Romanian Biotechnological Letters *Vol. 23, No. 5, 2018*

ORIGINAL PAPER

Biochemical analysis of some vegetal extracts obtained from indigenous spontaneous species of *Thymus serpyllum L.*

DOI: 10.26327/RBL2018.179

Received for publication, October, 20, 2016
Accepted, April, 7, 2017

AUGUSTINA PRUTEANU*[1], CARMEN POPESCU [2, 3], VALENTIN VLADUT[1], GEORGETA GAGEANU[1]

[1]*National Institute of Research-Development for Machines and Installations Designed to Agriculture and Food Industry, Bucharest, Romania;*
[2] *S.C. HOFIGAL Export Import S.A., Bucharest, Romania;*
[3] *Faculty of Pharmacy, "Vasile Goldiş" Western University, Arad, Romania*
***Address correspondence to:** National Institute of Research-Development for Machines and Installations Designed to Agriculture and Food Industry, 6 Ion Ionescu de la Brad Blvd., 013813, Bucharest, Romania, Tel.:+04-021-269.32.55; Fax:+04-021-269.32.73;*
Email: pruteanu_augustina@yahoo.com

Abstract

After harvesting, medicinal and aromatic plants are appropriately processed or dried/dehydrated in order to obtain phytotherapeutic products of different use. The plant therapeutic action may be due to a group of substances or a phytochemical combination and sometimes to ballast substances that enhance the biologically active substances, thus increasing their activity and making them last. For obtaining optimum yield of bioactive substances it must take care of the procedure of extraction used, type of solvent and also the physical and mechanical features of vegetal product cells.

This paper presents the comparative biochemical and physicochemical analysis for four vegetal extracts obtained from aerial parts of thyme, Thymus serpyllum L harvested from Romania spontaneous flora as dried, minced and broken into four fractions (sorts). It was determinated the content of substances extracted in the three solvents for four-dimensional fraction of thyme then were identified the solvent and fraction out of which the maximum of bioactive substances was extracted. After that, four ethanol extracts obtained from the four fractions of thyme were analyzed at Gas-Chromatograph GC-MS. Biochemical analysis of ethanol extracts obtained from Breckland thyme (Thymus serpyllum L.) has shown the existence both of common biochemical compounds and different biochemical compounds.

Keywords: biochemical compounds, *Thymus serpyllum L,* herba, vegetable extracts

1. Introduction

The genus *Thymus* L. (belonging to the family Lamiaceae) consists of 928 species, native to Europe, and grown in the Mediterranean basin and northern Europe, as well as other parts of the world such as Asia, South America, and Australia. *Thymus serpyllum L* (thyme) is known as one of the most important species, and is used in food, cosmetic and pharmaceutical industries, with a large number of studies providing evidence for its antimicrobial effects under in vitro conditions (NABAVI & al., [1]; POPESCU, [33]). In Romania, *Thymus* genus contains one cultivated species and 18 wild species (MARCULESCU & al., [2]).

Essential oils are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, enfleurage or extraction but the method of steam distillation is most commonly used for commercial production of essential oils. Essential oils or their components have been

shown to exhibit antimicrobial, antibacterial, antiviral, antimycotic, antitoxigenic, antiparasitic, and insecticidal properties. These characteristics are possibly related to the function of these compounds in plants. The greatest use of essential oils in the European Union (EU) is in food (as flavorings), perfumes and pharmaceuticals (for their functional properties), (BURT, [3]).

The chemical constituents in the plants from the genus *Thymus* are known to be biologically active ingredients such as thymol, carvacrol, p-cymene and terpinene. In Romania (MARCULESCU & al., [2]; POPESCU, [33]; NECULA & al., [4]) there were analyzed polyphenolic compounds from the medicinal products obtained from species of *Thymus*. (FECKA & TUREK, [5]; BOROS & al., [6]) studied polyphenolic compounds by chromatographic techniques in *Thymus* species.

Five monoterpenes (carvacrol, p-cymene, linalool, α-terpinene, and thymol) derived from the essential oil of thyme were examined by (BYEOUNG-SOO & al., [7]) for their repellency against the mosquito. Thymol, and its main natural source, thyme, are employed for their positive antioxidant, anti-inflammatory, local anesthetic, antinociceptive, cicatrizing, antiseptic, antibacterial, and antifungal properties as well as for their beneficial effects on the cardiovascular system (MARCHESE & al., [8]).

Many phytochemical studies so far investigated the chemical composition of the essential oil from *Thymus* species and her activity such as: antimicrobial activity (BORUGA & al., [9]), antimicrobial and antioxidant activities (MANCINI & al., [10]), antimicrobial, antioxidant and antitumor activities (NIKOLIĆ & al., [12]), antiaflatoxin activity (RAZZAGHI-ABYANEH & al., [13]). Essential oils with high proportions of the phenolic components thymol and/or carvacrol showed the highest antioxidant activity (CHIZZOLA & al., [14]).

