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Ethanol extracts of thyme (*Thyme* sp.) obtained by static and ultrasound assisted extraction – chemical composition and antioxidant activity

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Abstract. The aim of this study is a comparative analysis of the chemical composition and antioxidant activity of extracts obtained with 70% ethanol from Thymus sp. collected from three different areas of Bulgaria by static and ultrasound assisted extraction. The extracts were analyzed using GC/MS. The main compounds were: thymol (32.20–82.35%), p-cymene (7.80–38.55%), γ-terpinene (0.74–5.60%), borneol (1.74–3.39%), carvacrol (1.48–4.83%), β-linalool (0.34–4.86%), and β-caryophyllene (0.75–3.43%). The total phenolic content was determined between 6.50 and 14.65 mg (GAE/g in the extracts from static extraction and between 3.24 and 13.73 mg GAE/g in the extracts from ultrasound assisted extraction. The antioxidant activities on the extracts measured by DPPH assay (between 12.47 and 13.59 mM TE/g for extracts from static extraction and between 12.24 and 12.43 mM TE/g for extracts from ultrasound assisted extraction) and ABTS assay (between 11.27 and 11,87 mM TE/g for extracts from static extraction and between 10.94 and 11.78 mM TE/g for extracts from ultrasound assisted extraction).

1 Introduction

Thyme has been known since ancient times for its aromatic and medicinal properties. It is known to be rich in biologically active compounds and has beneficial effects on health [1]. The biological activity of thyme is mainly due to two groups of organic secondary metabolites: essential oil and polyphenols. Their content in thyme varies widely, which is due to the great species diversity of the plant, its habitat, climatic conditions and other external factors [2].

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Thyme cleanses the respiratory tract, prevents bacterial growth, and acts as an effective antispasmodic, suppressing the cough reflex. The antimicrobial, antioxidant, and other biological properties of essential oils are attributed to their main chemical components, such as thymol, borneol, pinene, linalool, cineole, myrcene, limonene, and cymene. Phenolic compounds are naturally present in almost all plant materials and protect cells from oxidative damage caused by free radicals [3].

Thyme is rich in flavonoids, phenolic antioxidants such as zeaxanthin, apigenin, lutein, luteolin, and thymine. The biological activity depends on the type of polyphenolic compounds, the type of solvent, and the methods used to determine antioxidant activity [4].

Water and ethanol are widely used as solvents for extracting biologically active substances, as they are good solvents and, even in large quantities, do not cause health problems. The use of water as an extractant allows the degree of dissolution to be varied by changing the extraction temperature. Lipid oxidation and chemical degradation are key factors limiting the shelf life of food products. Phenolic compounds are known to affect the oxidation of lipids in foods by slowing down their oxidation without altering their organoleptic properties [4].

Methanolic extracts of thyme are rich in various phenolic compounds, especially flavonoids and phenolic acids. The main compound in these extracts is rosmarinic acid. It is commonly found in plants of the Lamiaceae family. Many authors describe the biological activity of rosmarinic acid, antioxidant, antibacterial, anti-inflammatory, antiviral, etc. Rosmarinic acid is also attributed to a number of therapeutic benefits for human health, such as combating diabetes mellitus, various neurodegenerative diseases, cancer therapy, etc. [5, 6].

Various wild thyme species are found in Bulgaria, such as the species T. vulgaris L. is cultivated and several varieties have been selected from it, differing in the content of the main components of the essential oil.

In order to extract both main groups of compounds, many authors [7, 8], including from Bulgaria, process thyme in various ways with solvents of different polarity.

In recent years, ultrasonic extraction has attracted increasing interest, as it preserves the biologically active substances to a greater extent than conventional methods, providing a higher yield with a low amount of solvent [9-11].

In Bulgaria, essential oils and medicinal plants are processed in industrial conditions mainly by static extraction with polar or non-polar solvents, resulting in liquid extracts, and after evaporation of the solvent – semi-liquid or dry ones. There are also industrial and semi-industrial apparatuses for processing by extraction with liquefied gases, on which a number of essential oils and medicinal plants have been processed.

