Pharmazie 51 (1996) 4

Institute of Pharmacognosy[1], Faculty of Pharmacy, Institute of Chemistry[3], Faculty of Science, Skopje, Macedonia and Institute for Medicinal Plant Research[2], Belgrade, Yugoslavia

Composition of the essential oil from *Thymus alsarensis* Ronn. growing in Macedonia

SVETLANA KULEVANOVA[1], M. RISTIC[2], T. STAFILOV[3], and T. RISTOV[1]

Trough the last few years investigations on *Thymus vulgaris* essential oil, the phenols thymol and carvacrol have been established as therapeutically important components with antibacterial and antimycotic activity [1–5]. On the other hand, a lot of data showed a great variation in the quantity of this components in thyme oil, thus several chemotypes of the oil are defined [6–8] and, especially in Spain, a non-phenolic type of thyme oil is also known [7]. A few other investigation have been performed on the other taxa of the genus *Thymus* L. In many cases their essential oils contain therapeutically useful agents [9–14]. Their potential for commercial explotation in the fields of pharmaceutics, perfumes and cosmetics, distilleries, flavour and aroma enhancers and the food industry, justifies the efforts devoted in recent years to the study of the various *Thymus* taxa.

Thymus alsarensis Ronn. a plant endemic in Macedonia. It is spread in a small limited area, around Alšar mine, near Kavadarci, in the south of Macedonia. The "loccus classicus" of the plant is found in a zone with a strong influence of Mediterian climate conditions, that contribute to the fast growth of the plant and early blooming that starts in April [15]. The taxa creates powerful, pure and mixed populations very often with other *Thymus* taxa, at an altitude of about 700 m. This taxon has not been investigated up to now and this paper presents results of an essential oil investigation of *T. alsarensis*.

Hydrodistillation, carried out in a Clevenger type apparatus, after separation of the oil and desiccation over anh. sodium sulfate, yielded 1.5% (v/w) dark yellow oil with following characteristics: refractive index (n_D^{20}) 1.457 and relative density (d_{20}^{20}) 0.943. A GC and GC-MS analysis of the oil showed 65 well separated peaks. Thirty-eight of them were identified which represents 96.19% of the oil (Table). The rest (3.18%) included 27 components presented in trace amounts. The most abundant components were phenols (51.52%) with 18.52% of thymol and 33.00% of carvacrol, and hydrocarbons (21.58%) with 8.92% of *p*-cymen. The other components, alcohols (9.14%), esters (7.42%), ethers (1.18%), ketons (0.44%) and sesquiterpens (4.93%) were present in lower concentrations. The percentage of each identified component, listed in the Table, showed that beside thymol, carvacrol and *p*-cymene, terpinyl acetate was also an important constituent of the oil (7.36%). Concentrations of α-thujene, β-pinene, γ-terpinene, sabinene hydrate, *o*-cymene, borneol, *trans*-caryophyllene and β-bisabolene ranged between 1% and 4%.

The commercial thyme oil obtained from *T. vulgaris* and *T. zygis*, contains thymol (20–60%), carvacrol and *p*-cymene as the most abundant components. *Thymus alsarensis* essential oil also contains all these components and has a potential to become an article of commerce.

Table: Composition of the essential oil of *Thymus alsarensis* Ronn.

Component	CII*	(%)
Hydrocarbons		
α-Thujene	938	1.06
α-Pinene	942	0.85
Camphene	954	0.78
Sabinene	976	0.74
β-Pinene	981	2.22
α-Phellandrene	1002	0.16
orto-Cymene	–	1.14
para-Cymene	1020	8.92
Linonene	1030	0.83
γ-Terpinene	1057	3.56
Ethers		
1.8-Cineole	1021	0.96
Methylthymol	–	0.22
Alcohols		
Sabinene hydrate	–	1.26
Linalool	1092	3.21
exo-Borneol	1164	1.68
endo-Borneol	–	0.84
α-Terpineol	1185	2.46
cis-Dihidrocarveol	–	0.81
Nerol	1218	0.04
Geraniol	1243	0.10
Aldehydes		
e-Citral	1222	0.11
Ketons		
Camphor	–	0.44
Phenols		
Thymol	1287	18.52
Carvacrol	1297	33.00
Acetates		
Terpinyl acetate	1333	7.36
Geranyl acetate	1398	0.06
Sesquiterpens		
α-Copaene	–	0.05
β-Bourbonene	1406	0.05
trans-Caryophyllene	1428	1.76
α-Cubebene	–	0.04
α-Humulene	1465	0.06
β-Cubebene	1475	0.13
α-Elemene	–	0.05
Calarene	–	0.05
β-Bisabolene	1501	1.78
γ-Cadinene	1518	0.20
δ-Cadinene	1524	0.06
Caryophyllene oxide	1563	0.25
Total		96.19