PÎRVULESCU, [11] studied comparative evaluation of antioxidant capacity of *Thymus vulgaris* by different methods and ANGELOV, [15] studied the potential use of different green solvents, namely ethanol, limonene and ethyl lactate to extract thymol from thyme plants. In this work, the three green solvents have shown good capacity to extract thymol from thyme plants. (CHIZZOLA & al., [14]) obtained in his studies the best results with 60% ethanol as extracting. Also, (HOSSAIN & al., [16]) studied various crude extracts using different polarities of solvent which they was quantitatively evaluated of total phenol, flavonoids contents and phytochemical screening of *T. vulgaris*.

HUDAIB & al., [17] and RASOOLI & MIRMOSTAFA, [18] investigated variation in the chemical composition of the essential oil of thyme from the aerial parts at different growth stages, during pre and flowering. (SAFAII & al., [19]; KRÓL & al., [20] and CHAUHAN & al., [21]) have described the effect of harvesting stages and time, on growth, yield and herb quality of thyme.

TOROGLU, [22] studied in vitro interactions between antibiotics and *Thymus eigii* essential oil antimicrobial activity and antagonistic effect of essential oils from plant species of *Thymus* from southern part of Turkey. Similarly, (DE MARTINO & al., [23]) studied in vitro antimicrobial and mutagenic activities of essential oils from *Thymus vulgaris* in Italy. Also, (AFFONSO, [24]) studied in vitro influence of growth regulators in biomass production and volatile profile of plantlets of *Thymus vulgaris* L.

Recently, (NEMA & al., [25]) established the antiulcerogenic and wound healing activities of ethanolic extract of *Thymus vulgaris* and thus, justifies the ethnic uses of the plant. However, further studies are required to isolate active compounds from the potent extract and to elucidate the exact mechanism of action. KREMSH ALASADIY, [26]) suggested in his study that alcoholic extract of *Thymus vulgaris* is useful in infected man with the protozoan *Toxoplasma gondii,* (JAFARISANI & al., [27]) investigated antioxidants

effects of *Thymus vulgaris* ethanol extract for the treatment of female endocrine disorders, (KOMAKI & al., [28]) studied the effect of extract *of Thymus vulgaris* on anxiety.

This paper aims to evaluate the capacity of indigenous plant of *Thymus serpyllum L* were a source of essential oils rich in terpene compounds, having an important role for obtaining pharmaceutical products, food supplements, food, body lotions, antiseptics and cosmetics.

Thyme *Thymus serpyllum L* is an herbaceous plant with red-purple or white flowers and aromatic leaves, used in medicine, under the name of thyme.

The current paper aims to identify and quantify the content of total polyphenols expressed in chlorogenic acid and respectively caffeic acid, total flavonoids expressed in rutin and essential components from the essential oil of *Thymus vulgaris.* Ethanolic extracts of *Thymus serpyllum L* were obtained from aerial part of dried broken herb separated into four dimensional fractions, for identifying which fraction releases through extraction the biggest quantity of bioactive substances.

2. Materials and Methods

Thyme (*Thymus serpyllum L*) used for study was harvested from spontaneous flora and identified compared to microscopic, morphological and biological characteristics of species (TĂMAŞ & al., [29]; BOJOR, [30]). Herb was naturally dried in shadow until it reached the suitable humidity (maxim 13 %), cleaned of foreign bodies (inorganic materials or other plants, damaged parts) according to provisions from Romanian Pharmacopoeia [31], European Pharmacopoeia [32] and (POPESCU, [33]), then it was chopped in bulk by the equipment of TIMATIC type, designed to break the medicinal plants and separated by the sieve classifier of Rietsch AS 200 type, resulting in four fractions visible in figure 1.

Group I Group II Group III Group IV

Figure 1. Distribution of dimensional fractions of thyme

After establishing the fractions size, 20 g of vegetable material from each fraction obtained after separation addition 250 mL ethanol of 96 %, respectively water and ether for extracting the bioactive substances, were weighed in the analytical balance. The samples were manually agitated for ten minutes till the vegetal product got imbibed with solvent and homogenized, after which they were put on the rotary evaporator water bath at 50^0 C temperature and bottle rotation speed of 100 rpm, for achieving a continuous agitation for 120 minutes. Maceration extracts obtained were filtered on a filter paper and vegetal matter was dried in the oven at 105^0C, for 3 hours.

Then, the vegetable extracts were concentrated for all the fractions and samples were weighed and the quantity of bioactive substances extracted out of each fraction was determined, thus finding out the fraction and solvent with active substances maximum yield.

After this phase, the content of total polyphenols, total flavonoids and chromatographic profiles of thyme essential oil from extracts in ethanol of each fraction, was analyzed in the laboratory [34], in accordance with Romanian Pharmacopoeia [31] and Europeean Pharmacopoeia [32].

Methods of analysis used:

1. Content of total polyphenols expressed as caffeic acid equivalent chlorogenic acid

Product behavior in terms of total polyphenols expressed in caffeic acid, % and/or chlorogenic acid, is very important to be determined, as their content is directly proportional with the products drying method, and total polyphenols content (giving the non-enzyme antioxidant features) increases along with drying, controlled by stages. This test was performed by spectrophotometry UV - VIS in compliance with the method developed in S.C. HOFIGAL EXPORT IMPORT S.A. laboratory [31].

