

ANALYSIS OF VOLATILE COMPOUNDS FROM INFUSION AND HYDRODISTILLATE OBTAINED FROM THE SPECIES *THYMUS PULEGIOIDES* L. (*LAMIACEAE*)

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Abstract

The volatile compounds present in the species belonging to the *Thymus* genus (*Lamiaceae*) are responsible for the two well defined therapeutic effects demonstrated for these plants: antiseptic and spasmolytic effects. The essential oil which contains the majority of these compounds is hardly accessible and, as consequence, it is rarely used by the patient. That's why an analysis of the volatile compounds in other pharmaceutical forms, such as infusion, the most often used one, and hydrodistillate obtained by a simple and fast method - open circuit distillation - was necessary.

After the extraction on solid phase (C₁₈ cartridge) of the volatile compounds from infusion and hydrodistillate their identification was performed using GC-MS. The main compounds identified in infusion were: carvacrol (38.96 %), thymol (25.17 %), linalool (9.75 %) and thymoquinone (7.27 %), and in hydrodistillate: carvacrol (63.20 %), linalool (16.95 %) and thymol (15.55 %).

Rezumat

Compușii volatili prezenți în speciile genului *Thymus* (*Lamiaceae*) sunt responsabili de cele două efecte terapeutice bine definite, demonstrate pentru aceste plante: efectul antiseptic și cel spasmolitic. Uleiul volatil care conține majoritatea acestor compuși este mai greu accesibil și în consecință se folosește mai rar de către pacient. De aceea este necesară analiza compușilor volatili în alte forme farmaceutice, ca: infuzia, cel mai des utilizată și hidrodistilatul obținut prin metoda simplă și rapidă de distilare în circuit deschis.

După extracția pe fază solidă (cartuș cu C₁₈) a compușilor volatili din infuzie și hidrodistilat, identificarea acestora s-a făcut prin GC-MS. Compușii majoritari identificați în infuzie au fost: carvacrol (38,96 %), timol (25,17 %), linalol (9,75 %) și timochinonă (7,27 %), iar în hidrodistilat: carvacrol (63,20 %), linalol (16,95 %) și timol (15,55 %).

Keywords: *Thymus*; infusion; hydrodistillate; thymol; thymoquinone

Introduction

The uses of thyme, *Thymus vulgaris* and other *Thymus* species are well known all over the world. Thyme has changed from a traditional herb to a serious drug in rational phytotherapy. This is due to many pharmacological *in vitro* experiments carried out during the last decades.

The studies have revealed well defined pharmacological activities of both essential oils and the plant extracts, the antibacterial and spasmolytical properties being the most important ones. These activities are in part due to their volatile and semi volatile content [1, 4 - 6, 11, 12, 15, 16, 21].

A large amount of data concerning the chemical composition of essential oil obtained from *Thymus pulegioides* L. are presented in the scientific literature [6, 8 - 10, 17]. However, in therapeutics are used other forms, such as infusions and tinctures, which are much easier to obtain. Few data about the volatile content of these forms obtained from different *Lamiaceae* species can be found in the scientific literature [13, 14].

The aim of this study was to identify some volatile and semi volatile compounds in two aqueous forms obtained from one of the most spread and easy to collect species of *Thymus* in our country - *T. pulegioides* L. Previous studies presented analyses regarding the chemical composition of these species essential oil [9, 22].

Materials and methods

As raw material we used the herbage of *Thymus pulegioides* L. dried at room temperature away from daylight. The plant was harvested in the flowering stage from Azuga (July 2005).

Infusion preparation

100 ml of boiling water was added to samples of 5 g of vegetal drug (covered flask). After approximately 30 minutes the cold extracts were filtered on cotton filter. The residue from the filter was rinsed three to four times with distilled water, and the final filtrated infusion was adjusted to 100 mL.

Hydrodistillate preparation

30 g of raw plant material along with approximately 300 ml water were submitted to water distillation using a simple open circuit distillation apparatus. The operation was conducted until 100 ml extract was obtained.

Solid-phase extraction of infusion and hydrodistillate

A 500 mg C₁₈ solid-phase extraction (SPE) cartridge was conditioned by eluting it with 2 x 4 ml dichloromethane, followed by 2 x 4 ml of methanol. The cartridge was allowed to dry after each flush. Then 2 x 4 ml of distilled water was passed through the cartridge (the cartridge should not be allowed to dry before sample application). By applying a slight vacuum, the obtained infusion (100 ml) was allowed to pass through the cartridge at a flow of 1 - 2 ml/min. The cartridge was then dried for 5 - 10 min with nitrogen. The compounds retained on C₁₈ cartridge were eluted

with 2 x 1 ml dichloromethane. The solution was concentrated to 0.5 ml using nitrogen atmosphere before being injected.

For solid-phase extraction of the hydrodistillate, 50 ml were allowed to pass through the C₁₈ cartridge, and after that the same method used for the infusion was applied without sample concentration.

Gas chromatography - mass spectrometry

GC-MS analyses were carried out with a Fisons Instrument GC 8000, equipped with mass selective detector with quadrupole analyser, MD 800. The electron ionisation energy was 70 eV, ion-source temperature 200°C and the interface temperature 280°C. A split - splitless injection (split ratio 1: 30) at 280°C injector temperature was employed.

