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THE ESSENTIAL OIL COMPOSITION OF *CUNILA MICROCEPHALA* AND *CUNILA FASCICULATA**

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Key Word Index—*Cunila fasciculata*; *C. microcephala*; Lamiaceae; essential oil; GC–MS; limonene; menthofuran; poejo.

Abstract—The essential oils of cultivated and wild-growing *Cunila microcephala* and *C. fasciculata* from southern Brazil were analysed by GC, GC-mass spectrometry and ^{13}C NMR spectroscopy. The main constituent in both species was found to be menthofuran (82.3–85.1% in *C. microcephala* and 71.6–76.4% in *C. fasciculata*). Other compounds in significant amounts were limonene (2.1–3.8% in *C. microcephala* and 8.6–11.2% in *C. fasciculata*) and β-caryophyllene (3.3–3.9% in *C. microcephala* and 3.5–4.2% in *C. fasciculata*). The chemotaxonomic and toxicologic aspects of menthofuran accumulation are discussed. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The genus *Cunila* Royen ex L., subfamily *Nepetoideae*, tribe *Mentheae* [1] consists of *ca* 22 species with two centres of distribution: Mexico with 10 species [2] and the southern parts of South America (southern Brazil, Argentina, Paraguay and Uruguay) with 12 species. The latter species are divided into three sections (*Incana*, *Incisa* and *Spicata*) [3]. The species analysed here, *Cunila microcephala* and *C. fasciculata* belong to the section *Spicata*.

Cunila microcephala is native to southern Brazil, Argentina and Uruguay. It is a perennial herb with prostrate or ascendent branches growing in dense groupings at the edge of gallery forest and swamps. Flowers and fruits are found from September to December. Its leaves and flowers are used in the form of tea, internally as a stimulant, aromatic, antispasmodic and emenagogue and in the treatment of chronic cough and respiratory infections [4]. An ethnobotanical survey showed that the plant is one of the most widely used medicinal plants in the region of Porto Alegre, Rio Grande do Sul (RS), Brazil [5]. It is one the few native medicinal plants cultivated in gardens in various states in Brazil and it is currently commercialized by herbalists in Porto Alegre and other cities in RS. *Cunila microcephala* is also included in the first Brazilian Pharmacopoeia [6], and is popularly known as 'poejo', due to its morphological resemblance to pennyroyal (*Mentha pulegium* L.), a European plant cultivated in Brazil.

The other species examined—*C. fasciculata*—is endemic in RS. Until now, samples have been collected only in two sites of the state. It is an erect or ascendant herb *ca* 0.5 m in height, growing in dense groups in sandy soils of inundatable fields and swamps. No popular use is referred to in the examined literature. However, in the region of occurrence it is also called '*poejo*' and used in popular medicine as for *C. microcephala*. Furthermore, it is also used in culinary practice as flavour for candies made from milk and sugar cane.

Continuing our investigation on the essential oil of *Cunila* species [7], the results concerning *C. microcephala* and *C. fasciculata* are presented. Chemotaxonomic aspects and possible health hazards in the use of these plants are also discussed.

RESULTS AND DISCUSSION

The oil obtained from *C. microcephala* revealed an intense flavour upon tasting. The yield (v/w) of wild *C. microcephala* was 0.16% (sample M1) and 0.34% (M2). The sample from the cultivated plant of *C. microcephala* was significantly higher in yield, 0.80% (M3). The light-yellow oil from *C. fasciculata* has a flavour similar to that of *C. microcephala*. The yields from three wild samples were between 0.65 and 0.74% (0.65, 0.70 and 0.74% for samples F1, F2 and F3, respectively).