Reagents: sodium wolframate R; phosphoric acid R; water R; sodium phosphorwolframate solution (Folin Reagent): 10 g sodium wolframate R 10 mL phosphoric acid R and 75 mL water R are heated to reflux, for 2 hours, and after cooling it is completed with water R at 100 mL; sodium carbonate solution R 200 g/L; caffeic acid R/chlorogenic acid R.

Standard solutions: caffeic acid solution 20µg/mL; caffeic acid solution 30µg/mL; caffeic acid solution 40µg/mL; caffeic acid solution 50µg/mL; caffeic acid solution 60µg/mL; caffeic acid solution 70µg/mL; caffeic acid solution 80µg/mL; caffeic acid solution 90µg/mL; etanol 50% v/v.

Analysis sample: product as powder, alcoholic extract and product as a powder mixture of different percent.

Test solution: 10 g of *analysis sample* are weighed with analytical balance, after which they are brought into a balloon of 150 - 200 mL, 100 mL *ethanol R 50 % v/v* are added and water bath heated to boiling point, to reflux, during 30 minutes. The hot solution is filtered through cotton, in a volumetric flask and after cooling is completed up to 100 mL, by cleaning the residue with the same solvent *(solution A).* 5.0 mL solution A are brought to 50 mL in a volumetric flask with *ethanol R 50 % v/v.*

Working technique: At 5.0 mL *test solution* are added 5.0 mL *sodium phosphor-wolframate solution R, are agitated and filtered;* the first 2 mL of filtered solution are removed. 2.5 mL of filtrate are diluted by *sodium carbonate solution R 200 g/L* at 25 mL, in a graded balloon. The solution absorbance is determined at 660 nm, using as blank solution prepared of 2.5 mL filtrate and 25 mL water, in a graded balloon. Total polyphenols concentration of sample to be analyzed is calculated by means of a calibrating curve, working with: 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5 mL standard solution of *caffeic acid R 0.1 g/L,* after that are added 4.0; 3.5; 3.0; 2.5; 2.0; 1.5; 1.0 and 0.5 mL *water R* and then 5.0 mL *sodium phosphor-wolframate solution R* for each standard solution. From each units obtained, 2.5 mL are taken out and are graded and levelled in a balloon of 25 mL with *sodium carbonate R 200 g/L* freshly separated. The solution absorbance is determined at 660 nm, using as blank solution prepared from 2.5 mL out of each sample brought to level with *water R* in a volumetric flask of 25 mL.

2. Dry residue [%]

Determination of dry residue is performed according to European Pharmacopoeia, edition 9, chapter 2.8.16.

Working technique: In a weighing bottles about 50mm in diameter and about 30mmin height, introduce 1.0 g of extract. Evaporate to dryness on a water-bath and dry in an oven at 100 -105^{0}C for 3 hour. Allow to cool in a desiccator over *diphosphorus pentoxide* R or *anhydrous silicagel* R and weight. Results are expressed in mass percentage.

3. Determination in total flavonoids expressed in rutin

It is performed according to the analysis method elaborated after Romanian Pharmacopoeia, edition in force, considering a sample of 1.0 g to be analyzed.

3.1. Equipment:

- spectrophotometer UV-VIS

3.2. Reagents:

- *methanol R;*
- *methanol R, solution 50%, (v/v);*
- *sodium acetate R, solution 100 g/L;*
- *aluminium chloride R, solution 25 g/L;*
- *rutin (s.r.), solution 0,01% in methanol R;*
- *solution A*: 1.0 g sample to analyze is brought into a flask of 150-200 mL, 100 mL *methanol R, solution 50%, (v/v)* is boiling on water-bath, to reflux, during 30 minutes. The hot solution is filtered in a volumetric flask and after cooling is completed up to 100 mL, by cleaning the residue with the same solvent *(solution A).* 5.0 mL solution A are brought to 50.0 mL in a volumetric flask with *ethanol R 50 % v/v.*
- *test solution:* 5.0 mL *solution A* are diluted to 100 mL with m*ethanol R* in a volumetric flask.

Volumetric flask is strongly agitated with break of 10 minutes. The solution is filtered, removing the first parts of limpid filtrate and 5.0 mL are measured into a 25-mL volumetric flask, 5.0 mL of sodium acetate *R, solution 100 g/L* and 3.0 mL *aluminium chloride R, solution 25g/L are added,* continuously agitating after each reagent supplement, after which it is brought up to mark with *methanol R*, after which the solution obtained is strongly agitated;

- compensation *solution:* 5.0 mL *test solution* diluted at 25 mL with *methanol R*.

3.3. Working technique

Sorbing agent of *test solution* against the *blank solution* is measured 20 minutes after at spectrophotometer at 430 nm, into the cuvettes with 10 mm width. When the solution absorbance is bigger than 0.7 the appropriate dilution is performed.

Flavonoids quantity is calculated in comparison with the rutin calibrating curve, established such as: in three 25 ml graded balloons 1; 2 and 3 mL of *rutin solution are dropped 0.01% into methanol R*. In each graded balloon, 5 ml of sodium acetate *R, solution 100 g/L* and 3 mL *aluminium chloride R, solution 25g/L,* are added, while agitating after every reagent supplement. The balloons are completed up to the mark with *methanol R* and are strongly agitated. Solutions obtained are read at spectrophotometer at 430 nm, in the cuvettes with 10 mm width.

