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COMPOSITION AND VARIATION OF ESSENTIAL OIL CONSTITUENTS IN THREE *MENTHA* L., (LABIATAE) SPECIES IN YUGOSLAVIA

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Abstract

The essential oil components piperitone, linalool, menthol, geraniol, limonene, carvone, pulegone, caryophyllene, menthone, isomenthone, neoisomenthone *P.* cymene, borneol, piperitenone an a- terpinene were determined by GLC in 3 wild species of the genus *Mentha* collected from the River Danube ravine north east of Yugoslavia. Considerable variation was observed among some essential oil components; commercially this variation could be useful.

Introduction

The genus *Mentha* L., is very complex due to different sex forms and ploidy levels, long history of clonal cultivation and naturalisation of cultivated forms, followed by hybridisation with native species and morphological plasticity (Harley, 1972). The taxa under study are distributed in the temperate regions of the old world; like many Mediterranean labiates they are rich in volatile oils. These oils are found in glands which are distributed mainly on the leaves.

Essential oils of *Mentha* species in the past have been the object of numerous investigation (Jancic, 1984; Martinieus & Croteau, 1981). This is the first study where the essential oil components of wild *Mentha* species of Yugoslavian origin have been analysed. The taxa analysed by GLC show very clearly that essential oil components e.g., linalool, menthol and piperitone variation could be important.

Material and Methods

Voucher specimens of the different species analysed have been deposited in the herbaria of the Institute of Botany, Faculty of Pharmacy, Belgrade University. Leaves and inflorescences were dried at room temperature and essential oil components were extracted by steam distillation. The extracts were analysed qualititatively and quantitatively by GLC. Steel column, packed with carbowax 20 M, 5% on chromo-

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Table 1. Distribution of essential oil components from different geographical locations in *Mentha arvensis*.

Essential oil components in order of their Rt.	% of total oil in different geographical location			
	U.S.A.*	China*	Yugoslaviat**	Japan*
a - pinene	0.6	1-4	0.04	0.2-1.5
Ḇ - pinene	0.6-0.91	1-4	0.02	0.9-1.3
myrcene	0.38-0.70	0.2-1.0	0.02	---
limonene	0.66-6.20	1.0-4	0.17	4.3-6.0
P-cymene	---	---	0.03	---
sabinene hydrate	0.25	0.2	0.57	---
menthofuran	0.25	0.2	0.57	---
menthone	3.4-14.7	10.0	1.10	9.9-17.1
isomenthone	1.9-4.82	---	0.21	2.7-4.2
menthyl acetate	0.95-2.57	1-4	5.18	4.7-6.9
neomenthol	0.86-1.55	4-10	34.35	1.6-2.2
neoisomenthol	0.3	---	4.89	---
menthol	65.36-80.29	10	33.37	61.5-68.6
isomenthol	---	---	2.88	---
pulegone	0.20-0.50	0.2-1.0	5.08	0.2-1.1
piperitone	0.2-1.67	4-10	2.60	1.8-2.7

* Data relates to cultivated species or different races of the same species from literature, Rt = retention time, ** average of five samples.

sorb W AW 80 - 100 mesh; oven temperature was linear programmed from 75°C - 220°C, (3°/min). Detection and injector temperature were 225°C and 250°C. Carrier gas Helium with a flow rate of 25 ml/min. was used. Flame ionization detector was used. Quantitative identification of compounds was carried out by disc integration digital printer attached to the gas chromatograph. The different fractions were identified against authentic markers and comparisons of retention times from the literature.

Results and Discussion

Yield percentages from different geographical locations of 16 identified oil components in *M. arvensis* L., are presented in Table 1. In our investigations it was found that menthol 33.47%, neomenthol 34.35%, pulegone 5.08%, menthyl acetate 5.18%, isomenthol 2.88% and piperitone 2.60% were the main components, menthol and neomenthol being at the highest percentage levels. The high level of neomenthol is most likely due to the activation of specific dehydrogenase which converts menthone to neomenthol (Martinicus & Croteau, 1981) and probably due to the maturation of plants and some other environmental factors (Murray *et al.*, 1972). On the whole our results were comparable with findings from other geographical areas of

Table 2. Distribution of essential oil components from different geographical locations in *Mentha longifolia*.

