Comparative essential oils study of *Thymus longidens* Velen. *var. lanicaulis* Ronn. and *thymus longidens* Velen. *var. dassareticus* Ronn.

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Pervenuto in Redazione l'8 luglio 1995

SUMMARY

Using GC and GC-MS techniques, two different varieties of *Thymus longidens* Vel. (*var. lanicaulis and var. dassareticus*) have been examined on essential oils composition. Big differences in oil composition were noticed. *Var. lanicaulis* contained 35.65 41.56/33.66% of thymol/carvacrol, 11.96-31.52% of geraniol and 9.46-9.66% of terpynil acetate as main components while the essential oil from *var. dassareticus* contained very low amounts of thymol (3.95%) and carvacrol (1.98%) and higher quantities of linalool (14.81%), geraniol (14.49%), α-terpineol (15.62%) and terpynil acetate (16.23%) as major constituents. Lemon like odor of second oil was noticed, originating from presence of *e*-citral (5.56%) and *z*-citral (3.60%) that were determined. First analyzed taxa belongs to the phenol type while the second one is lemon type of genera *Thymus* L.

RIASSUNTO

È stata esaminata la composizione di olii essenziali di due differenti varietà di *Thymus longidens* Vel. (*var. lanicaulis e var. dassareticus*) mediante GC e GC-MS. *Var. lanicaulis* contiene 35.65-41.56/33.66% di timolo/carvacrolo. 11.96-31.52% di geraniolo e 9.46-9.66% di terpinilacetato come componenti principali mentre l'olio essenziale da *Var. dassareticus* contiene quantità molto piccole di timolo (3.95%) e carvacrolo (1.98%) e quantità maggiori di linalolo (14.81%), geraniolo (14.49%), α-terpineolo (15.62%) e terpinilacetato (16.23%), come costituenti principali. L'odore di limone del secondo olio deriva dalla presenza di e-citrale (5.66%) e z-citrale (3.60%). La prima varietà analizzata è di tipo fenolico, mentre la seconda è del tipo limone della varietà del genere *Thymus* L.

KEY WORDS: ***Thymus, T. longidens var. lanicaulis, T. longidens var. dassareticus*, Essential oil, GC and GC-MS analysis.**

INTRODUCTION

Two taxa of genera *Thymus* L. (*T. vulgaris* and *T. zygis*) have been established as plant source for commercial thyme oil appreciated because of its expectorant, antibacterial, antimycotic and spasmolytic efficiency [1-7]. Those effects are due to presence of phenolic constituents, thymol and carvacrol, although few recent studies point out that alcohols, especially geraniol, could be also responsible for antimicrobial activity [4]. Investigation that has been carried out in the last few years showed that the content of this phenols varied widely depending on the origin [8,9], vegetation period [10], way of collection, preparation of plant material, and other factors [11]. Examinations of some other taxa of genera *Thymus*, very often point at occurrence of other species of this genera that contain large amount of essential oil with high percentage of thymol and carvacrol [12-14]. Few studies on essential oil composition of *T. serpyllum* [15-17] showed that this taxa contain essential oil similar to that from *T. vulgaris* which provides an official use of *Thymi serpylli herba* [18]. Two taxa of genera *Thymus*, wild growing in Republic of Macedonia have been examined by GC and GC-MS techniques in intention to determine the essential oil composition and check the possibilities for their use in pharmacy or in some other fields.

EXPERIMENTAL

Plant material was collected during the summer of 1993 and 1994. Samples of *Thymus longidens* Velen. *var. lanicaulis* Ronn. were collected near Skopje. Two samples at village Sonje (**A** and **B**) and one sample at village Banjani (**C**). Sample of *Thymus longidens* Velen. *var. dassareticus* Ronn. was collected at Karadzica mountain (**D**). The identity of the taxa was confirmed by Dr. V. Matevski from the Institute of Biology, Faculty of Natural Sciences, Skopje. The voucher specimen were deposited at the Herbarium in the same Institute of Biology.
Plant material was air-dried, put in paper bags and stored at dark dry and cool place until it was used for examination.

Isolation of essential oil

Isolation of oils was carried out by hydrodistillation in Clavenger type apparatus for 5 hours. Obtained oils were dried over anhydrous sodium sulfate.

Gas chromatography (GC)

The oils were analyzed on a Hewlett-Packard gas chromatograph, model 5890, series II, equipped with split-splitless injector, fused silica capillary column PONA (crosslinked methyl silicone), 50 m, 0.2 mm I.D., 0.5 mm film thickness, and flame ionization detector (FID). Sample solutions in ethanol (1.0%) were injected in split mode (1:100) at 250°C. Detector temperature was 300°C (FID) while

the column temperature was linearly programmed from 40-280°C, 2°C/min.