Flavonoids content, expressed in rutin is calculated by the formula:

$$\text{Flavonoids content} = \frac{C \cdot V_1}{m_p \cdot V_2} \cdot 10^{-4} \cdot F$$

where:

C = concentration read on the standard curve, in μg/mL;

m_p = mass of *sample to analyze*, in g;

V_1= volume of graded balloon used, in mL;

V_2= volume of *solution A* taken into consideration, in mL;

F = dilution factor;

4. Chromatographic profile of thyme essential oil Thymus serpyllum L from the four fractions

4.1. Equipment

Gas Chromatography device equipped with:

- detector: mass spectrometer;
- splitting injector;

- automated system of injecting the sample to analyze;
- automated system of integrating the dropping areas from chromatography obtained;
- Macrogol column 20 000 R (film thickness 0.25 μm; l = 30 m; Ø = 0.25 mm.

Working conditions:

- transporting gas – helium for chromatography R with rate flow of 1.5 mL/minute;
- splitting report 1/50;
- temperature:

	Time, (minute)	Temperature, (^{0}C)
Column	0 -50	40 40→280
Injector		250
Detector		287

- injected volume: 1μL.

4.2. Reagents:

- hexane R;
- test solution: sample to analyze is diluted with hexane, if is necessary and is dried on a small quantity of anhydrous sodium sulfate R and is filtered through a filter of 0.2μm;
- anhydrous sodium sulphate R;
- reference solution**:** 5 μL absinthin R are dissolved in 5 mL hexane R.

4.3. Working technique:

Test solution and reference solution are injected and the retention time is recorded.

Elution order: order indicated by the reference solution.

Using the retention time obtained by chromatography of reference solution, its components are located in the chromatography obtained with test solution. The percentage content of test solution components is determined by the normalization procedure.

3. Results and Discussions

In order to obtains the dimensional fractions of dry and chopped plant material, were analyzed five samples, each sample was weighed precision scales a quantity of 120 g of plant material, which sieve sift classifier, the amplitude of 50 mm for 5 minutes. On each sieve was found a quantity of material which has represented the totality of fragments with sizes smaller than the mesh the upper screen and larger than the mesh through which passed. For chopped thyme to 2 mm sieve setting was: collector - 2.2 - 3.15 - 4.5 - 6.3 mm.

Limits of thyme dimensional fractions obtained after separating the dry vegetable matter chopped are shown in table 1.

Table 1 – Distribution per dimensional fractions of thyme fragments

Fraction I	Fraction II	Fraction III	Fraction IV
0.1- 2.2 mm	2.21-3.15 mm	3.16-4.5 mm	4.6-6.3 mm

After setting the dimensional fractions, was passed to the extraction of bioactive substances in each fraction.

Percentage content of substances extracted is calculated by the formula:

$$\text{Substances extracted content [\%]} = \frac{M_2 - \cdot M_1}{M} \cdot 100$$

where:

M_1 = mass fraction before extraction, in g;
M_2 = mass fraction after extraction, in g;
M = mass fraction, in g.

Percentage content of substances extracted in the three solvents for each fraction (sort) is shown in figure 2.

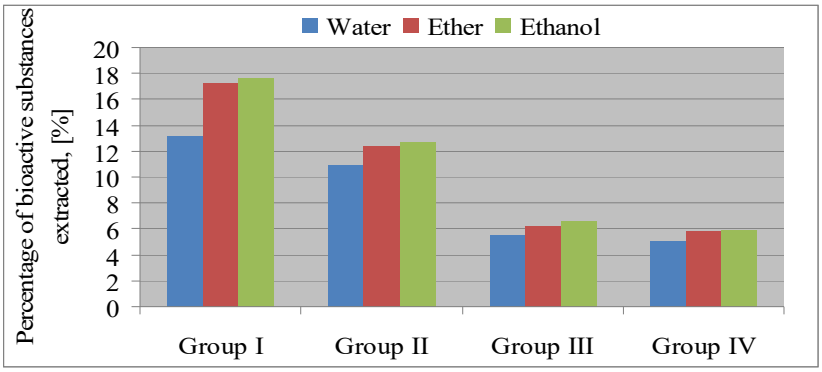


Figure 2 – Percentage of substances extracted using three solvents for four fractions of dry thyme

From Figure 2 it is observed that the fraction I with size of 0.1-2.2 mm were extracted 17.67% bioactive substances in ethanol, 17.24% in ether, and 13.17% water, and from Fraction IV-sized fragments vegetable 4.6-6.3 mm were extracted 5.92% bioactive substances in ethanol, 5.81% in ether, and 5.04% water. So in terms of the maximum content of bioactive substances obtained for all three types of extracts (ethanol extract, ether extract and aqueous extract) is observed descending order for the dimensional characteristics of the four fractions:

Group I > Group II > Group III > Group IV

Given the three solvents used in the extraction of volatile oil of the four fractions of vegetable chopped was a mining best in alcohol, followed by the effected in ether and extraction relatively low in the water, regardless of the dimensional characteristics of the fragments thyme.

