anti-pyretic, anti-septic, and as a anti-dandruff in topical form [4]. In recent years, therapists have used the oil on patients suffering with cancer, promote hair growth, therefore is used mainly by the cosmetic industries to produce creams and hair shampoo. Also suggested that the essential oil can modulate macrophages activated [5-7]. To the best of our knowledge, the essential oils of the aerial parts of this plant in Lorestan area have not been considered before. The matters on hand of this study were the determination of the percentage volatile oil molecules by nanoscale injection

### 2. Materials and methods

#### 2.1. Plant material

The plants were identified and authenticated by Prof. Dr. Nasser Akbari at the Department of Agronomy, faculty of agriculture, University of Lorestan. The voucher specimens have been deposited at the Herbarium of the Lorestan university. The aerial parts of the plant were collected from Lorestan university campus area in May 2009 and dried at 18-22 °C for 2 days without applying any heat treatment to minimize the loss of active components. Dried materials were kept in deep freeze until use.

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## Instruments and GC/MS Operating Conditions and procedure

### 2.2. GC–MS analysis

A Shimadzu 17 A GC system, coupled with a Shimadzu QP5050 quadrupole mass spectrometer was used. The extracted compounds were separated on a DBX-5 capillary column (30m×0.25mm I.D., 0.25 µm film). Split injection was employed for hydro distillation with a ratio of 20:1. The column oven temperature was programmed to rise from an initial temperature of 40 °C to 115 °C at 15 °C /min, 115 °C – 160 °C at 3 °C /min, 160 °C – 200 °C at 4 °C /min, then to 270 °C at 70 °C /min, and held at 270 °C for 2 min. The injection temperature and ion source temperature were 260 and 230 °C, respectively. The amount of the sample injected was 1.0 nL (diluted 1.0 µL of sample in 1000 ml of *n*-hexane, v/v) in the splitless mode. Helium was used as the carrier gas with a flow rate of 1.9 ml/min. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 40–500 amu. Compounds in the *Achillea millefolium* L essential oil were identified using the Wiley 5.0 (Wiley, New York, NY, USA) Mass Spectral Library, and retention indices. The three active compounds of 1,8-cineole, camphor, borneol in the *Achillea millefolium* L essential oil were main compounds.

### 2.3. Chemicals and Reagents

Helium, 99.999%, used as carrier gas was purchased from Roham Gas Company (Tehran, Iran). Alkane mixture consisting of the C8-C20 alkanes (concentration of 40 mg/mL in hexane) was purchased from Fluka. All other chemicals were of the highest purity available from Merck or Fluka. Doubly distilled deionized water was used

### 2.4. Hydro-distillation

The sample (100 g of dried material was charged with a particle size of about 500 µm) was submitted to hydro-distillation for 2.5 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia (1975). The volatile distillate was collected over anhydrous sodium sulphate and after filtration, immediately injected to GC/MS. The yield of the oil was 1.23% v/w based on dry plant weight.

### 2.5. Qualitative and quantitative analyses of bioactive

Most constituents were identified by gas chromatography through comparison of their retention indices (RI) with those of the literature [8] or with those of authentic compounds available in our laboratories. The retention indices (RI) were determined in relation to a homologous series of *n*-alkanes (C8–C24) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature [8]. Component relative concentrations were calculated based on GC peak areas without using correction factors.

## 3. Results and discussion

Thirty-six compounds were identified and constituted 97.21% of the total oil. The essential oil of *Achillea millefolium* was characterized by a high number of terpenes.  $\alpha$ -pinene(2.53%), linalool (3.15%), geraniol(33.43%), neryl acetate(17.48%), faranesol(7.61%) and benzyl benzoate(6.08%) were the principal components comprising the 70.7% of the essential oil (Table 1) Changes in the composition of *Achillea millefolium* essential oil were reported as being related to maturation of the plant, with increasing amounts of monoterpenes in relation of the sesquiterpenes. Eucalyptol, camphor, and/or  $\alpha$ -terpineol have been found as major compounds in many other *Achillea* species [9-11]

Table 1. Chemical composition of the essential oil from *Achillea millefolium* subsp. *Millefolium*

No.	Compound	TR	RI <sub>cal</sub>	RI <sub>exp</sub>	%
1	$\alpha$ -pinene	11.27	939	939	2.53
2	Sabinene	12.83	980	975	0.34
3	$\beta$ -Pinene	13.10	987	979	0.68
4	Limonene	15.20	1039	1029	5.38
5	1,8-Cineole	15.39	1044	1031	1.93
6	Linalool	18.07	1110	1097	3.15
7	Neral	18.38	1118	1109	2.93
8	Chrysanthenone	19.20	1139	1128	1.77
9	Nerol oxide	20.10	1162	1158	0.54
10	Camphor	20.32	1168	1156	0.27
11	$\alpha$ -Terpineol	22.08	1213	1189	0.57
12	Geraniol	23.38	1247	1253	33.43
13	Geranial	24.74	1283	1267	0.11
14	Neryl acetate	27.88	1371	1362	17.48
15	cis-Jasmone	29.38	1413	1391	0.47
16	Nerolidol	34.60	1573	1550	2.66
17	$\delta$ -Elemene	35.47	1600	1566	1.00
18	farnesol	36.78	1643	1673	7.61
19	Geranyl butyrate	37.32	1661	1698	2.67
20	Nerolidol	39.55	1737	1745	5.61
21	Benzyl benzoate	41.62	1810	1760	6.08
					97.21

The studied essential oil displayed different chemical profile from those observed from *Achillea millefolium* plants of other geographical origin. *Achillea millefolium* essential oil from Cuba [12] was found to include caryophyllene oxide at a percentage of 20%, while *Achillea millefolium* oil from five clones from Russia [13] were characterized by sesquiterpenes with high chamazulene contents (46–74%) in the four clones and high  $\beta$ -caryophyllene content (38–45%) in the one clone. Eucalyptol (1,8-cineole) and camphor are well-known chemicals with their pronounced antimicrobial potentials [14-15] Antimicrobial

### Acknowledgement

This work was supported by a grant from the Research Council of Lorestan University.

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