Gas chromatography-mass spectrometry (GC-MS)

For GC-MS analysis Hewlett-Packard 5890 Series II gas chromatograph equipped with HP 5971 MS detector was used.

The chromatographic conditions were as above. Transfer line was heated at 280°C.

The identification of components was achieved by comparison of their retention times with those of analytical standards of terpenoides under the same chromatographic conditions, and matching mass spectral data of essential oil constituents with those from Wiley library of MS spectra.

RESULTS AND DISCUSSION

Dried herbs from *T. longidens var. lanicaulis* and *T. longidens var. dassareticus* contained 1.20-1.65% (v/w) and 1.0% (v/w) of essential oil, respectively. Obtained oils were dark yellow colored liquids with specific odor, reminiscenting to that of lemon for *var. dassareticus.*

Results from GC and GC-MS analyses, listed in Table I, showed big differences in the composition of essential oils between those two taxa. *Var. lanicaulis* contained large amount of phenols that ranged from 34.52% to 45.09%. While samples **B** and **C** contained mainly thymol (36.65-41.56%), sample **A** had carvacrol as the most abundant component. Important group of components were alcohols (16.38-36.10%). Content of geraniol was 31.52%, 11.96% and 18.17% in this fraction from samples **A**, **B** and **C**, respectively. Hydrocarbons were present in the range from 13.84% to 17.91%, and the most abundant was *p*-cymene (6.94-8.95%). Sample **A** did not contain terpenyl acetate. It was different from **B** and **C** which contained 9.46-9.65% of terpenyl acetate. *Trans*-caryophyllene (0.99-1.79%) and β-bisabolene (3.10-3.47%) were the most abundant sesquiterpens for samples **A**, **B** and **C**.

Summarizing results obtained for samples **A**, **B** and **C**, it appears that sample **A** belongs to the geraniol-carvacrol chemotype while the samples **B** and **C** belong to thymol-geraniol-terpenyl acetate chemotype of *T. longidens var. lanicaulis*. We expect that further investigation will show occurrence of other chemotypes of this taxa, unknown until now. At the moment we are working on that subject. Differences between two chemotypes of the taxa are shown in Fig. 1. Concentration indices (CI) were plotted in relation with relative retention times to thymol (RRT).

Fig. 1. Bar graph GC-patterns of different chemotypes of *T. longidens var. lanicaulis*