Thyme extract in ethanol corresponded stipulations of Romanian Pharmacopoeia, which appears as a dark green opalescent liquid with a characteristic taste and smell aromatic.

The chemical reaction of identifying the polyphenols and flavonoids was positive.

The physical and chemical analysis of the four varieties consisted in determining the following parameters: residues by drying,%; total polyphenols expressed as caffeic acid,%; total polyphenols expressed in chlorogenic acid ,%; total flavonoids expressed in rutin, % and it is shown in table 2.

Table 2 – Physical and chemical analysis of thyme ethanolic extracts

	Dry residues, %	Total polyphenols expressed in caffeic acid ,%	Total polyphenols expressed in chlorogenic acid ,%	Total flavonoids expressed in rutin, %
Fraction I	5.8	0.48	0.96	0.20
Fraction II	5.8	0.41	0.82	0.23
Fraction III	4.5	0.40	0.78	0.15
Fraction IV	3.4	0.19	0.39	0.28

From physical and chemical analysis of ethanol extracts can be seen that the extraction percentage of total polyphenols decreases with increasing the size fractions, and in case of the total flavonoid the extraction process increases with increasing of the size fractions.

Chromatographic profiles achieved at thyme ethanolic extracts have emphasized 22 compounds in the essential oil analyzed (table 3).

Table 3 - Chromatographic profiles of the ethanol extracts from *Thymus serpyllum L*

Den. No.	*Volatile compound*	*Retention time [min]*	**Group I** *Area [%]*	**Group II** *Area [%]*	**Group III** *Area [%]*	**Group IV** *Area [%]*
1	Cineole	14.18	0.72	0.81	-	-
2	Terpinene γ	16.04	4.51	3.55	-	-
3	Ocimene	17.05	16.76	24.98	17.03	19.89
4	Terpineol	23.37	1.69	1.82	1.11	1.30
5	Caryophylene β	26.61	3.21	2.52	2.60	3.25
6	Thymol methyl ether +	26.73	1.43	1.91	1.03	1.41
7	Terpinen-4-ol	26.91	0.69	-	-	-
8	Thymol methyl ether -	27.00	0.93	1.17	1.36	1.06
9	Borneol	29.24	3.99	5.02	3.92	3.73
10	Bisabolene	29.79	0.93	0.87	-	1.26
11	Copaene	30.45	0.66	-	1.04	-
12	Caryophylene α	30.85	2.29	1.29	2.60	2.76
13	Nerol	32.53	2.48	3.22	2.10	2.07
14	Caryophylene oxide	35.21	1.24	2.11	1.39	1.03
15	Thymol +	39.28	54.84	36.30	43.60	48.87
16	Carvacrol	39.80	3.63	3.05	3.02	3.02
17	Hydroquinona tert butil	45.15	-	10.25	-	-
18	Phytol	46.42	-	1.14	-	-
19	3 Carene	16.03	-	-	3.55	5.07
20	Butyl butyryl lactate	30.29	-	-	1.03	-
21	Tert butylcatechol	45.28	-	-	7.65	-
22	Dibutyl phtalate	48.01	-	-	6.98	3.24
23	Methyl butanol	14.84	-	-	-	1.05
24	Cetyl chloride	26.89	-	-	-	0.99

The ethanol extract of thyme obtained from *fraction I* consists in 16 compounds, out of which the most preponderant is thymol with a percentage of 54. 84 %, but also ocimene with 16.76 %, percentage, carvacrol with 3.63 % percentage and terpinene -γ 4.51 % percentage.

Essential oil comprised: borneol 3. 99 %, terpineol 1. 69 %, bisabolene 0.93 %, cineole 0.72 %. Single compound identified only within this extract is terpinene-4-ol 0. 69 %.

In thyme ethanol extract obtained from *fraction II* 16 compounds were identified, terpinene-4-ol and copaene were not found, but, in exchange two new compounds, namely tertiary butyl hydroquinone 10.25 % and phytol 1.14 % were extracted.

At the same time, there are compounds that have higher percentages than those of extract obtained in the previous fraction, such as ocimene 24.98 % in comparison with 16.76 % in fraction I, borneol 5.02 % in comparison with 3.99 %, nerol 3,.22 % in comparison with 2.48 %, but there are also compounds which values are close, such as: cineole, terpineol, thymol methyl ether + and -.

In thyme ethanol extract obtained from *fraction III* 16 compounds were identified according to retention time, the first being 3 Carene in proportion of 3.55 %, a new compound that cannot be found in the first two extracts.

Furthermore, three chemical organic compounds of high percentage, appear, namely: butyl butyryl lactate 1.03 %, 4 tert butylcatechol 7,65 % and dibutyl phtalate 6.98 %.

As small percentage we find: terpineol 1.11 %, thymol methyl ether + 1.03 %, nerol 2.10%.

In high percentage were find thymol methyl ether – 1.36 %, borneol 3.92 %, caryophylene α 2.6 %, thymol +43.6 %, carvacrol 3.02 %.

