

Short communication

Essential oil composition of the leaves and stems of *Meum athamanticum* Jacq., from Spain[☆]

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Abstract

The essential oil of the leaves and stems of *Meum athamanticum* Jacq., has been extracted by steam distillation and analysed by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC–MS). The monoterpene fraction was predominant while the sesquiterpene one was practically absent. The principal constituents have been identified as (*E*)- β -ocimene (29.6%), γ -terpinene (17.9%), terpinolene (17.0%) and *p*-cymene (9.7%). Our results show that the chemical composition of the essential oil obtained of the leaves and stems of *M. athamanticum* from Spain is different to that obtained from plants of Germany, Italy and France.

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1. Introduction

The genus *Meum* Miller belongs to the Apiaceae family, one of the most widely distributed around the world. *Meum athamanticum* Jacq., commonly named “spignel” is the only species currently recognized in this genus. It is a glabrous, strongly aromatic perennial plant which grows wild in the Western and Central Europe mountains [1].

The botanical and ecological aspects of this species have been previously study [2,3] and it has been widely investigated from a chemical point of view in order to explain its use in popular medicine [4]. Some of the components isolated from the extracts of *M. athamanticum* were cinnamic acid esters (methyl ferulate, methyl caffeate, quinic acid and feruloyl quinic acid) [5], phthalide derivatives (ligustilide, butylidenphthalide and butylphthalide) [4] and sterol derivatives (cerylalcohol and β -sitosterine) [6].

The essential oils of *M. athamanticum* have been also previously reported. The first report about the root

oils of a German specimen showed ligustilide (40.2%), δ -3-carene (12.6%) and viridene (12.6%) as main constituents [7,8]. Besides, other components typical of liverworts (β -bazzanene, α -barbatene, β -barbatene and their derivatives) were identified from the steam distilled oil of this species [9]. The essential oil of this species growing in Middle Appennin (Italy) showed (*E*)- β -ocimene (34.9%), *p*-cymene (12.1%), (*Z*)- β -ocimene (10.2%) and δ -3-carene (6.2%) as main constituents from the epigeous part and (*Z*)-ligustilide (36.2%), (*E*)- β -ocimene (14.4%) and (*Z*)-3-butylidene phthalide (6.3%) from the hypogeous one [10]. The volatile components of this species extracted by solid-phase microextraction (SPME) from France showed differences depending of the month of collection. The sample gathered in July had α -acoradiene (32.0%) and β -pinene (10.0%) as main constituent, while δ -elemene (41.0%) and β -pinene (14%) were the major compounds in the September recollection [11]. Recently, a study of this species has detected a new phthalide compound (3-but-2-enylidene-4,5,6,7-tetrahydro-3-H-isobenzofuran-1-one), (*Z*)-ligustilide and sedanoic acid lactone as main constituents [12].

In the present work, we report on the chemical composition of the oil from the leaves and stems of *M. athamanticum* from Spain extracted by hydrodistillation and analyzed by

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gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC–MS). As far as we know it is the first report about the analysis of a Spanish specimen.

2. Material and methods

2.1. Plant material

The leaves and stems of one population of *Meum athamanticum* were gathered in Salientes (León province, Spain) during late August of 2001. A voucher specimen (MACB-83201) has been lodged at the Herbarium of the Faculty of Biology, Complutense University, Madrid, Spain.

2.2. Oil isolation

The oils from the air-dried leaves and stems were isolated by steam distillation with cohobation for 8 h according to the method recommended in the Spanish Pharmacopoeia. The oils were dried over anhydrous sodium sulphate and stored at 4 °C in the dark. The sample yielded 0.6% of a yellow pale oil (based on dry mass etc.).

2.3. GC analysis

Analytical gas chromatography was carried out on a Varian 3300 gas chromatograph fitted with a fused methyl silicone DB-1 column (50 m × 0.25 mm), 0.25 µm film thickness. Temperature was programmed from 95 to 240 °C at 4 °C/min. Injection was performed at 250 °C in the split mode (1:100). Nitrogen was used as the carrier gas (1.5 ml/min). Flame ionization detection (FID) was performed at 250 °C. Injection volume for the sample was 0.1 µl.