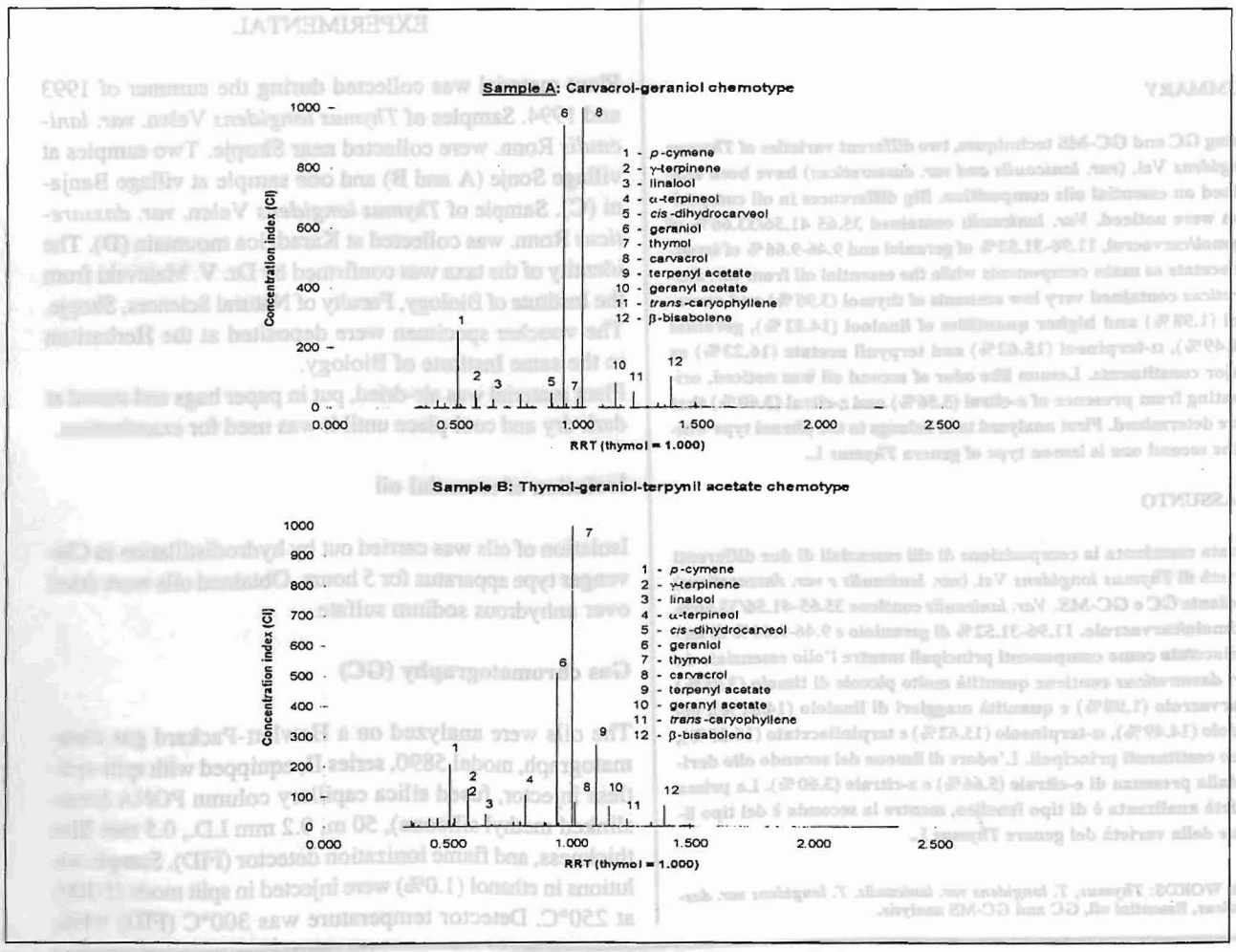


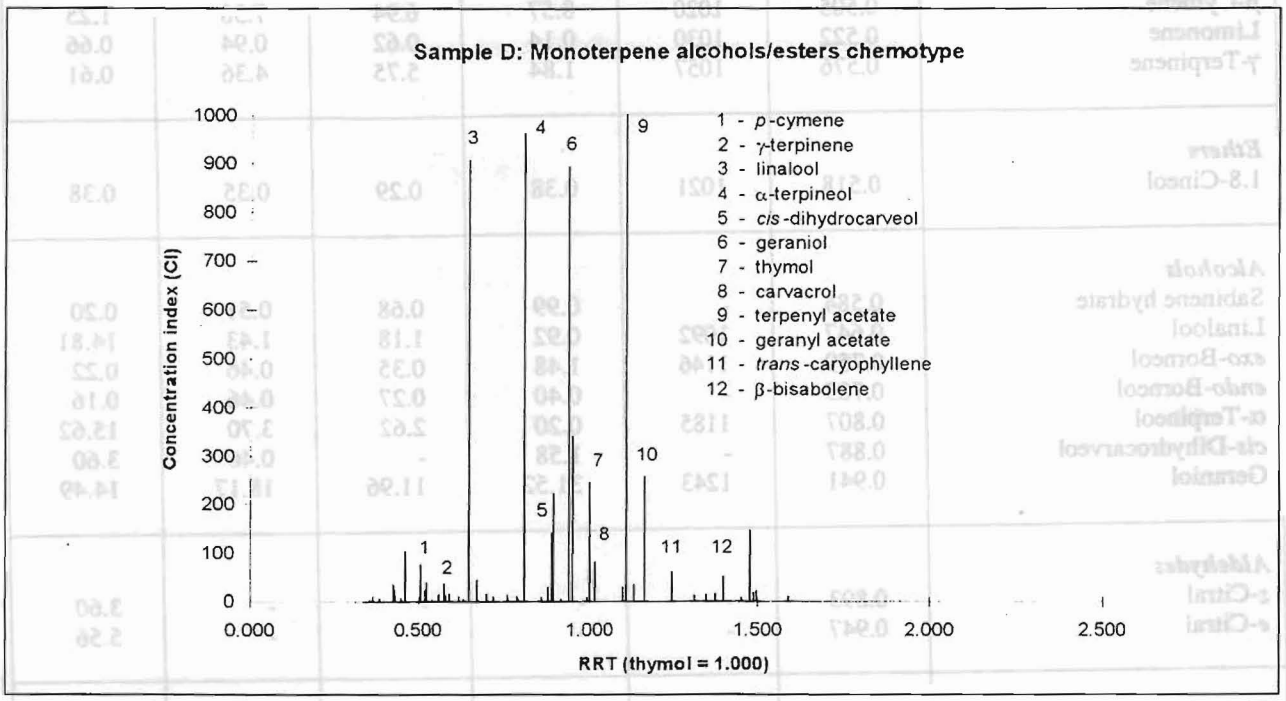
Table I. The composition of essential oils from *Thymus longidens var. lanicaulis and var. dassareticus*