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Table 1. Constituents identified in the essential oils of *Cunila microcephala* and *C. fasciculata*

Components	Percentage in samples*						Identification method	Mass fragments [*m/z* (intensity)] of tentatively or unidentified compounds
	M1	M2	M3	F1	F2	F3		
α-Pinene	0.48	0.46	0.51	0.74	0.48	0.43	a,b	
Camphene	tr	tr	tr	0.55	0.19	0.25	a,b	
β-Pinene	0.43	0.41	0.47	0.47	0.44	0.36	a,b	
Sabinene	0.15	0.19	0.24	0.16	0.14	0.18	a,b	
β-Myrcene	0.39	0.41	0.36	0.43	0.53	0.51	a,b	
Limonene	3.8	2.1	3.2	8.6	11.2	9.16	a,b,c	
1,8-Cineole	0.76	0.9	1.3	0.37	0.28	0.35	a,b	
(*Z*)-β-Ocimene	tr	0.1	tr	tr	tr	tr	a,b	
γ-Terpinene	tr	tr	tr	tr	tr	tr	a,b	
Unidentified acetic acid ester derivative	0.13	0.1	0.1	0.54	—	—	a	43(100%), 55(12%), 70(10%), 83(12%), 101(18%), 112(7.5%), 143(2.3%)
Octen-1-ol acetate†	0.15	tr	tr	tr	—	—	a	43(100%), 54(22%), 67(22%), 72(12%), 81(9%), 91(15%), 99(26%), 128(2.5%)
3-Octanol	tr	0.1	tr	tr	tr	tr	a,b	
1-Octen-3-ol	0.23	0.17	0.1	0.1	tr	0.13	a,b	
Menthofuran	84.24	85.1	82.3	71.65	76.42	72.31	a,c	
Linalool	0.5	0.43	0.71	0.46	0.36	0.57	a,b	
β-Caryophyllene	3.92	3.54	3.3	4.42	3.85	3.5	a,b	
Pulegone	tr	tr	tr	tr	tr	tr	a,b	
α-Terpineol	0.41	0.15	tr	0.89	0.47	0.2	a,b	
Germacrene D	0.64	1.32	0.93	0.26	0.24	1.25	a,b	
Germacrene B†	1.23	1.50	0.84	1.79	1.77	2.4	a	41(47%), 55(26%), 67(37%), 79(58%), 93(100%), 107(61%), 121(93%), 136(15%), 147(4.8%), 161(19%), 189(7.9%), 204(22%)
Unidentified benzofuran derivative (*M*, 176)	0.51	0.38	0.4	0.75	0.68	0.67	a	77(28%), 103(15%), 105(15%), 115(12%), 145(16%), 161(50%), 176(100%)
Caryophyllene oxide	0.28	0.29	0.3	1.27	0.22	0.7	a	
(−)-Spathulenol	0.21	0.21	0.4	0.91	0.12	0.7	a,b	
Unidentified sesquiterpene (*M*, 220)		—	—	1.5	0.48	1.9	a	41(97%), 55(46%), 69(94%), 79(84%), 91(100%), 105(53%), 117(23%), 133(24%), 145(12%), 159(13%), 187(13%), 205(5.2%), 220(0.9%)

*Samples: *C. microcephala* M1: aerial parts, flowering branches; M2: aerial parts, sterile branches; M3: aerial parts, post-flowering stage; F1: *C. fasciculata* leaves of the plants at the flowering stage; F2: inflorescences; F3: aerial parts of sterile plants.

†Tentative identification; tr <0.1%.

a = Comparison of mass spectra to those from the NIST library [8]; b = GC retention data comparison with standards; c = ^{13}C NMR data comparison with those of ref. [9].

The constituents of the essential oils from *C. microcephala* and *C. fasciculata* are presented in Table 1. Identification of these compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library [8] and by comparison of the retention values with those of standards. Furthermore, the ^{13}C NMR spectra of the essential oils were considered for the additional confirmation of menthofuran. The ^{13}C NMR spectra signals have been assigned based on the reported individual spectrum of menthofuran [9], considering their chemical shifts and signal intensities.