The aim of this study is a comparative analysis of the chemical composition of 70% ethanol extracts obtained by static and ultrasound assisted extraction from cultivated and wild thyme growing in different regions of Bulgaria and determination of their antioxidant effect with a view to their future application in various functional foods.

2 Materials and methods

2.1 Materials

Thyme with all plant part was harvested in 2024 from three different locations in Bulgaria in full bloom, and this stage of the growing season was in May-June:

- Sample 1: The plants, cv. "German Winter" were grown in the experimental field of the Institute of Rose, Essential and Medicinal Plants in the town of Kazanlak. This region is located in Central Bulgaria (42°63'48.3"N 25°38'85.6"E, altitude 407 m). The soil type is cinnamon-forest, with a pH of 5.6. Soil care with fertilization is a guarantee for the favorable development of plants and their supply of the necessary nutrients

- Sample 2: The plants were wild and collected from town of Yambol, Kurtkaya area. This region is located in Southeastern Bulgaria (42°29'23.4"N 26°27'56.1"E, altitude 114 m). The soil cover is diverse, with chernozem resins predominating.
- Sample 3: The plant were wild and collected from village of Mogila. This region is located in Southeastern Bulgaria (42°29'50.51"N 26°35'46.81"E, altitude 195 m), near the Tundzha River. The soil is black earth and alluvial - meadow.

The belonging of plants to the genus *Thyme* was deter-mined by a botanist from the Institute of Rose, Essential and Medicinal Plants, Kazanlak by their morphological features. The plants harvested from the field are stored for 20 days in dry and ventilated rooms in order to avoid contamination and reduce the essential oil content. They were then stored in paper bags in a dark, dry place. The moisture content of the stored samples (6.04% for sample 1, 4.96% for sample 2, and 4.29% for sample 3) was determined by drying at 105℃, and the plants were pre-cut to size between 0.5 and 1 cm.

2.2 Methods

2.2.1 Extraction procedure by static extraction

The extraction was carried out with 70% ethanol, as a periodic process, without stirring, with a raw material:solvent ratio=1:10, a temperature of 60°C and a process duration of 60 min. The choice of technological parameters hydromo-dule, temperature and duration is based on our unpub-lished data, and these conditions are gentle on both the sol-vent and the aromatic biologically active substances. It is known that 70% ethanol has the best biological properties, in addition, it is widely used in the food industry as a solvent for various substances and is part of a number of foods and beverages.

2.2.2 Extraction procedure by ultrasound assisted extraction

The extraction was carried out under the same technological parameters (solvents, raw material:solvent ratio, temperature, and duration) as the static one in order to compare the extracts obtained by chemical composition and antioxidant activity. The extraction procedure was performed in an ultrasonic bath Elmasonic P30H (Elma Schmidbauer GmbH, Gottlieb, Germany), operating at a frequency of 35 kHz and power of 300 W.

The temperature was monitored with a contact thermometer and kept constant (60°C) during the process.

All obtained extracts were filtered and stored at 4-6°C until analysis.

2.2.3 Chemical composition and activity

Quantitative and qualitative measurements of phenolic acids, flavonoids, total phenolic acids, and antioxidant activity in the thyme extracts were determined by standart methods.2.2.5. Antioxidant activity of extracts

2.3. Chemicals

All reagents and standarts for chemical analysis were purchased from Sigma–Aldrich Chemie GmbH (München, Germany) and were of analytical quality.

The ethanol (95%) was purchased from Fillab LtD (Plovdiv, Bulgaria).

2.4. Statistical Analysis

All experiments were performed in triplicate. The values in the tables are averaged, and represented with their mean and standard deviation (SD). The means are compared using Duncan's multiple range tests, with a significance of $p < 0.05$.

3 Results and discussion

The extracts are yellow-green liquids with a characteristic thyme odor, with different shades in individual samples.

