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CONTRIBUTIONS AT PHYTOCHEMICAL STUDY OF THYMUS VULGARIES L. SPECIES FROM LEBANON

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ABSTRACT

The paper follows aspects subject to the content in active principles of vegetal products *Thymy folium* harvested from *Thymus vulgaris L*. species from Lebanon and *Thymy herba* harvested from Romania. It was also followed the determination of chemo-variety type through gas-chromatographic analyze of volatile oils obtained in laboratory conditions. Volatile oil resulted from harvested species from Lebanon is rich in carvacrol (66.64%) and that resulted from Romania contain p-cymene (30.26%) and thymol (29.74%).
Key-words: *Thymus vulgaris L., volatile oil, carvacrol, thymol.*

INTRODUCTION

Thymi herba is used in phyto-therapy for anti-infectious action and anti-helmintic (thymol and carvacrol), stomachic (bitter principles and volatile oil), diuretic (flavones, triterpenes), carminative, anti-spasmodic (volatile oil, poly-phenol derivates), in treatment of respiratory affections (bronchitis), digestive (fermentative colitis, bilious cramps, intestinal parasites raw material in order to obtain the tincture and of volatile oil; food spicy (Istudor, 2001).

In Lebanon, thyme is used as flavor and as infusion, with predilection, in digestive disorders treatment.

Taking into account the fact that *Thymus vulgaris L*. species presents more chemo-varieties (gerianol in the oil of *T.vulgaris geranoliferum;* linalool in *T.vulgaris linaloliferum*; p-cymene in *T.vulgaris paracymeniferum*; thujan in *T.vulgaris thujanoliferum*; thymol in *T.vulgaris thymoliferum*; carvacrol in *T.vulgaris carvacroliferum*) (Franchomme et al., 1990), present study propose to determine its type for the vegetal product *Thymi folium* harvested from Lebanon.

MATERIALS AND METHODS

Materials: *Thymi folium* resulted from Lebanon and *Thymi herba* resulted from Romania, volatile oils obtained from the two types of vegetal products in laboratory conditions at Neoclevenger apparatus.

Methods: pharmacognosy analyze (Ciulei et al., 1996) applied on vegetal raw material (with the purpose of quantitative and qualitative chemical detection of active principles) and gas-chromatographic analyze applied on volatile oils (with the purpose to determine majority compounds).

Conditions of analyze

GC-MS analyses was performed on a Fisons Instrument GC 8000 equipped with an electron impact quadropole, MD 800 mass spectrometer detector. The electron ionization energy was 70 eV, ion-sources temperature 200°C and the interface temperature 280°C.

A fused silica capillary column 5% phenyl-poly-dimethyl-siloxane (DB-5MS 30m x 0,32 mm i.d and 0,25 μm film thickness, J&W Scientific) was used. The column temperature was programmed as follows: from 40°C (3 min hold) raised at 4°C/min to 250°C and finally hold at 250°C for 10 min. A plit-splitless injection (split ratio 1:30) at 280°C was employed. The carrier gas (helium) flow rate was 2 mL/min. Two μL of sample was injected. Data acquisition was performed with MassLab software for the mass range 30-600 u with a scan speed of 1 scan/s. The identification of compounds was performed by comparing their mass spectra with data from Adams [2], US National Institute of Standards and Technology (NIST, USA), WILEY 1996 Ed. Mass spectra library and a personal library of 600 spectra. The identification of compounds was also based on the Kovats retention indices.

The Kovats retention indices were calculated by using n-*alkanes* C_8-C_{20} and C_{21}-C_{40} and the experimental values were compared with those reported in the literature (www.flavornet.com, 2004; www.pherobase.com, 2003-2005; Pavel et al., 2009; Radulescu et al., 2009).