Essential oil components in order of their Rt.	% of total oil in different geographical location			
	Greece	Netherlands	Germany	Yugoslavia
a - pinene	0.6.1	0.17	0.46	0.01
β - pinene	1-1.5	1.93	1.7	0.08
myrcene	0.7-1.2	0.53	---	0.13
limonene	0.66-6.20	1.0-4	0.17	4.3-6.0
s- terpinene	0.4-0.6	---	---	0.64
P-cymene	0.1-0.2	---	----	1.55
menthone	0.4-0.8	---	---	0.14
isomenthone	1.0-2.3	---	---	0.62
1-terpinen-4-ol	0.1	---	---	0.22
isomenthone	1.9-4.82	---	0.21	2.7-4.2
Ƀ-caryophyllene	4.9-6.7	---	---	4.52
borneol	---	---	---	1.16
pulegone	---	---	---	0.33
piperitone + piperitone oxide	59.3-66.4	62.24	75.79	73.16
geraniol	---	---	---	2.95
piperitenone	1-1.2	1.5	---	4.87
piperitenone oxide	2.3-2.9	1.75	---	0.68

* average of five samples. Rt. = retention time.

this species except isomenthol and neoisomenthol were present and identified against authentic markers in our analysis but were not reported by other workers and the percentage amounts of essential oil components, limonene, menthone, isomenthone, were reported to be higher than in our investigation (Hefendehl & Zeigler, 1975; Handa *et al.*, 1964; Lawrence, 1981). In *M. arvensis* the menthol content was always at the highest level whereas in *M. longifolia* (L.) Hudson menthol was absent and in *M. spicata* L., it was found to be only 3.51%. Pulegone in *M. arvensis* was 5.08% whereas in *M. longifolia* and *M.spicata* this component was found to be less than 1%.

The results of 16 identified essential oil components, their yields from different geographical locations in *M. longifolia* are presented in Table 2. The main essential oil components in this species were piperitone + piperitone oxide 73.16%, piperitenone 4.87%, β caryophyllene 4.52%, geraniol 2.95%, limonene 3.47% and linalool 2.97% were the main components; piperitone + piperitone oxide and piperitenone being at the highest level. This was also true for samples analysed from different geographical locations of this species (Table 2) except borneol, geraniol and pulegone were not reported by other workers and limonene was found to be at a higher level in samples from Germany when compared with samples from Greece, Holland and Yugoslavia (Karting & Still, 1975; Kokkini & Papageorgiou, 1988) a- terpinene,

Table 3. Distribution of essential oil components from different geographical locations in *Mentha spicata.*

Essential oil components in order of their Rt.	% of total oil in different geographical location					
	Japan	Argentina	U.S.A.	Poland	Greece	Yugoslavia
a - pinene	0.7	—	0.8	0.2	0.01-0.15	0.02
β - pinene	2.3	2.3	0.7	0.3	0.19-0.31	0.05
myrcene	—	—	2.5	—	4.73-5.90	0.10
limonene	20.20	22.90	9.3	1.9	0.27-0.31	1.39
P-cymene	—	—	0.2	0.2	0.01-0.31	0.17
sabinene hydrate	—	—	1.3	—	—	0.30
fenchone	—	—	—	—	—	—
menthone	0.4	5.2	—	—	—	—
linalool	1.1	—	0.1	86.3	65.21-75.31 81.25	
menthyl-acetate	—	—	—	—	—	2.05
B-caryophyllene	0.3	—	30.9	1.2	4.39-6.32	0.32
menthol	0.5	—	0.2	—	—	3.51
berneol	—	—	—	—	—	1.02
pulegone	—	3.5	—	—	—	0.42
carvone	63.6	60.5	67.6	—	0.01-0.03	2.87
dehydrocarvone	1.8	—	1.5	1.3	0.09-0.12	1.20
Caryophyllene oxide	—	—	—	—	—	—
Piperitenone	—	—	—	—	—	0.27
Piperitenone oxide	—	—	—	—	—	0.38

Average of five samples. Rt = retention time.