Comparison of the data in Table 1 demonstrates that there are no major qualitative and quantitative differences between the composition of the essential oils from the examined samples. The main component in both oils was menthofuran with 82.3–85.1% in *C. microcephala* and 71.6–76.4% in *C. fasciculata*. Other compounds in significant amounts were limonene with 2.1–3.8% in *C. microcephala* and 8.6–11.2% in *C. fasciculata*, and β-caryophyllene with 3.3–3.9% in *C. microcephala* and 3.5–4.2% in *C. fasciculata*. Only traces (< 0.1%) of pulegone were detected in the samples.

Up to now, the accumulation of menthofuran as the main component in essential oils has been reported only for the *Capitatae* group of the section *Mentha* from the genus *Mentha* [10]. According to Lawrence [11] it has been reported as the main constituent in *M. aquatica* (85%), *M. arvensis* L. f. *piperacens* Holmes (40%), *M.* × *verticillata* L. (70%), *M.* × *dumetorum* Schultes (25%) and *M.* × *maximiliana* F. M. Schultz (35%). The occurrence of menthofuran as a main constituent of essential oil is therefore reported here for the first time outside of the genus *Mentha*. Considering the published results on the essential oil of *C. lythrifolia* [12], *C. angustifolia* [13], *C. incisa* [6] and our own unpublished results for other *Cunila* species from southern Brazil, the occurrence of menthofuran seems to be limited to both of the species from the section *Spicata* examined here.

The occurrence of menthofuran in high concentrations is also interesting from the toxicological point of view. Menthofuran is considered a hepatotoxin that is metabolically activated to reactive intermediates capable of covalent binding to cellular proteins [14]. Actually, it is considered to form the same electrophilic metabolite as pulegone, a well known hepatotoxic substance which is responsible for the toxicity of pennyroyal oil (*M. pulegium*). The oral administration of menthofuran to rats once a day caused dose and time-dependent increase in serum glutamic pyruvic transaminase and decrease in the level of liver microsomal cytochrome P_{450} reductase activities [15]. Therefore, we consider that the utilization of these species in popular medicine should be viewed with the same concern as the utilization of pennyroyal [16]. The presence of *C. microcephala* in the Brazilian Official Pharmacopoeia should be re-evaluated in the light of these results.

EXPERIMENTAL

Plant material. Wild *C. microcephala* Bentham samples were collected in Amaral Ferrador (RS) in October 1993 (M1) and May 1994 (M2). The cultivated *C. microcephala* sample was collected in Viamão (RS) in December 1993 (M3). *C. fasciculata* Bentham samples were collected in Paraíso do Sul (RS) in October 1993 (F1 and F2) and May 1994 (F3). Herbarium specimens (voucher leg. Bordignon 1306 (M1), Bordignon and Salazar 1335 (M2), Bordignon *et al.* 1312 (M3), Bordignon 1300 (F1 and F2) and Bordignon 1338 (F3) are deposited in the Herbarium of the Federal University of Rio Grande do Sul, Brazil (ICN).

Analysis of the essential oils. Fresh aerial parts of the plant were subjected to steam distillation for 2 hr using a Clevenger-type apparatus. The obtained essential oil was sepd from H_2O and dried over Na_2SO_4. Volatile oils were analysed by GC and GC-MS using a fused silica capillary column coated with Carbowax 20M (60 m × 0.25 mm × 0.25 μm). The column temp. was programmed from 40–220° at 3° min^{-1}. Chromatographic peaks from GC-MS analysis were checked for homogeneity with the aid of mass chromatograms of characteristic fragment ions. Further analyt. conditions were the same as those described previously [6].

^{13}C NMR spectroscopy. ^{13}C NMR spectra of the oils were recorded in $CDCl_3$ soln (1:1) at 50 MHz with TMS as into standard. The following parameters were used: pulse width 45°, acquisition time 1.5 sec for 64 K data table with spectral width of 250 ppm, number accumulations was 10 000. The presence of menthofuran in the ^{13}C NMR spectrum of essential oils could be proved by the signals at δ 150.51, 136.70, 119.41, 117.24, 31.42, 31.33, 29.58, 21.43, 19.83 and 7.98.

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