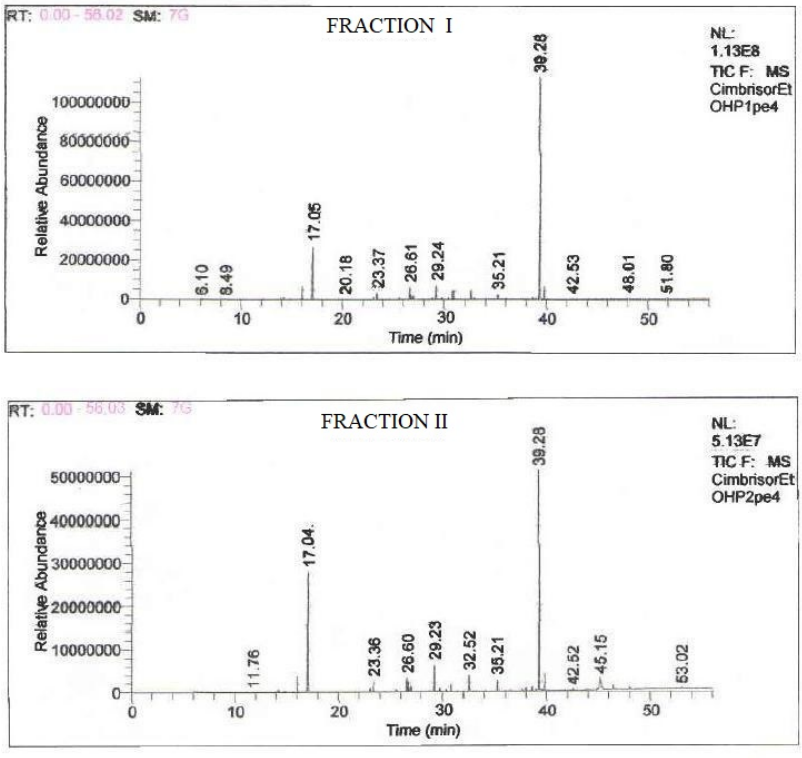
There are also compounds that cannot be found in this extract, such as: bisabolene, terpinen-4-ol, terpinene γ, cineole.

At the same time, a big percentage of copaene 1.04 % appears in comparison with 0.66 % in extract 1.

Thyme ethanol extract obtained from *fraction IV* comprises 16 compounds and additionally appear methyl butanol 1.05 % and cetyl chloride 0,99 %. It predominate thymol + 48.87 %, ocimene 19.89 %, 3 carene 5.07 %, borneol 3.73 %, caryophylene β 3.25 %.

In comparison with the compounds identified in previous extracts, in extract IV the following compounds were not found: cineole, terpinene γ, terpinen-4-ol, copaene, phytol and hydroquinona.

The qualitative and quantitative report of fraction components is shown below in chromatograms obtained for the four ethanol extracts of thyme depending on each fraction and is presented in figure 3.



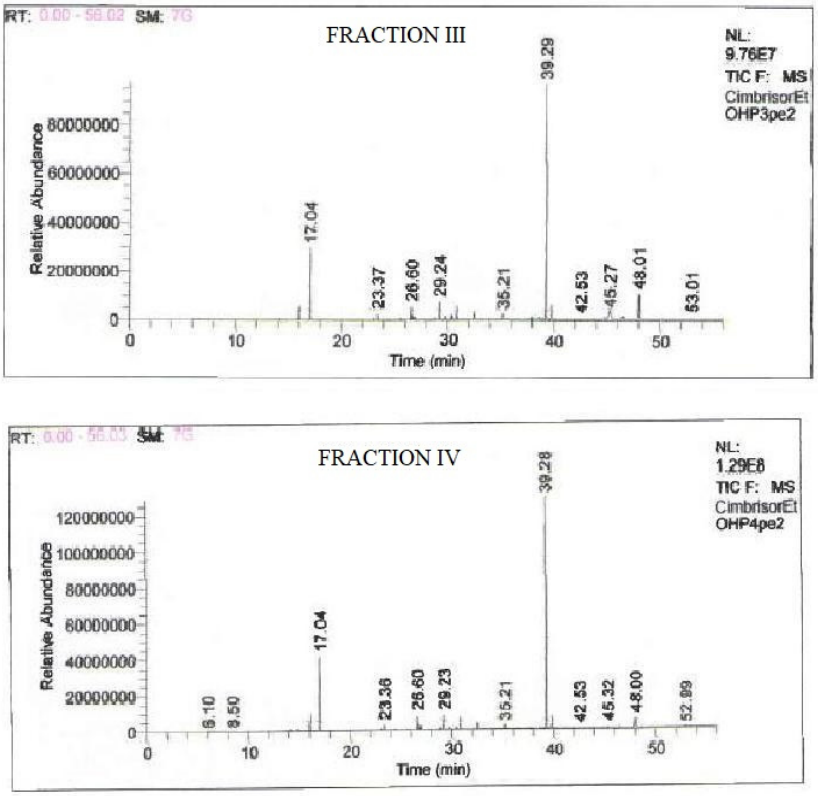


Figure 3. Chromatographic profiles of the four ethanol extracts obtained out of different size fractions

Chromatogram graphically registers the signal transmitted by detector, where the components of mixture separated, released at different times, are.