The chemical composition of the extracts is presented in Table 1. The data shows that between 99.26 and 99.88% of the components were identified in all extracts. The main components in all extracts (over 2%) are: oxygenated phenyl propanoids thymol and carvacrol, phenyl propanoids hydrocarbon p-cymene, oxygenated monoterpenes β-linalool, borneol, and *cis*-sabinene hydrate, monoterpene hydrocarbons α- and γ-terpinene, and sesquiterpene hydrocarbon β-caryophyllene. The main component in all studied ethanol extracts, regardless of the method of production and origin of raw material, is thymol, which assigns them to the thymol chemotype. The amount of thymol is higher in extracts obtained from wild thyme in both extraction methods, and in static extraction its content is more than twice higher as in cultivated thyme extract.

Comparative analysis of the data from Table 1 shows that in the extract from sample 1, regardless of the type of extraction, the thymol content is the lowest. In static extraction, its amount is highest in samples 2 and 3 (the values are statistically indistinguishable) and almost one and a half times lower in ultrasound assisted extraction. For comparison, the thymol content in the essential oils from the same samples [51] is 53.55% for sample 1, 44.09% for sample 2 and 42.88% for sample 3. This difference in the amount of the main component thymol between the essential oils and the obtained extracts can be explained by the method of processing, which was also found in other essential oils extracted from raw materials. The amounts of p-cymene and γ-terpinene in the obtained extracts differ from those found in the essential oils [12], which can be explained by the cyclization and/or isomerization and/or oxidation reactions that occurred. During distillation, aromatic substances evaporate, and with an increase in temperature (up to 100°C), their vapor pressure increases which allows them to pass from the plant cell to the vapor space. The extraction of aromatic substances depends on their solubility in polar or non-polar dilutors, and the process takes place at a much lower temperature than distillation.

Conventional extraction techniques (Soxhlet extraction, maceration, percolation, *etc*.) are known to consume more solvent, time, thermal energy, and are associated with a number of drawbacks. New unconventional extraction methods (extraction with supercritical fluids, ultrasound-assisted extraction, microwave extraction, enzyme extraction, pulsed electric field extraction, *etc*.) are increasingly preferred in the extraction of aromatic and biologically active substances from plant sources, mainly due to the relatively high yield, short extraction time and competitive energy efficiency. The use of an environmentally friendly "green" extraction method also contributes to the development of sustainable technologies that are beneficial to the environment. Of course, differences in the composition of extracts can also be due to other factors, explainable by the different functional groups of compounds.

The distribution of components by compound groups is presented in Fig. 1. The distribution of aromatic compounds by groups shows that:

- For static extraction, oxygenated phenyl propanoids predominated in the extracts (36.46% for sample 1, 88.31% for sample 2, and 79.60% for sample 3), followed by phenyl propanoid hydrocarbons (30.71% for sample 1, 0.47% for sample 2, and 7.82% for sample 3), monoterpene hydr-car-bons (12.54% for sample 1, 0.97% for sample 2, and

3.87% for sample 3), oxygenated monoterpenes (12.50% for sample 1, 0.34% for sample 2, and 6.56% for sample 3), sesqui-terpene hydrocarbons (3.77% for sample 1, 0.76% for sample 2, and 1.85% for sample 3), oxygenated sesquiter-penes (1.79% for sample 1, 0.16% for sample 2, and 0.30% for sample 3), and oxygenated aliphatics (2.23% for sample 1).

- For ultrasound assisted extraction, oxygenated phenyl propanoids predominated in the extracts (39.42% for sample 1, 44.93% for sample 2, and 48.97% or sample 3), followed by phenyl propanoid hydrocarbons (32.30% for sample 1, 38.74% for sample 2, and 29.64% for sample 3), monoter-pene hydrocarbons (10.46% for sample 1, 6.03% for sample 2, and 7.90% for sample 3), oxygenated mono-terpenes (10.90% for sample 1, 6.30% for sample 2, and 8.25% for sample 3), sesquiterpene hydrocarbons (3.35% for sample 1, 1.93% for sample 2, and 2.53% for sample 3), oxygenated sesquiterpenes (1.60% for sample 1, 0.92% for sample 2, and 1.21% for sample 3), and oxygenated aliphatics (1.97% for sample 1, 1.15% for sample 2, and 1.50% for sample 3).

