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The Essential Oil Composition of *Achillea millefolium* L. Cultivated under Tropical Condition in India

M.M. Nadim, Afaq Ahmad Malik, Javed Ahmad and S.K. Bakshi

Department of Botany, Jamia Hamdard, New Delhi 110062, India

Abstract: The present study was carried out to evaluate the effect of tropical climatic conditions on essential oil content and composition of the temperate plant, *Achillea millefolium* L. 30 components were identified in its essential oil by GC and GC-MS, making up 93.43% of the oil content (0.70±0.05%). The predominant constituents were sabinene (17.58%), 1,8-cineole (13.04%), borneol (12.41%), bornyl acetate (7.98%), "-pinene (6.28%), $-pinene (6.26%), terpinine-4-ol (6.17%) and chamazulene (5.28%). The present trial, first effort in this direction, resulted in slight change in *A. millefolium* essential oil profile, suggesting its ability and plasticity to resist the change in climatic conditions.

Key words: *Achillea millefolium* % Biranjasif % Yarrow % Essential oil

INTRODUCTION

The genus *Achillea* grows in temperate climates in dry or semi-dry habitats. It belongs to family Asteraceae and comprises of about 115 species [1]. The most important among them having pharmacological significance is *Achillea millefolium* Linn. [2, 3] commonly known as yarrow or milfoil [4]. It is a very important medicinal plant in *Unani* (Greco-Arab) system of Medicine under the name of Biranjasif [5, 6] and has been used in traditional medicine for hundreds of years by various cultures [7], internally as herbal teas for headaches, hepato-biliary disorder, gastrointestinal complaints and as an appetite enhancing drug and externally as lotions and ointments against skin inflammations, wounds, cuts and abrasions [4, 8-14]. *A. millefolium* is diaphoretic, astringent, tonic, stimulant and mild aromatic and produces a group of active compounds including isovaleric acid, salicylic acid, asparagine, sterols, flavonoids, tannins and coumarins. The most medicinally active parts of the plant are the flowering tops containing essential oil. *A. millefolium* essential oil has an economical importance due to its anti-inflammatory and disinfectant properties, used mainly in colds and influenza and as a haemostatic [12-14]. Major components in its oil are 1,8-cineole, camphor, borneol and $-pinene [14, 15].

Secondary metabolites response to change in environment can be useful measurement to determine the quality of the product as they are frequently utilized in medicine as therapeutic agents. As reported, the biosynthesis of secondary metabolites including the volatiles in medicinal and aromatic plants are influenced by various agricultural practices and environmental factors namely the soil mineral fertilization, the climate conditions and the culture site [16-22].

We undertook the present study to analyse the chemical profile of the essential oil of *A. millefolium* under the tropical climatic conditions of Delhi, which has otherwise been extensively studied under natural temperate conditions.

MATERIALS AND METHODS

Plant Material and Field Experiments: Planting material (bulbs) of *A. millefolium*, obtained from a local market in Shimla, Himachal Pradesh, India (temperate climate), were transplanted in the experimental field of Jamia Hamdard, New Delhi, India (tropical climate). 49 plants were transplanted in blocks of 5m × 5m size with plant to plant and row to row spacing of 70 cm. The soil of the experimental field was sandy loam with neutral pH (7.1). The organic carbon content of the soil was 0.28% (w/w). The soil had 128 kg/ha available nitrogen, 24 kg/ha

Corresponding Author: Javed Ahmad, Department of Botany, Jamia Hamdard, New Delhi 110062, India.
Tel: +91-9312524573.

available phosphorus and 120 kg/ha available potassium. Irrigation, throughout the whole process of growth and development, was carried out at times dependent upon the rainfall.

Fully grown plant specimens were identified by plant taxonomists at Jamia Hamdard, New Delhi. Voucher specimen No. DM/JH/A-0115 has been housed in the Drug Museum of Department of Botany, Jamia Hamdard, New Delhi (India).

Isolation of Essential Oil: For the isolation of essential oil of aerial parts, the plants were harvested at full bloom stage. Aerial parts (500 g) were collected and subjected to hydrodistillation in a Clevenger-type apparatus (23) for 4 h. The oil after extraction was collected in screw capped glass vials and dried over anhydrous Na_2SO_4. The oil was analyzed by GC and GC/MS.

Instrumentation and Analysis of Oil: GC analyses were carried out on a Shimadzu-17A gas chromatograph equipped with a flame ionization detector (FID) and a DB-5 capillary column packed with 5% phenyl polysiloxane (30 m, 0.25 mm i.d.; 0.25 µm film thickness). The oven temperature was held at 60°C for 2 min, then programmed to rise at 7°C/min to 230°C and held for 20 min. Other operating conditions were as follows: carrier gas, helium at a flow rate of 30 ml/min; oxidant, oxygen at a flow rate of 300 ml/min.; fuel, hydrogen at a flow rate of 30 ml/min; injector temperature, 240°C; detector temperature, 260°C, injection volume was 0.1 µl.

GC/MS analyses were performed on a HP-6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP5-MS capillary column packed with fused silica (60 m, 0.25 mm i.d.; 0.25 µm film thickness). The oven temperature program was initiated at 40°C, held for 1 min then raised at 3°C/min to 250°C and held for 60 min. Other operating conditions were as follows: carrier gas, helium at a flow rate of 1 ml/min; injector temperature, 250°C; split ratio, 1:50; injection volume was 0.1 µl. Mass spectra were recorded at 70 eV. Mass range was from *m*/*z* 40 to 600 amu.

