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# Volatile Constituents of *Achillea millefolium* L. ssp. *millefolium* from Iran

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The volatile constituents of *Achillea millefolium* L. ssp. *millefolium* growing wild north-east of Teheran, Iran, were studied by GC and GC–MS. Twenty-one constituents were identified. The main components were α-bisabolol, spathulenol, *cis*-nerolidol, *cis*-carveol and *trans, trans*-farnesol. The percentage of sesquiterpenic components was high, attaining 55.4% of the total oil.

KEY WORDS: *Achillea millefolium* L. ssp. *millefolium*; Compositae; volatile oil; α-bisabolol

## INTRODUCTION

*Achillea millefolium* L. (yarrow) is a rhizomous herbaceous medicinal plant which is commonly found growing wild in the British Isles, southern Europe, Asia, Australia and North America.[1] In Iran, it grows in several areas,[2-4] and its essential oil is used to treat neuralgia and rheumatic pain.[4] No previous investigation of the volatile constituents of the *A. millefolium* ssp. *millefolium* growing in Iran has been carried out but the composition of the essential oil of this and closely related species growing in other regions of the world has been studied by several investigators.[5-13]

## EXPERIMENTAL

### *Plant Material*

Aerial parts of flowering plants of *A. millefolium* L. ssp. *millefolium* were collected in May 1992 from a population growing wild near Polur (north-east of Teheran, Iran) at an altitute of 1900–2220 m. The plant material was identified in the Botany Department of Sciences, University of Isfahan, Iran and a voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Iran.

### *Isolation of the Volatile Oil*

The air-dried plant material was grossly pulverized and the volatile fraction was isolated by hydrodistillation for 4 h according to the *British Pharmacopoeia*.[14] The isolated pale yellow oil was collected in 1 ml of pentane and then used for gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analyses.

### *Gas Chromatography*

GC analyses were performed using a Hewlett Packard 5890 gas chromatograph fitted with a flame ionisation detector and a megabore cross-linked methyl silicone fused-silica column (OV-1, J. & W. Scientific 30 m × 0.31 mm i.d., film thickness 3 μm). The carrier gas was nitrogen with a flow rate of 2.5 ml/min. The oven temperature was programmed from 70°C to 280°C at 10°C/min. Injector and detector temperatures were 270°C.

### *Gas Chromatography–Mass Spectrometry*

A Kratos Concept 25 spectrometer was used, equipped with a Sun Mash 3 computer data out-

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put. The same OV-1 column was used and the GC operating conditions were as above but using helium as the carrier gas. Mass spectrometer conditions were the following: ionisation potential, 70 eV; ion source temperature, 150°C; resolution, 1000; scan time 1 s. The identification of the constituents was based on computer matching against the library spectra built up using pure substances and components of known essential oils, and MS literature data.[15,16] For further identification of the constituents, co-injections with some authentic components were carried out using a Chrompack 438 gas chromatograph, fitted with a flame ionisation detector and a CP SIL-5 (Chrompack) column (25 m × 0.32 mm i.d., film thickness 0.25 μm). The carrier gas was helium with a flow rate of 2.5 ml/min. The oven temperature was programmed from 50°C to 280°C at 6.5°C/min. Injector and detector temperatures were 270°C and 280°C respectively.

## RESULTS AND DISCUSSION

Twenty-one constituents representing 80.4% of the total oil were identified (Table 1). The main components, α-bisabolol, spathulenol, *cis*-nerolidol, *cis*-carveol, and *trans, trans*-farnesol amounted to 50.0% of the oil.

The monoterpene fraction amounted to 17.6%; *cis*-carveol (5.0%), *trans*-carveol (3.7%), and *cis*-sabinol (2.5%), being the most abundant constituents. It is noteworthy that this fraction occurred in higher amounts (up to 83%) in the oils isolated from flowers and from leaves of the same subspecies, collected during the flowering period in the Botanical Garden of Lisbon. 1,8-Cineole (25%) and *trans*-sabinene hydrate (10%) were reported to be the most abundant components of the oil from the leaves, while 1,8-cineole (29%) and sabinene (15%) were found to be the major constituents of the oil obtained