The data shows that in all extracts, except for the one obtained by static extraction from samples 2 and 3, the content of oxygenated phenyl propanoids and phenyl propanoid hydrocarbons is close. In the extracts obtained from sample 1, regardless of the processing method, the amount of monoterpenes (hydrocarbons and their oxygen derivatives) is the highest.

The type and amount of extracted polyphenolic compounds is presented in Table 2. From the comparative analysis of the groups of components in the extracts, the following conclusions can be drawn. The content of oxygenated phenyl propanoids are highest in extracts obtained from wild thyme (samples 2 and 3) obtained by static extraction. However, the amount of phenyl propanoid hydrocarbons in them is significantly lower. In the extract obtained from sample 1, the content of the groups of compounds, regardless of extraction, is comparable. There are no oxygenated aliphatics in the extracts obtained from samples 2 and 3 by static extraction. In extracts obtained by ultrasound assisted extraction, the amount of phenylpropanoids (hydrocarbons and oxygenated compounds), regardless of the type of sample, is comparable.

This difference in the content of the groups of components can be explained by the way the raw material is grown – sample 1 is cultivated, and samples 2 and 3 – are wild. The soils on which the plants grow also have different compositions, which also affects the biosynthesis of the various aromatic substances, as has been found in other essential oil plants. Differences in the composition of extracts may also be due to other factors, for example, the transformation of some compounds during extraction, such as isomerization, oxidation or degradation [13].

The high thymol content in the extracts obtained indicates that both cultivated and wild-growing thyme are suitable raw materials for processing by extraction.

The data (Table 2) shows that the total amount of polyphenolic acids and flavonoids is:

In static extraction: Sample 1: polyphenolic acids (2.608 g/mL), flavonoids (1.152 g/mL); Sample 2: polyphenolic acids (1.279 g/mL), flavonoids (1.088 g/mL); Sample 3: polyphenolic acids (1.703 g/mL), flavonoids (3.109 g/mL).

When extracted with ultrasound assisted extraction: Sample 1: polyphenolic acids (2.377 g/mL), flavonoids (1.353 g/mL); Sample 2: polyphenolic acids (0.965 g/mL), flavonoids (0.832 g/mL); Sample 3: polyphenolic acids (0.517 g/mL), flavonoids (0.630 g/mL).

The total polyphenol content of cultivated thyme extracts is comparable in both extraction methods (3.76 g/mL and 3.73 g/mL, respectively). Ethanol extracts of wild thyme obtained by static extraction are richer in polyphenols than those obtained by ultrasound assisted extraction. This is probably due to the disintegration of plant tissue and the impediment, of diffusion processes.

Table 1. Chemical composition of garden thyme extracts (% of TIC [a]).