RESULTS AND DISCUSSIONS

From macroscopic and microscopic point of view, harvested product from Lebanon is inscribed in morphological and anatomic peculiarities described in specialty literature [9]. However, it may be mentioned that *Thymi folium* from Lebanon has much more dimensions in comparison with those resulted from Romanian species, and the odor is much more intense and pregnant. Microscopically it were identified both elements common to the family (glandular octo-cellular hair, stomata of diacit type) and specific to vegetal product (tector geniculate hair).

Qualitative chemical, active principles were identified in the solutions resulted by exhausting of vegetal raw material with ether, alcohol and water. Chemical reactions intensity is dependent on raw material nature used in research (table 1).

Table 1-Result of qualitative chemical screening (apolar and bipolar solution)

Active principle	**Remarks**
Cumarinic aglycones	Low fluorescence in UV light
Carotenoids	Intense positive reaction, possible tetraterpenes of hydrocarbon type
Flavone aglycones	Positive reaction, compounds of type flavonol
Reducing compounds	Intense positive reaction
Osis and poliosis	Intense positive reaction
Poliholozides	Positive reaction, compounds of type mucilage
ODP's	Intense positive reaction, formation of caffeic acid oxime
Flavones	Positive reactions
Proantocians	Positive reactions
Tannin	Intense positive reaction tannin, of catehic type
Saponosides	Positive reactions, triterpene saponosides

Quantitative chemical it was determined the content in poly-phenol derivates of type caffeic acid and rutoside, volatile oil; preliminary the losses by drying and the content in substances soluble in water were determined (table 2).

Table 2 - Results of quantitative chemical determinations

Determination	**Vegetal raw material**	
	Thyme-Lebanon	**Thyme-Romania**
Humidity g%	6.78	8.78
Soluble substances g% (in water)	23.34	15.67
ODP's g% caffeic acid	0.897	0.297
Flavones g% rutoside	1.345	0.450
Volatile oil mL/100 g pv	8.9	0.9

Remarks: it is stated that the product resulted from Lebanon is much richer in active principle, fact explained by presence of the body (*caulis*) at Romanian product, which usually is poor in active principles.

Significant differences were remarked as consequence of gas-chromatographic analyzes (table 3, 4 and figures 1,2) of volatile oils, thus in the oil obtained from Lebanon provenience product prevails carvacrol (66.64%), fact that explains different aroma in comparison with similar Romanian product; in volatile oil obtained from Romanian product prevails p-cymene (30.26%) and the thymol (29.74%).

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Table no. III.

Chemical compounds from thyme volatile oil

No.	Compound	RT[a] (min)	KI	Area %	
				Lebanon	Romania
1	Tryciclene	6.25	921	-	0.04
2	α-Thujene	6.41	926	1.00	1.00
3	α-Pinene	6.60	933	0.40	1.76
4	Camphene	7.09	949	0.03	1.15
5	Sabinene	7.90[b]	973	0.08	-
6	β-Pinene	7.99	976	0.08	0.28
7	Vinyl amyl ketone	8.16	981	-	0.04
8	l-Octen-3-ol	8.28	984	-	0.65
9	β-Myrcene	8.51	990	0.92	1.20
10	3-Octanol	8.87	999	-	0.06
11	α-Phellandrene	8.97	1002	0.16	0.20
12	3-δ-Carene	9.05	1005	0.04	0.09
13	α-Tertipene	9.33	1015	2.06	1.89
14	p-Cymene	9.61	1025	2.94	**30.26**
15	Limonene	9.73	1029	0.26	2.47
16	1,8-Cineole	9.81	1031	-	0.98
17	β-Ocimene	10.39	1050	0.04	0.03
18	γ-Tertipene	10.71	1060	23.43	11.06
19	β- Tertipene	11.11	1071	-	0.25
20	α-Terpinolene	11.58	1084	-	0.12
21	Linalool	12.11	1098	0.05	2.46
22	α-Thujone	12.23	1102	-	0.10
23	Camphor	13.47	1145	-	0.44
24	Borneol	14.28	1170	-	1.22
25	1-Terpinen-4-ol	14.57	1179	0.05	0.82
26	α-Terpineol	15.04	1193	-	0.21
27	Thymol methyl ether	16.11	1230	-	0.94
28	Carvacrol methyl ether	16.37	1239	-	0.50
29	Gerianol	16.83	1255	-	0.03
30	Bornyl acetate	17.72	1284	-	0.31
31	Thymol	18.01	1293	0.11	**29.74**
32	Carvacrol	18.19	1299	**66.64**	3.96
33	Terpenyl acetate	19.43	1345	-	0.04
34	β-Bourbonene	20.43	1381	-	0.03