Table 1. Volatile constituents of dried aerial parts of *Achillea millefolium* L. ssp. *millefolium* growing wild north-east of Tehran, Iran

| Constituents | Kovats indices* | Identification | % | |
|---|---|---|---|---|
| Phenol | 1062 | GC–MS | 3.9 | n.r |
| *cis*-Sabinol | 1140 | RI,GC–MS | 2.5 | n.r |
| *trans*-Carveol | 1215 | RI,GC–MS | 3.7 | |
| *cis*-Carveol | 1225 | RI,GC–MS | 5.0 | |
| Bornyl acetate | 1281 | RI,GC–MS,Co-I | 1.3 | |
| $C_{10}H_{18}O_2$ | 1298 | GC–MS | 3.5 | |
| Geranyl acetate | 1365 | RI,GC–MS | 0.9 | n.r |
| Neryl acetate | 1375 | RI,GC–MS | 0.7 | |
| Caryophyllene | 1425 | RI,GC–MS | 0.9 | |
| β-Himachalene | 1443 | RI,GC–MS | 1.2 | n.r |
| *cis*-β-Farnesene | 1448 | RI,GC–MS | 2.7 | n.r |
| α-Patchoulene | 1445 | RI,GC–MS | 2.2 | n.r |
| 2-Pentyl-5-propylresorcinol | 1480 | GC–MS | 1.8 | n.r |
| Spathulenol | 1575 | RI,GC–MS | 12.4 | n.r |
| *cis*-Nerolidol | 1585 | RI,GC–MS,Co-I | 5.7 | |
| α-Bisabolol | 1685 | RI,GC–MS,Co-I | 22.9 | n.r |
| *trans, trans*-Farnesol | 1710 | RI,GC–MS | 4.0 | n.r |
| Campherenone | 1715 | RI,GC–MS | 1.7 | n.r |
| 4-Oxo-3,4-dihydro-2,3-diazaphenoxathiin | 1775 | GC–MS | 0.9 | n.r |
| $C_{15}H_{26}O$ | 1805 | GC–MS | 1.7 | |
| 6,10,14-trimethyl pentadecan-2-one | 1905 | GC–MS | 0.8 | n.r |
| Grouped constituents: | | | | |
| Oxygen-containing monoterpenes | | | 17.6 | |
| Sesquiterpene hydrocarbons | | | 7.0 | |
| Oxygen-containing sesquiterpenes | | | 48.4 | |
| Non-terpenic constituents | | | 7.4 | |
| Volatile oil yield (ml/100 gm dry weight) | | | 0.4 | |

* = on OV-1

n.r = Not previously reported in the volatile oil of *Achillea millefolium* ssp. *millefolium*; RI = Retention index; Co-I = Co-injection.

from the flowers of the Portugese subspecies.[5] We were unable to detect the oygenated monoterpene 1,8-cineole in the oil isolated from the Iranian flowering plant. However, this monoterpene was only reported as a minor constituent of the oil obtained from leaves of *A. millefolium* collected during the vegetative phase[5] and the oil obtained from the same flowering plant growing wild in Kerepes (Hungary).[12] On the other hand 1,8-cineole has been reported to be the major constituent in the oils of several *Achillea* species.[19–21]

No thujane monoterpenes were detected in the volatile oil of the Iranian *A. millefolium* ssp. *millefolium*. Components of this type have been found in relatively high amounts in the Portugese *A. millefolium* ssp. *millefolium*,[5,6] *A. abrotanoides* and *A. grandifolia*.[17]

The oil isolated from Iranian *A. millefolium* possessed a high percentage (55.4%) of sesquiterpenes α-bisabolol (22.9%), spathulenol (12.4%), *cis*-nerolidol (5.7%), and *trans, trans*-farnesol (4.0%) being the major components of this fraction. The percentage of sesquiterpenes in the oil obtained from flowers and leaves of the plants grown in Portugal was much lower (up to 8.5%) and was dominated by germacrene-D. In contrast, a high percentage of sesquiterpenes was found in the oils of *A. millefolium* ssp. *collina* Becker,[18] *A. distans* W. et K. ex Willd,[19,20] *A. ochroleuca* Ehrh.[20], *A. nobilis* L. and *A. ptarmica* L.[21]

No azulenes were detected in the volatile oil of the Iranian *A. millefolium* ssp. *millefolium*. However, Lamaison *et al.*[10] have reported the presence of trace amounts of azulene in the French *A. millefolium* ssp. *millefolium*. A correlation between the ploidy level and the ability of *Achillea* species to synthesize proazulenes has been shown.[9,21] Although in a few cases, proazulenes have been detected in diploids of the *millefolium* group,[22] the majority of the diploid *Achillea* taxa are not able to produce proazulene compounds.[21,23]

As can be seen in Table 1, two monoterpenic, seven sesquiterpenic and four non-terpenic compounds were not previously reported in the essential oil of *A. millefolium* ssp. *millefolium* growing in other parts of the world.

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