No peak	RT [2], min	RI [3]	Compounds	Static extraction			Ultrasound assisted extraction		
				Sample 1	*Sample 2*	*Sample 3*	*Sample 1*	*Sample 2*	*Sample 3*
1.	8.81	922	α-Pinene	0.59±0.0 [a]	nd [4]	nd	0.53±0.0 [a]	0.30±0.0 [b]	0.40±0.0 [c]
2.	9.32	929	Camphene	0.61±0.0 [a]	nd	nd	0.35±0.0 [b]	0.20±0.0 [c]	0.26±0.0 [c]
3.	10.10	970	Sabine	0.77±0.0 [a]	nd	nd	0.49±0.0 [b]	0.28±0.0 [c]	0.37±0.0 [d]
4.	10.22	974	β-Pinene	0.22±0.0 [a]	nd	nd	0.29±0.0 [b]	0.17±0.0 [c]	0.22±0.0 [a]
5.	10.45	981	1-Octen-3-ol	1.71±0.15 [a]	nd	nd	1.52±0.12 [a]	0.88±0.0 [b]	1.16±0.09 [c]
6.	10.58	985	3-Octanone	0.14±0.0 [a]	nd	nd	0.12±0.0 [a]	0.07±0.0 [b]	0.09±0.0 [b]
7.	10.70	988	β-Myrcene	0.67±0.0 [a]	nd	nd	0.60±0.0 [a]	0.35±0.0 [b]	0.46±0.0 [c]
8.	11.01	981	3-Octanol	0.36±0.0 [a]	nd	nd	0.32±0.0 [a]	0.19±0.0 [b]	0.25±0.0 [c]
9.	11.96	1027	α-Limonene	0.38±0.0 [a]	nd	nd	0.34±0.0 [a]	0.19±0.0 [b]	0.26±0.0 [c]
10.	12.03	1033	1,8-Cineole	1.12±0.01 [a]	nd	nd	1.00±0.08 [a]	0.58±0.0 [b]	0.76±0.0 [c]
11.	12.28	1015	α-Terpinene	2.00±0.01 [a]	0.23±0.0 [b]	0.49±00 [c]	1.78±0.15 [a]	1.03±0.0 [d]	1.35±0.010[e]
12.	12.34	1023	p-Cymene	30.48±0.28[a]	9.45±0.08 [b]	7.80±0.07 [c]	32.12±0.31 [a]	38.55±0.35 [d]	29.60±.025[a]
13.	13.40	1057	γ-Terpinene	5.60±0.05 [a]	0.74±0.0 [b]	3.37±0.03 [c]	4.58±0.04 [d]	2.65±0.02 [e]	3.48±0.03 [c]
14.	13.72	1083	Terpinolene	1.61±0.01 [a]	nd	nd	1.44±0.01 [a]	0.83±0.0 [b]	1.09±0.01 [b]
15.	13.78	1067	*cis*-Sabinene hydrate	nd	nd	3.00±0.03	nd	nd	nd
16.	14.25	1099	β-Linalool	4.86±0.04 [a]	0.34±0.0 [b]	0.65±0.0 [c]	4.12±0.04 [d]	2.38±0.02 [e]	3.13±0.03 [f]
17.	14.57	1105	*trans*-Sabinene hydrate	0.89±0.0 [a]	nd	nd	0.80±0.0 [a]	0.46±0.0 [c]	0.60±0.0 [c]
18.	15.86	1145	Camphor	0.90±0.0 [a]	nd	nd	0.80±0.0 [b]	0.46±0.0 [c]	0.61±0.0 [d]
19.	16.64	1180	Terpinen-4-ol	0.68±0.0 [a]	nd	nd	0.60±0.0 [a]	0.35±0.0 [b]	0.46±0.0 [c]
20.	16.88	1171	Borneol	3.39±0.03 [a]	nd	2.90±0.02 [b]	3.01±0,03 [c]	1.74±0.01 [c]	2.29±0.02 [d]
21.	17.32	1195	α-Terpineol	0.57±0.0 [a]	nd	nd	0.51±0.0 [a]	0.30±0.0 [b]	0.39±0.0 [b]
22.	18.04	1228	Thymol methyl ether	0.29±0.0 [a]	0.91±0.0 [b]	1.28±0,01[c]	0.26±0.0 [a]	0.15±0.0 [d]	0.20±0.0 [d]
23.	20.31	1293	Thymol	32.20±0.31[a]	82.35±0.81[b]	76.67±0.75[b]	35.65±0.34 [a]	42.66±0.41 [c]	46.21±0.45[c]
24.	20.59	1300	Carvacrol	3.54±0.03 [a]	4.83±0.04 [b]	1.48±.0.01 [c]	3.15±0.03 [a]	1.82±0.01 [d]	2.39±0.02 [e]
25.	21.27	1350	Thymyl acetate	0.16±0.0 [a]	nd	nd	0.14±0.0 [a]	0.08±0.0 [b]	0.11±0.0 [b]
26.	23.64	1420	β-Caryophyllene	3.43±0.03 [a]	0.75±0.0 [b]	1.85±0.01 [c]	3.05±0.03 [d]	1.76±0.01 [c]	2.32±0.02 [e]
27.	24.73	1480	Germacrene D	0.31±0.0 [a]	nd	nd	0.28±0.0 [a]	0.16±0.0 [b]	0.21±.00 [b]
28.	28.32	1581	Caryophyllene oxide	1.78±0.01 [a]	0.16±0.0 [b]	0.30±0.0 [c]	1.59±0.01 [a]	0.92±0.0 [d]	1.21±0.01 [d]