2.4. GC–MS analyses

A Hewlett-Packard 5890 gas chromatograph fitted with a fused silica SE-30 capillary column (50 m × 0.22 mm), 0.25 µm film thickness, coupled to a HP 5971A mass-selective detector was used for qualitative determination. Column temperature was programmed from 70 to 220 °C at 4 °C/min. Results were confirmed in a Hewlett-Packard 6890 gas chromatograph coupled to a HP 5973 mass-selective detector, fitted with a fused silica HP INNOWax polyethyleneglycol capillary column (50 m × 0.20 mm), 0.20 µm film thickness. In both cases, helium was used as carrier gas and mass spectra were recorded in the scan mode at 70 eV.

2.5. Qualitative analyses

Most constituents were tentatively identified by GC by comparison of their retention indices with those of authentic standards available in the author's laboratory or with

retention indices in close agreement with [13–18]. Further identification was achieved by GC–MS. Other constituents were either synthesised or identified in oils of known composition. The fragmentation patterns of mass spectra were also compared with those stored in the spectrometer data base using the WILEYL built-in libraries. Mass

Table 1

Essential oil composition from the leaves and stems of *M. athamanticum* Jacq.

Compound	<i>I</i>	%	Methods
α-Thujene	926 (1066)	0.1	GC–MS, <i>I</i> _s
α-Pinene	934 (1071)	2.1	GC–MS, <i>I</i> _s
α-Fenchene	950 (10976)	0.1	GC–MS, <i>I</i> _s
3-Methylcyclohex-3-en-1-one	961	<i>t</i>	GC–MS, <i>I</i> _b
Sabinene	970 (1140)	0.5	GC–MS, <i>I</i> _s
β-Pinene	978 (1133)	1.4	GC–MS, <i>I</i> _s
3- <i>p</i> -Menthene	979	<i>t</i>	GC–MS, <i>I</i> _s
Myrcene	981 (1171)	1.3	GC–MS, <i>I</i> _s
α-Phellandrene	1003 (1186)	1.4	GC–MS, <i>I</i> _s
δ-3-Carene	1010 (1169)	0.4	GC–MS, <i>I</i> _s
α-Terpinene	1012 (1202)	<i>t</i>	GC–MS, <i>I</i> _s
<i>p</i>-Cymene	1015 (1296)	9.9	GC–MS, <i>I</i> _s
Limonene	1022 (1221)	3.5	GC–MS, <i>I</i> _s
(<i>Z</i>)-β-Ocimene	1024 (1244)	8.6	GC–MS, <i>I</i> _s
(<i>E</i>)-β-Ocimene	1034 (1264)	29.7	GC–MS, <i>I</i> _s
γ-Terpinene	1050 (1269)	17.6	GC–MS, <i>I</i> _s
<i>m</i> -Cymenene	1070 (1456)	<i>t</i>	GC–MS, <i>I</i> _s
α-Terpinolene	1082 (1309)	16.9	GC–MS, <i>I</i> _s
1,3,8- <i>p</i> -Menthatriene	1102 (1383)	<i>t</i>	GC–MS, <i>I</i> _s
<i>allo</i> -Ocimene	1113 (1388)	<i>t</i>	GC–MS, <i>I</i> _s
(<i>Z</i>)-Myroxide	1125 (1493)	<i>t</i>	GC–MS, <i>I</i> _b
(<i>E</i>)-Sabinol	1130 (1493)	<i>t</i>	GC–MS, <i>I</i> _s
(<i>E</i>)-Myroxide	1142 (1484)	0.3	GC–MS, <i>I</i> _b
<i>neo-allo</i> -Ocimene	1142	<i>t</i>	GC–MS, <i>I</i> _s
Viridene	1157 (1416)	0.6	GC–MS, <i>I</i> _b
(2-penten-1-yl-3-cyclohexadiene)			
<i>p</i> -Cymen-8-ol	1170 (1826)	<i>t</i>	GC–MS, <i>I</i> _s
Myrtenal	1178 (1473)	<i>t</i>	GC–MS, <i>I</i> _s
Myrtenol	1179 (1493)	<i>t</i>	GC–MS, <i>I</i> _s
<i>p</i> -Cymen-7-ol	1190 (1558)	<i>t</i>	GC–MS, <i>I</i> _s
Citronellol	1213 (1488)	<i>t</i>	GC–MS, <i>I</i> _s
(<i>E</i>)-Chrysanthenyl acetate	1223 (1804)	<i>t</i>	GC–MS, <i>I</i> _s
Thymol	1278 (2113)	<i>t</i>	GC–MS, <i>I</i> _s
(<i>E,Z</i>)-2,4-Decadienal	1288 (1429)	<i>t</i>	GC–MS, <i>I</i> _b
β-Elementene	1390 (1584)	<i>t</i>	GC–MS, <i>I</i> _s
β-Caryophyllene	1418 (1615)	2.0	GC–MS, <i>I</i> _s
(<i>Z</i>)-β-Farnesene	1441 (1662)	<i>t</i>	GC–MS, <i>I</i> _s
α-Humulene	1450 (1690)	<i>t</i>	GC–MS, <i>I</i> _s
<i>epi</i> -Bicyclosesquiphellandrene	1452 (1730)	0.1	GC–MS, <i>I</i> _s
Bicyclogermacrene	1492 (1760)	0.4	GC–MS, <i>I</i> _s
Spathulenol	1568 (2127)	0.8	GC–MS, <i>I</i> _s
(<i>Z</i>)-Nerolidol	1570 (1934)	<i>t</i>	GC–MS, <i>I</i> _s
Caryophyllene oxide	1574 (1998)	0.1	GC–MS, <i>I</i> _s
β-Oplophenone	1601 (2129)	<i>t</i>	GC–MS, <i>I</i> _b
Butylidene-phthalide(<i>Z</i>)-3	1649 (2023)	<i>t</i>	GC–MS, <i>I</i> _b
(<i>Z</i>)-Ligustilide	1714 (1417)	2.3	GC–MS, <i>I</i> _b
(<i>E</i>)-Ligustilide	1783 (1467)	<i>t</i>	GC–MS, <i>I</i> _b