Retention time or *retention is a qualitative characteristic of the respective components* where the maximum drop, measured when introducing the sample, appears. *The drop height or area* are quantitative characteristics, which are proportional to the quantity of sample component.

4. Conclusions

In this work has been studied the influence of the thyme grinding degree from 0.1 mm to 6.3 mm, on bioactive substances contain, by using different solvents (water, ether, ethanol).

The presented results demonstrate that there is a proportionally direct connection among vegetable product, solvent and extracted bioactive substances, namely, among extracted, vegetable fractions, used solvent type and extracted bioactive substances.

In the three extracts type (watery, etheric and ethanolic) have been identified common biochemical compounds, but also different, extraction superiority has been proved in case of ethanol for all four dimensional fractions and significant bioactive substances contain has been proved for small fractions, indifferently of the used solvent.

Thyme is an indigenous medicinal plant, that abundantly and spontaneous grows in diverse places within the country. Modern therapeutic research revealed that this plant may be used as raw material source for food supplement with antioxidant and hepatoprotective effects, but especially in drags and cosmetics or in products for health care with large antiseptic and antimicrobial spectrum.

References

1. S. M. NABAVI ., A. MARCHESE ., M. IZADI ., V. CURTI , M. DAGLIA ,S.F. NABAVI, Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy , *Food Chemistry* 173 (2015) 339–347
2. A. MĂRCULESCU, L. VLASE, D. HANGANU, C. DRĂGULESCU, I. ANTONIE, O. NELI-KINGA, Polyphenols Analyses From *Thymus* Species, *Proc. Rom. Acad.*, Series B, 2007, *3*, p. 117–121
3. S. BURT, Essential oils: their antibacterial properties and potential applications in foods—a review, *International Journal of Food Microbiology,* 94 (2004) 223– 253
4. R. NECULA, C. APROTOSOAIE, O. CIOANCA, E. GILLE, U. STANESCU, Contributions to the study of the biochemical variability of the polyphenols existing in 8 *Thymus pulegioides* populations from Northern Moldavia, *Bulletin of the Transilvania University of Brasov,* Series VI: Medical Sciences • Vol. 4 (53) No. 2 - 2011
5. I. FECKA, S. TUREK, Determination of polyphenolic compounds in commercial herbal drugs and spices from Lamiaceae: thyme, wild thyme and sweet marjoram by chromatographic techniques, *Food Chemistry* 108 (2008) 1039–1053
6. B. BOROS, S. JAKABOVÁ, A. DORNYEI, G. HORVÁTH, Z.PLUHÁR, F. KILÁR, A. FELINGER, Determination of polyphenolic compounds by liquid chromatography–mass spectrometry in *Thymus* species, *Journal of Chromatography A*, 1217 (2010) 7972–7980
7. P. BYEOUNG-SOO, C. WON-SIK, K. JEONG-HAN, K. KAP-HO, L. SUNG-EUN, Monoterpenes from thyme (*Thymus vulgaris*) as potential mosquito repellents, *Journal of the American Mosquito Control Association*, 21(1):80-83. 2005
8. A. MARCHESE, I. E. ORHAN, M. DAGLIA, R. BARBIERI, A.DI LORENZO , S.F. NABAVI, O. GORTZI , M. IZADI, S. M.NABAVI, Antibacterial and antifungal activities of thymol: A brief review of the literature, *Food Chemistry* 210 (2016) 402–414
9. O. BORUGĂ, C. JIANU, C. MIŞCĂ, I. GOLEŢ, AT. GRUIA, FG. HORHAT, *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity , *J Med Life*. 2014; 7(Spec Iss 3): 56–60.
10. E. MANCINI, F. SENATORE, D. DEL MONTE, L.DE MARTINO, D. GRULOVA, M. SCOGNAMIGLIO, M. SNOUSSI, V. DE FEO, Studies on Chemical Composition, Antimicrobial and Antioxidant Activities of Five *Thymus vulgaris* L. Essential Oils, *Molecules,* 2015, *20*, 12016-12028
11. L. PÎRVULESCU, Comparative evaluation of antioxidant capacity of herbal plants by different methods, *JOURNAL of Horticulture, Forestry and Biotechnology ,* Volume 19(4), 9- 12, 2015
12. M. NIKOLIĆ, J. GLAMOĆLIJA, I. C.F.R. FERREIRA, R. C. CALHELHA, Â. FERNANDES, T. MARKOVIĆ, D. MARKOVIĆ, A. GIWELI, M. SOKOVIĆ, Chemical composition, antimicrobial, antioxidant and antitumoractivity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils, *Industrial Crops and Products* 52 (2014) 183– 190
13. M. RAZZAGHI-ABYANEH, M. SHAMS-GHAHFAROKHI, M.-B.REZAEE, K. JAIMAND, S. ALINEZHAD, R. SABERI, T. YOSHINARI, Chemical composition and antiaflatoxigenic activity of *Carum carvi* L.,*Thymus vulgaris* and *Citrus aurantifolia* essential oils, *Food Control* 20 (2009) 1018–1024
14. R. CHIZZOLA , H. MICHITSCH , C. FRANZ, Antioxidative properties of *Thymus vulgaris* leaves: comparison of different extracts and essential oil chemotypes, *J Agric Food Chem.,* 2008 Aug 27;56(16):6897-904
15. I. ANGELOV, D. V. BERMEJO, R. P. STATEVA1, G. REGLERO, E. IBAÑEZ, T. FORNARI, Extraction of thymol from different varieties of thyme plants using green solvents, *III Iberoamerican Conference on Supercritical Fluids* Cartagena de Indias (Colombia), 2013
16. M. A. HOSSAIN, K. A. S. AL-RAQMI, Z. H. AL-MIJIZY, A. M. WELI, Q. AL-RIYAMI, Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris* , *Asian Pac J Trop Biomed,* 2013; 3(9): 705-710
17. M. HUDAIB, E. SPERONI, AM. DI PIETRA , V. CAVRINI, GC/MS evaluation of thyme (*Thymus vulgaris* L.) oil composition and variations during the vegetative cycle, *Journal of Pharmaceutical and Biomedical Analysis,* 29 (2002) 691–700
18. I. RASOOLI, S.A. MIRMOSTAFA, Antibacterial properties of *Thymus pubescens* and *Thymus serpyllum* essential oils, *Fitoterapia* 73, 2002, 244-250
19. L. SAFAII, E. S ASHOORABADI, S. D. EMAMI, D. AFIUNI, The effect of harvesting stages on quantitative and qualitative characters of essential oil and phenolic yield composition in two thyme species (*Thymus daenensis* Celak & *T. vulgaris* L.) in Iran, *Intl J Agri Crop Sci.* Vol., 7 (13), 1346-1351, 2014