[1] total ion current; [2] retention time, min; [3] retention index (Kovat's); [4] lower than 0.05% of TIC or not identified; Values with different letters in the same row indicate significant differences ($p < 0.05$).

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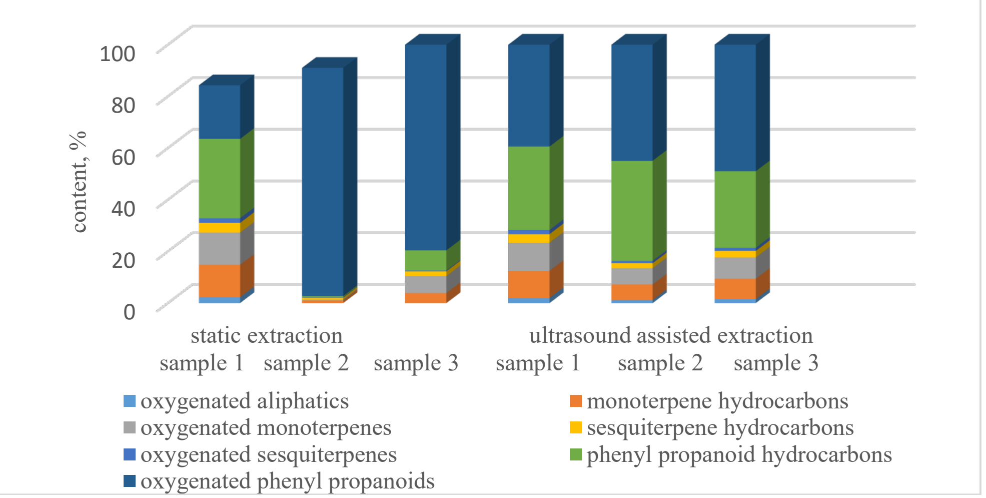

Fig. 1. Group of components in thyme extracts, %.

The data in Table 2 shows that regardless of the sample and the method of its processing, in the extracts of polyphenolic acids, rosmarinic acid predominates, and from flavonoids – rutin, followed by quercetin. The amount of rosmarinic acid is less compared to the research of Sik et al. [14] explainable by the extraction method and the type of solvents. The content of the remaining identified polyphenolic compounds is lower, and their amount varies. It is difficult to make a comparison with the data from the literature due to the different origin of the raw material for analysis and the conditions of the extraction. It is known that the high efficiency of ultrasound assisted extraction, in terms of yield and composition, is achieved by increasing the power, decreasing the humidity of the plant matrix to improve the solvent-solid contact and optimizing the temperature. The most suitable frequencies are between 20 and 40 kHz, at higher frequencies it is more difficult to induce cavitation, since cavitation bubbles need a little delay to be initiated. The polyphenolic acids and flavonoids contained in the extracts are a prerequisite for their use in various functional dairy foods, such as cheese, yellow cheese, *etc*.