35	Trans-caryophyllene	21.39	1416	0.11	2.64
36	α-Humulene	22.33	1452	-	0.14
37	β-Cadinene	22.88	1473	-	0.20
38	Germacrene D	22.99	1477	-	0.03
39	Bicyvlogermacrene	23.41[b]	1492	0.05	-
40	α-Muurolene	23.51	1496	-	0.06
41	α-Farnesene	23.85	1508	-	0.25
42	γ-Cadinene	24.01	1516	-	0.51
43	α-Bisabolene	24.58[b]	1540	0.10	-
44	t-Cadinol	27.02	1640	-	0.15
45	α-Muurolol	28.05[b]	1648	0.03	-
46	α-Cadinol	27.33	1654	-	0.06
47	Agarospirol	27.73	1671	-	0.04
Identified from total area				**99.98**	**98.41**

Legend: **RT[a]** = retention time for thyme volatile oil from Romania, [b] from Lebanon
KI = experimental Kovats retention indices.

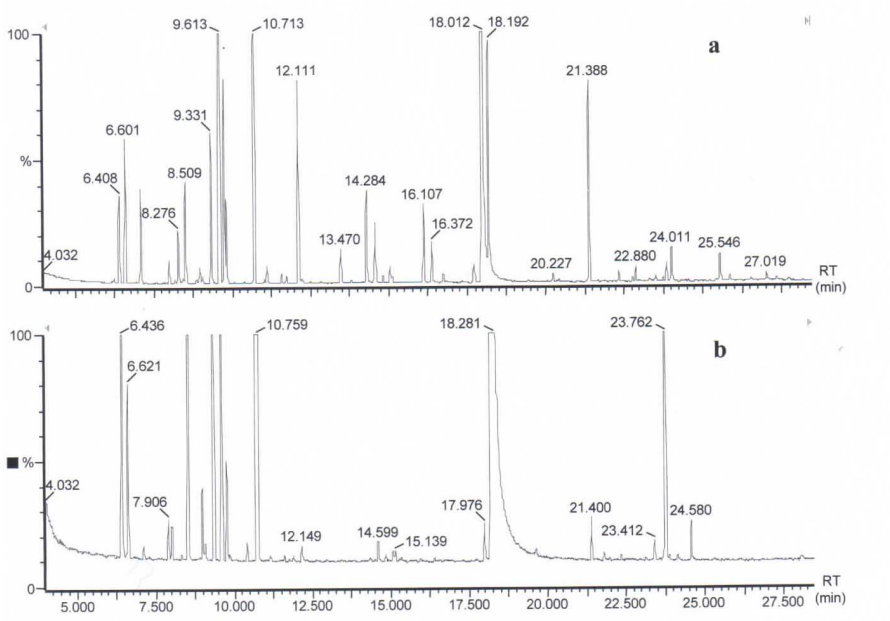

Figure 1-*Chromatogram of volatile oils, Romanian thyme* **(a)** *and Lebanese thyme* **(b).**

CONCLUSIONS

Gas-chromatographic analyze of volatile oil has determined that the product harvested from Lebanon results from *Thymus vulgaris* species, *carvacroliferum* chemo-variety and that obtained from Romanian product may result from *Thymus vulgaris* species, *paracymeniferum* or *thymoliferum* chemo-variety.

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