A fused silica column 5% phenyl-poly-dimethyl-siloxane (DB-5MS 30m x 0.32 mm i.d. and 0.25µm film thickness, J&W Scientific) was used. The oven temperature was programmed as follows: from 40°C (3 min hold) raised at 4°C/min to 280°C (20 min hold). A sample of 2 µl was injected. Data acquisition was performed with MassLab software for the mass ranges 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, 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 using n-alkanes C₈ - C₂₀ and the experimental values were compared with those reported in literature [18 - 20].

Results and discussion

In figure 1 the chromatograms of infusion (a) and hydrodistillate (b) are presented. Table 1 shows the relative content of volatile compounds from infusion, expressed as percentage from total area.

It can be seen that 27 volatile compounds were separated from infusion, but only 18, adding up to 92.26 % of the total area were identified. The most important compounds were the two terpenoid phenols isomers, thymol (25.17 %) and carvacrol (38.96 %). Their percentage (64.13 %) constitutes the majority of the volatile content which can be found in *T. pulegioides* infusion. The percentage of monoterpenoid alcohols is also important (11.6 %), the most abundant compound from this class being linalool (9.75 %). Smaller percentages of borneol (1.51 %) and terpinene-4-ol (0.34 %) were found.

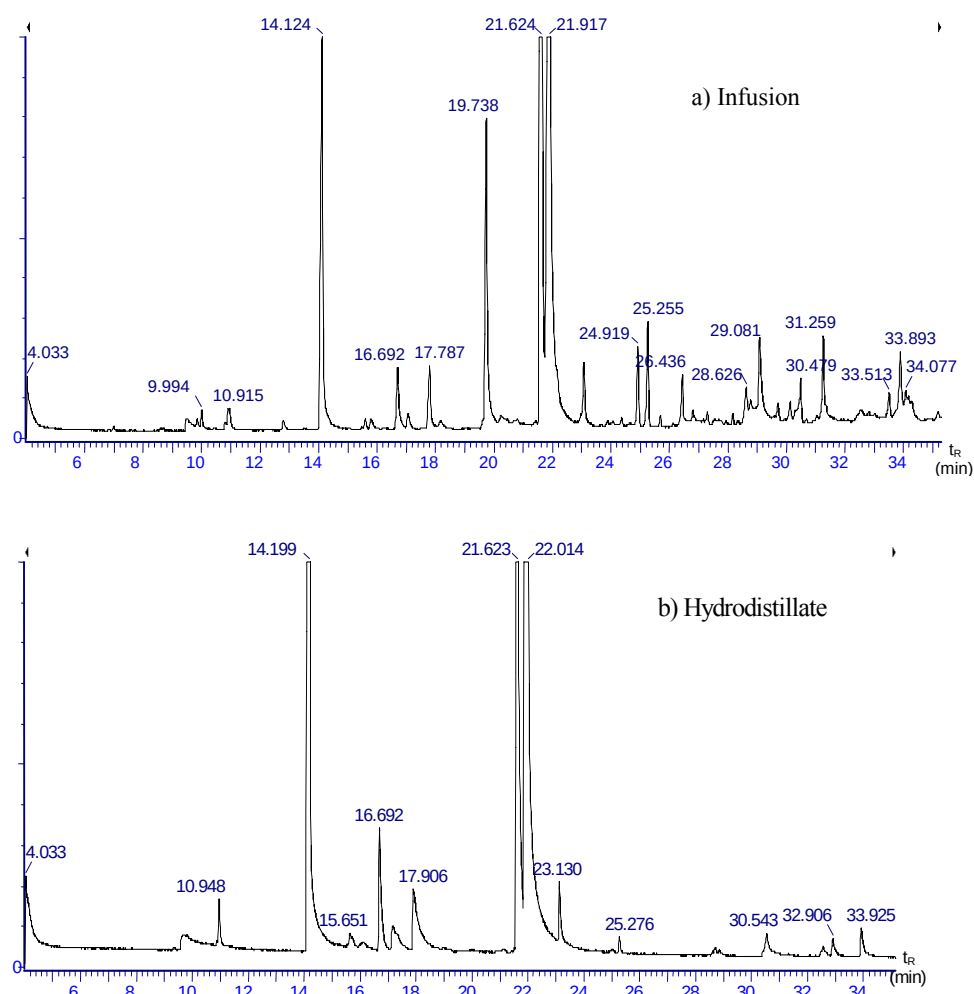


Figure 1
The chromatograms of infusion (a) and hydrodistillate (b)

Table I
Chemical composition of *Thymus pulegioides* infusion

No.	Compound	RT (min)	KI	Area %
1	n.i.	9.474	972	0.66
2	n.i.	9.853	979	0.23
3	δ-3-carene	9.994	981	0.33
4	ocimene	12.791	1052	0.25
5	linalool	14.124	1088	9.75
6	camphor	15.793	1135	0.28
7	borneol	16.692	1160	1.51

(continued)