t = Trace (<0.1%); *I* = Kováts retention index according to *n*-alkanes on DB-1 column and on INNOWax polyethyleneglycol column in parenthesis; *I*_s = Kóváts retention index according to authentic standards; *I*_b = Kóváts retention index according to bibliography; principal compounds on bold characters.

spectra data of viridene were in good agreement with the [8].

3. Results and discussion

Forty six compounds have been identified in the essential oil extracted from the leaves and stems of *M. athamanticum*; their retention indices and percentage composition are given in Table 1, where the components are listed in the order of their elution on the DB-1 column. The monoterpene fraction amounts 94.4% of the oil while the sesquiterpene fraction was lower than 6.0%. The principal components were identified as (*E*)- β -ocimene (29.6%), γ -terpinene (17.9%) and α -terpinolene (17.0%). Other minor constituents were found to be *p*-cymene (9.7%), (*Z*)- β -ocimene (9.2%), limonene (3.0%) and (*E*)-caryophyllene (1.9%).

Our results agree with those of [7–12], the essential oils from the aerial parts of *M. athamanticum* being richer in monoterpenes than in sesquiterpenes. Nevertheless, the Spanish sample studied by us show different major compounds that those found for German, Italian and French plants [9–12], although comparisons are difficult since some of these plants [11] were extracted by SPME. As the volatile composition of this species has been shown to change during the year, it should be necessary a seasonal study of different populations in order to establish if differences have been caused by seasonal effects or they can correspond to chemotypes.

It is worth mentioning that some of the identified compounds [viridene, (*Z*)-ligustilide and (*E*)-ligustilide] that have been reported as main constituents of the root oils of this species, in our analysis they only appeared as minor constituents. The identification of viridene (2-penten-1-yl-3-cyclohexadiene) was confirmed using the spectral data of [8] identified in although has been also identified in other species as *Ligusticum mutellina* (L.)

Crantz [19]. We did not detect the new compound recently isolated in *M. athamanticum* from Germany [12].

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