Component	RRT	RI[a]	A[b]	B[b]	C[b]	D[b]
Hydrocarbons						
α-Thujene	0.353	938	0.20	0.46	0.69	0.06
α-Pinene	0.363	942	0.18	0.43	0.42	0.17
Camphene	0.382	954	0.29	0.13	0.18	0.11
Sabinene	0.426	976	0.82	0.79	0.95	0.57
β-Pinene	0.458	981	0.51	1.13	0.09	1.67
α-Phellandrene	0.477	1002	0.15	0.11	0.77	0.02
o-Cymene	0.500	-	0.28	0.98	0.38	0.07
p-Cymene	0.505	1020	8.57	6.94	7.38	1.25
Limonene	0.522	1030	0.14	0.62	0.94	0.66
γ-Terpinene	0.576	1057	1.84	5.75	4.36	0.61
Ethers						
1.8-Cineol	0.518	1021	0.38	0.29	0.35	0.38
Alcohols						
Sabinene hydrate	0.584	-	0.99	0.68	0.51	0.20
Linalool	0.647	1092	0.92	1.18	1.43	14.81
exo-Borneol	0.759	1146	1.48	0.35	0.46	0.22
endo-Borneol	0.785	-	0.40	0.27	0.46	0.16
α-Terpineol	0.807	1185	0.20	2.62	3.70	15.62
cis-Dihydrocarveol	0.887	-	1.58	-	0.46	3.60
Geraniol	0.941	1243	31.52	11.96	18.17	14.49
Aldehydes						
z-Citral	0.893	-	-	-	-	3.60
e-Citral	0.947	-	-	-	-	5.56
Phenols						
Thymol	1.000	1287	0.91	41.56	35.65	3.97
Carvacrol	1.015	1297	33.61	3.53	2.84	1.38
Acetates						
Terpenyl acetate	1.105	1333	-	9.46	9.65	16.23
Geranyl acetate	1.164	1398	2.78	1.42	2.35	4.13
Sesquiterpens						
α-Copaene	1.176		0.06	0.09	0.04	0.04
β-Bourbonene	1.188	-	0.32	0.11	0.13	0.02
trans-Caryophyllene	1.248	1406	1.79	0.99	0.89	0.96
α-Cubebene	1.256	1428	-	0.06	0.03	0.04
α-Humulene	1.286	-	0.11	0.06	0.12	0.06
γ-Murolene	1.353	1465	0.12	0.23	0.20	-
Calarene	1.366	1475	0.45	0.41	0.41	-
β-Bisabolene	1.378	-	3.47	3.10	3.17	0.85
γ-Cadinene	1.396	1501	0.23	0.36	0.31	0.10
Caryophyllene oxide	1.485	1524	0.59	0.13	0.42	0.34
Total %:			**95.80**	**96.09**	**97.92**	**82.82**

[a]Kovats's retention index
[b]Samples of oils (see text)

Essential oil from *T. longidens var. dassareticus* (sample D) contained alcohols (45.30%) as the most important constituents, with linalool (14.81%), α-terpineol (15.62%) and geraniol (14.49%) as the most abundant. Besides, 16.23% of terpenyl acetate were also registered. This sample contained very low percentage of phenols (5.35%) and hydrocarbons (5.39%). Bars (Fig. 2) corresponding to linalool, α-terpineol and geraniol are almost equal to that of terpenyl acetate. These four components are the most important for recognition of this oil.

Fig. 2. Bar graph GC-pattern of oil of *T. longidens var. dassareticus*



Specific lemon-like odor is due to presence of *e*-citral (5.56%) and *z*-citral (3.60%) that were determined, also. Because of this, the taxa belongs to the "lemon" type of the taxa of genera *Thymus*, similar to *T. beaticus* Boiss. from Spain [19] and hybrid between *T. pulegioides* and *T. vulgaris* (*T. x citriodorus*) [20] that contain very similar quantities of citral and geraniol. However, there are few other taxa containing only traces of citral (*T. Hyemalis* [21], *T. serpylloides ssp. gadorensis* [22]).

CONCLUSION

Herbaceous plant *T. longidens var. lanicaulis* thymol-geraniol-terpenyl acetate chemotype could be used in pharmacy because of high amount of essential oil, high percentage of thymol and its potential to act antimicrobial [1-4].

The second determined chemotype, carvacrol-geraniol, could be also used in pharmacy as strong antimycotic agent because of high percentage of carvacrol [21]. *T. longidens var. dassareticus* could not be used in pharmacy for preparation of some therapeutic forms but has a potential to be used for correction of odor or as a kind of flavor or fragrance.

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