Table I (continued)

No.	Compound	RT (min)	KI	Area %
8	terpinene-4-ol	17.050	1171	0.34
9	n.i.	17.787	1189	1.43
10	n.i.	18.177	1199	0.23
11	thymoquinone	19.738	1249	7.27
12	thymol	21.624	1304	25.17
13	carvacrol	21.917	1314	38.96
14	neryl acetate	23.076	1348	1.33
15	n.i.	24.919	1399	1.34
16	trans-caryophyllene	25.255	1410	1.84
17	α -humulene	26.436	1448	0.97
18	2,6-di-tert-butylquinone	26.794	1459	0.25
19	n.i.	28.626	1513	0.73
20	acetovanillone	28.777	1520	0.25
21	dihydroactinidiolide	29.081	1530	1.99
22	germacrene B	29.709	1551	0.30
23	nerolidol	30.121	1564	0.34
24	n.i.	30.479	1576	0.87
25	n.i.	31.259	1600	1.79
26	n.i.	33.513	1678	0.47
27	β -farnesol	33.893	1692	1.13

Legend: RT = retention time; KI = experimental Kovats retention indices; n.i. = not identified.

The infusion lacks hydrocarbons with monoterpenoid structure (0.58 %). This result is in concordance with previous research studies performed on infusions obtained from other plants [13, 14]. Sesquiterpenoid hydrocarbons vary among 0.30 % (germacrene D), 0.97 % (α -humulene) and 1.84 % (trans-caryophyllene).

Two sesquiterpenoid alcohols were identified: nerolidol (0.34 %) and β -farnesol (1.13 %). Camphor, a carbonyl compound with monoterpenoid structure, is also present in a small quantity (0.28 %).

The presence of thymoquinone (7.27 %) in the infusion is notable. This compound was not identified in the essential oil [9] or the hydrodistillate, so we conclude that it is an "artefact" probably formed during the process of infusion preparation. Recent *in vitro* studies showed that thymoquinone has important pharmacological properties (anti-inflammatory, hepatoprotective and anticancer), so its presence in forms such as infusions could be of great interest [2, 3, 7]. It remains to prove the necessary concentrations for a required therapeutic effect.

In table II the chemical composition of hydrodistillate is shown. The number of separated compounds (14) is smaller in this case compared

to the infusion. Eleven compounds, representing 99.44 % of the total area were identified.

The monoterpenoid phenols, thymol and carvacrol are the most important compounds in this case, too. Their total percentage is 78.75 %, carvacrol being the most representative, with 63.20 %, while thymol only 15.55 %. Monoterpenoid alcohols constitute the other important class of volatile compounds, reaching up to 19.51 % of the total area. The majority of this percentage is attributed to linalool (16.95 %); the rest is represented by borneol (1.16 %), terpinene-4-ol (0.39 %) and α -terpineol (1.01 %).

Table II
Chemical composition of *Thymus pulegioides* hydrodistillate

No.	Compound	RT (min)	KI	Area %
1	1,8-cineole	10.948	998	0.23
2	linalool	14.199	1088	16.95
3	borneol	16.692	1160	1.16
4	terpinene-4-ol	17.158	1171	0.39
5	n.i.	17.364	1178	0.15
6	α -terpineol	17.906	1192	1.01
7	thymol	21.623	1304	15.55
8	carvacrol	22.014	1314	63.20
9	neryl acetate	23.130	1348	0.42
10	trans-caryophyllene	25.276	1410	0.09
11	n.i.	30.543	1578	0.27
12	n.i.	32.570	1646	0.14
13	τ -cadinol	32.906	1658	0.16
14	β -farnesol	33.925	1692	0.28

Legend: RT = retention time; KI = experimental Kovats retention indices; n.i. = not identified.

Very small percentages of sesquiterpenoid compounds were found: trans-caryophyllene (0.09 %), τ -cadinol (0.16 %) and β -farnesol (0.28 %).

A single ester, neryl acetate, was identified just like in the case of infusion.

Monoterpenoid hydrocarbons are not present in hydrodistillate. This fact is probably due to their high volatility which leads to the loss of these compounds during the long process of obtaining (around 1.5 hours).

The monoterpenoid ether 1,8-cineol is present in hydrodistillate (0.23 %) but not in infusion.

The most important volatile constituents of infusion and hydrodistillate, thymol, carvacrol and linalool are very efficient antiseptics [1, 4, 5, 10 - 12, 16]. Future studies will try to establish a correlation between those active substances and a possible antibacterial or antifungal effect of the aqueous pharmaceutical forms.

Conclusions

In infusion, an aqueous pharmaceutical form, 17 volatile compounds with monoterpenoid (86.62 %) and sesquiterpenoid (3.45 %) structures were identified. During the process of obtaining the infusion, chemical reactions may take place, which determine the appearance of new compounds (e.g. thymoquinone) not found in hydrodistillate.

The hydrodistillate contains high percentages of monoterpenoid phenols and alcohols (thymol, carvacrol, linalool), but not highly volatile compounds like the monoterpenoid hydrocarbons. Sesquiterpenoid compounds are in very small percentages (0.53 %).

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