P. cymene, menthone, isomenthone, 1-terpinene-4-ol and B- caryophyllene were not reported in samples from Germany and Holland (Karting & Still, 1975) of this species. In our analysis piperitone + piperitone oxide were always more than 70%, whereas in *M. arvensis* this mixture was less than 3% and in *M. spicata* it was absent. Menthol was absent in *M. longifolia*.

In *M. spicata* 19 essential oil components from different geographical locations were identified, their yield percentages are presented in Table 3. The main essential oil in Yugoslavian race were linalool 81.25%, menthol 3.51%, carvone 2.87% and limonene 1.39%. Pulegone, caryophyllene were found to be less than 1% and piperitone + piperitone oxide was absent. Carvone was absent in other two species. Workers from Poland and Greece also confirm that linalool is the main essential oil in *M. spicata* (Gora & Kalemra, 1979; Kokkini & Papageorgiou, 1982). However, when data from Japan of essential oil components of *M. spicata* were compared (Nagasawa, 1974; Torres & Retaner, 1975) limonene percentage varied from 9.3 - 22.90 whereas the same component in European species was between 0.27 - 1.39. The essential oil percentage of linalool in plants from European locations varied between 65.21 - 86.3 whereas in cultivated species or different chemical races of the same

Table 4. Comparison of essential oil components of three *Mentha* species.

Essential oil components	M. arvensis	M. longifolia	M. spicata
Sabinene hydrate	(+)	-	(+)
menthofuran	+	-	-
menthone	+	(+)	(+)
isomenthone	(+)	(+)	-
menthylacetate	+	-	(+)
neomenthol	+	-	-
neoisomenthol	(+)	-	-
menthol	+	-	(+)
isomenthol	(+)	-	-
pulegone	(+)	+	+
piperitone + oxide	+	+	-
1-terpinene-4-ol	-	(+)	-
β-caryophyllene	-	(+)	(+)
borneol	-	(+)	(+)
geraniol	-	(+)	-
piperitone oxide	-	(+)	(+)
linalool	-	-	(+)
carvone	-	-	(+)
dehydrocarvone	-	-	(+)

+ = always present, (+) = variably present

species investigated in Japan, Argentina and USA the percentages were found to be between 0.1 - 1.1. Carvone from European plants was between 0.01 - 2.87% (Table 3) whereas in plants from Japan, Argentine and USA showed percentage levels which varied between 60.5 - 67.6, (Nagasawa, 1974; Torres & Retaner, 1975; Lawrence, 1980).

In previous work we have examined different genotypes of *M. piperita* L., cultivated in Yugoslavia because of the commercial interest, and its pharmacological activities (Tyler *et al.*, 1976). All essential oil components e.g., limonene, cineol, pulegone, piperitone in the genus *Mentha* contribute to the pharmacological activities (Wagner, 1980). Limonenem pulegone and linalool are commonly used as food flavours. Several species of *Mentha* including *M. spicata* and *M. longifolia* have also been studied for their chemotaxonomic applications (Tomas-Barberan *et al.*, 1988). To overcome the essential oils variability at least 5 individuals of each species were analysed. It was found that major essential oil studies carried out on some of these cultivated or different chemical races of taxa from different geographical locations (Table 2 and 3) show quantitative variations but also confirm that the major essential oil components were always present.

These results show that the essential oil components are widespread in the genus *Mentha* and the GLC analysis clearly indicates that menthofuran, piperitone, menthol, sabinene hydrate, isomenthone, neomenthol, methyl acetate have only a very limited distribution in these three *Mentha* species (Table 4).

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