20. B. KRÓL., A. KIEŁTYKA-DADASIEWICZ, Yield and herb quality of thyme (*Thymus vulgaris* L.) depending on harvest time, *Turkish Journal of Field Crops,* 2015, 20(1), 78-84
21. N. K. CHAUHAN, S. SINGH, H. LOHANI, S. Z. HAIDER, Effect of different harvesting time on growth, yield and quality of Thyme (*Thymus serpyllum* L.) under the agro-climatic conditions of Doon valley, Uttarakhand , *J. Chem. Pharm. Res., 2011, 3(6):982-986*
22. S. TOROGLU, In vitro antimicrobial activity and antagonistic effect of essential oils from plant species, *Journal of Environmental Biology,* July 2007, 28(3) 551-559 (2007)
23. L. DE MARTINO, V. DE FEO, F. NAZZARO, Chemical Composition and in Vitro Antimicrobial and Mutagenic Activities of Seven Lamiaceae Essential Oils, *Molecules* 2009, *14*, 4213-4230
24. VR. AFFONSO , HR. BIZZO , CL. LAGE , A. SATO , Influence of growth regulators in biomass production and volatile profile of in vitro plantlets of *Thymus vulgaris* L., *J Agric Food Chem,.* 2009 Jul 22;57(14):6392-5
25. SS. NEMA, MA. TOHAMY, HA. EL-BANNA, MR. ABEER, AA. EL-GENDY, IO. ASMAA, Phytochemical and pharmacological studies of ethanolic extract of *Thymus vulgaris, World Journal Of Pharmacy And Pharmaceutical Sciences,* Volume 4, Issue 10, 1988-2001, 2015
26. Y. D. KREMSH ALASADIY, Study of the biological effect of Thymus vulgaris extracts on spermatogenesis in experimentally infectedwhite mice Balb/c by Toxoplasma gondii , *International Journal of Scientific & Engineering Research*, Volume 5, Issue 10, October-2014, pp. 547-553
27. M. JAFARISANI, M. MASOOMIKARIMI, S.S KAZEMI, S. MIRZAEIDELAVIZ, Z. NADERI, R. AHMADI, Effect of Thymus Vulgaris Ethanol Extract, on Serum Total Antioxidant in Experimental Induced Poly Cystic Ovarian Syndrome (PCOS) Rats, *International Journal of Health Studies* 2016;2(1): 30-34
28. A. KOMAKI, F. HOSEINI, S. SHAHIDI, N. BAHARLOUEI, Study of the effect of extract of Thymus vulgaris on anxiety in male rats, *Journal of Traditional and Complementary Medicine* 6 (2016) 257-261
29. M. TĂMAŞ, D. BENDEC, I . ONIGA, O. ILIOARA, Ghid pentru recunoaşterea şi recoltarea plantelor medicinale, vol I, Flora spontană.*Editura Dacia*, Cluj-Napoca, 2005
30. O. BOJOR, Ghidul plantelor medicinale si aromatice de la A la Z, Ed. Fiat Lux, 2003
31. ***, Farmacopeea Română ed-a X-a. *Editura Medicală*, Bucureşti, reeditata 2008
32. ***, European Pharmacopoeia, 9th edition. *Editura EDQM Council of Europe*, Strassbourg, 2016
33. C.,V. POPESCU, *Ingredienţi activi naturali cu proprietăţi antimicrobiene*, Ed. Vasile Goldiş University Press, Arad, ISBN: 978-973-664-638-6, (2013),
34. ***, The method developed in the laboratory of SC HOFIGAL EXPORT-IMPORT SA, by UV - VIS, 2013