Identification of Essential Oil Constituents: The components of the essential oil were identified by comparing their retention indices and mass spectra fragmentation patterns with the literature values [24-29]. Further identification was made by matching their recorded mass spectra with those stored in our library.

RESULTS AND DISCUSSION

A. millefolium exhibited normal growth under the temperate climatic conditions of Delhi. The data for plant height (82.30±1.53 cm/plant), number of branches/plant (16.5±0.83) and herb fresh weight (312.55±10.59 g/plant) obtained in this study was at par to the reported data obtained in other studies on this plant under temperate conditions. The hydrodistillation of aerial parts yielded 0.70±0.05% essential oil, calculated on fresh weight basis, which is again at par to other studies on the same plant in temperate European countries [30]. The essential oil was blue in color. The essential oil yield was 4.29±0.35 ml/m^2 or 42 liters/ha. The quality and yield of essential oils have been reported previously to be influenced by environmental factors including nutrient status and the pH of soils [31, 32]. Fertilizers have been found to increase the yield of essential oil from established crops like *Artemisia annua* [22], *Chamomilla recutita* [33], *Rosmarinus officinalis* [34], *Valeriana officinalis* [35]. Other environmental factors namely the soil salinity level [36], the light intensity [37], the climate conditions [16] and the culture site [18] have also been reported to influence the biosynthesis of volatile oil. In the present study, the essential oil yield remained unchanged by changing the habitat of the plant.

Gas-chromatographic analysis of the composition of essential oil revealed very interesting profile of chemical constituents. The components of the oil, the percentage of each constituent and their retention indices are summarized in Table 1. Thirty components were characterized in the essential oil, representing 93.43% of the oil. The major constituents were sabinene (17.58%), 1,8-cineole (13.04%), borneol (12.41%), bornyl acetate (7.98%), "-pinene (6.28%), $-pinene (6.26%), terpinine-4-ol (6.17%) and chamazulene (5.28%).

Among the major common components isolated in other studies, 1,8-cineole, borneol and $-pinene were found in this study also as the major components but camphor was totally absent in this study. Sabinene (17.58%) emerged as the major component of the *A. millefolium* essential oil under tropical conditions, which is present in minute quantities under temperate environment except for a few studies reporting it as a major component [38]. This is not surprising as there are some supporting studies that environmental variations affect content and composition of volatile oil in medicinal and aromatic plants [16-22]. Percentage of 1,8-cineol

Table 1: Essential oil composition of *A. millifolium* cultivated under the tropical conditions of Delhi

S. No.	Component	RI	Percentage
1.	"-Pinene	931	6.28
2.	Camphene	939	2.07
3.	Sabinene	960	17.58
4.	$-Pinene	979	6.26
5.	Myrcene	988	0.81
6.	*p*-Cymene	1026	1.11
7.	Limonene	1030	1.27
8.	1,8-Cineole	1033	13.04
9.	(-Terpinene	1062	1.19
10.	"-Thujone	1089	1.00
11.	Thujanol	1100	0.54
12.	Methanol	1110	1.03
13.	Borneol	1165	12.41
14.	Terpinine-4-ol	1176	6.17
15.	"-Terpineol	1189	1.04
16.	Myrtenol	1191	0.13
17.	"-Thujenal	1195	0.16
18.	Dodecane	1199	0.18
19.	trans-Carveol	1202	0.44
20.	Cyclohexene	1250	0.12
21.	Bornyl acetate	1273	7.98
22.	Azulen	1299	0.85
23.	"-Copaene	1379	0.13
24.	$-Caryophyllene	1417	2.31
25.	Germacrene D	1465	1.49
26.	(-Cadinene	1527	0.94
27.	"-Cadinol	1718	0.90
28.	Chamazulene	1725	5.28
29.	Myristic acid	1774	0.23
30.	Pentadecanoic acid	1848	0.49

RI: Retention indices

(13.04%) obtained in this study is lower and that of borneol (12.41%) and "-pinene (6.28%) is higher than other studies under temperate conditions [14, 15, 39]. Percentage of $-pinene (6.26%) and chamazulene (5.28%) is almost in the same quantity as obtained under temperate conditions or slightly higher [40]. In another study, fourteen out of the twenty oils collected from five habitats in Lithuania, did not contain chamazulene [41].

In this study, it has been found that the essential oil of *A. millefolium* grown under tropical conditions is rich in sabinene, 1,8-cineole, borneol, bornyl acetate, "-pinene, $-pinene, terpinine-4-ol and chamazulene. Chamazulene is a sesquiterpene-generated compound probably responsible for the physiological activity of *A. millefolium* oil [40]. These compounds present a special interest because of their wide industrial applications; their effects on animal and human health have been reported by several investigators [8, 11].

CONCLUSION

A. millefolium essential oil content, yield and chemical profile were slightly affected under tropical climatic conditions of Delhi, suggesting its ability and plasticity to resist the change in climatic conditions. Tropical conditions resulted in the accumulation of more quantities of thujane type compounds. *A. millefolium* cultivated under tropical climate is characterized by its high sabinene, borneol and "-pinene contents which are otherwise slightly in lesser quantity under temperate conditions. Chamazulene, probably responsible for the physiological activity of *A. millefolium*, has been found in both tropical and temperate conditions. This suggests that climate and the environmental influences have a slight effect on the composition of *A. millefolium* essential oil.

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