* Kovats's retention index [16]

Experimental

1. Materials and instruments

Plant material – herb from *Thymus alsarensis*, was collected during the summer of 1994. A vaucher specimen was deposited at the Herbarium of Institute of Biology, Faculty of Natural Sciences, Skopje, Macedonia. The identity was conformed by Dr. V. Matevski, Institute of Biology, Faculty of Sciences, Skopje, Macedonia; anh. sodium sulfate (Merck).

Clevenger type apparatus for hydrodestillation; Hewlett-Packard 5890 series II gas chromatograph, with fused silica capillary column PONA (50 m × 0.2 mm I.D., 0.5 μm film thickness) split-splittless injector, FID; Hewlett-Packard model HP 5890 series II/HP 58971A GC/MSD system.

2. Destillation of oil

Air-dried plant material was submitted to hydrodestillation in Clevenger apparatus for 5 h. After being separated from water, the oil was dried over anh. sodium sulfate.

3. GC and GC-MS analysis

Sample solution in ethanol (1.0%) was injected in split mode (1 : 100) at 250 °C. The detector temperature was 300 °C (FID) while the column temperature was linearly programmed from 40–280 °C, with a rate of 2 °C/min. In the case of GC-MS analysis the same chromatographic conditions were used, detector operating in SCAN mode (mass range 50–300).

Identification of the components was based on comparison of their retention times with those of authentic samples on the same column and matching the mass spectral data with those from the Wiley library of MS spectra. The quantities of the components were obtained using "peak area 100% method".

References

1 Van den Broucke, C. O.: Fitotherapia **54**, 171 (1983)
2 Morozumi, S.; Wuake, T.; Kudoh, Y.; Hitokoto, H.: Bioact. Mol. **10**, 155 (1989)
3 Arass, G.; Grella, G. E.: Horticul. Sci. **67**, 197 (1992)
4 Aureli, P.; Constantin, A.; Zolea, S.: J. Food Prot. **55**, 344 (1992)
5 Juven, B. J.; Kanner, J.; Schved, F.; Weisslowicz, H.: J. Appl. Bacter. **76**, 621 (1994)
6 Rovesti, P.: Parfum, Cosmetic, Savon (France), **1**, 137 (1971)
7 Garcia-Martin, D.; Fernandez-Vega, F. I.; Lopez de Bustamante, F. N.; Garcia-Valley, C.: Contribution al estudio de las Acetes Essenciales Espanoles II. Acetes Essenciales de Provincia de Gvadalajara, Madrid, 1974
8 Gerharth, U.; Wolf, M.: Fleise wirt. **56**, 1305 (1976)
9 Velasco-Negueruela, A.; Perez-Alonso, M. J.; Burzaco, A.: Anal. Bromatol. **43**, 395 (1991)
10 Amparo-Blazquez, M.; Zafra-Polo, M. C.; Villar, A.: Planta Med. **59**, 198 (1989)
11 Cabo, M. M.; Cabo, J.; Castillo, M. J.; Cruz, T.; Jimenez, J.: Plant. Med. et Phytoth. **26**, 197 (1990)
12 Sattar, A.; Sariq-Malic, M.; Khan, S. A.: Pak. J. Sci. Ind. Res. **34**, 119 (1991)
13 Matela, C. S.; Taskinen, J.: J. Indian Chem. Soc., **17**, 1249 (1980)
14 Crespo, M. E.; Gomias, E.; Jimenez, J.; Navarro, C.: Planta Med. **54**, 161 (1988)
15 Matveski, V.: Ph.D Thesis, Faculty of Science, Skopje, 1987
16 Sandra, P.; Bicchi, C.: Capillary gas chromatography in essential oil analysis, p. 261, Dr. Alfred Huetig Verlag, Heidelberg–Basel–New York 1987

Received September 14, 1995
Accepted November 15, 1995

Svetlana Kulevanova
Institute of Pharmacognosy
Vodnjanska 17
91000 Skopje
Macedonia