It is known that essential oils contain aromatic substances that cause allergic reactions, manifested in edema, itching of the skin, redness of the eyes, etc.. These aromatic substances, as well as the essential oils that contain them, are described in the Regulation 2023/1545. This regulation specifies the permissible concentrations of allergens that can be included in various cosmetic preparations, with their limits being different for products that are washed off the skin and for products that remain on it for a longer time. According to this Regulation, the following allergens have been identified in the extracts, with their amounts varying with the thyme samples and the processing method of the raw material, as follows: α-pinene (0.30 to 0.59%), β-pinene (from 0.17 to 0.29%), α-limonene (from 0.19 to 0.38%), α-terpinene (from 0.23 to 1.78%), γ-terpinene (from 0.74 to 5.60%), terpinolene (from 0.83 to 1.61%), β-linalool (from 0.34 to 4.86%), camphor (from 0.46 to 0.90%), terpinen-4-ol (from 0.46 to 0.68%), α-terpineol (from 0.30 to 0.57%), and β-caryophyllene (from 0.75 to 3.43%). The comparative analysis shows that their amount is highest in the extracts obtained from sample 1, while in the static extraction in extracts obtained from samples 2 and 3 some of them are missing. Their distribution by structure and functional groups is: monoterpene hydrocarbons (α- and β-pinene, α- and γ-terpinene, limonene, and terpinolene), monoterpene oxygen derivatives: alcohols (linalool, α-terpineol, and terpinen-4-ol), ketones (camphor), and sesquiterpene hydrocarbons (β-caryophyllene). The lower allergen content in extracts obtained by ultrasound assisted extraction makes them more suitable for inclusion in cosmetic preparations, such as mouthwashes, lotions, creams, etc. Extracts obtained by static extraction can also be used in the above cosmetic products, but their content must be taken into account the concentration of allergens.

Table 2. Polyphenolic acids and flavonoids of garden thyme extracts.

Compounds	**Content, g/mL extract**					
	Static extraction			**Ultrasound assisted extraction**		
	Sample 1	***Sample 2***	***Sample 3***	***Sample 1***	***Sample 2***	***Sample 3***
Phenolic acids						
Caffeic acid	0.038±0.0 [a]	0.027±0.0 [a]	0.020±0.0 [a]	0.037±0.0 [a]	0.021±0.0 [a]	0.017±0.0 [a]
p-Coumaric acid	0.027±0.0 [a]	0.177±0.01 [b]	0.033±0.0 [a]	0.028±0.0 [a]	0.007±0.07 [c]	0.004±0.0 [c]
Ferulic acid	0.039±0.0 [a]	0.086±0.0 [b]	0.024±0.0 [a]	0.032±0.0 [a]	0.036±0.0 [a]	0.023±0.0 [a]
Salicylic acid	0.476±0.04 [a]	0.186±0.01 [b]	0.131±0.01 [c]	0.482±0.04 [a]	0.122±0.010 [c]	0.074±0.0 [d]
Rosmarinic acid	2.028±0.19 [a]	0.803±0.08 [b]	1.495±0.14 [c]	1.798±0.17 [d]	0.779±0.07 [b]	0.399±0.390 [e]
Flavonoids						
Rutin	0.858±0.08 [a]	0.643±0.06 [b]	2.448±0.23 [s]	0.867±0.08 [a]	0.559±0.05 [b]	0.359±0.03 [d]
Hesperidin	0.057±0.0 [a]	0.043±0.0 [a]	0.064±0.0 [a]	0.065±0.0 [a]	0.012±0.12 [b]	0.058±0.05 [c]
Quercetin	0.214±0.02 [a]	0.229±0.02 [a]	0.495±0.04 [b]	0.216±0.02 [a]	0.206±0.02 [a]	0.134±0.01 [c]
Kaempferol	0.023±0.0 [a]	0.173±0.01 [b]	0.102±0.01 [c]	0.205±0.02 [d]	0.055±0.0 [e]	0.079±0.0 [e]

[1] Values with different letters in the same row indicate significant differences ($p < 0.05$).

The values of total phenols and antioxidant activity of the obtained extracts are presented in Table 3. The data show that in static extraction the content of total phenols is highest in sample 1 and lowest in sample 3 (the values are statistically distinguishable). A similar trend is also found in the extracts obtained by ultrasond assisted extraction treatment of thyme, but the values are lower.

The antioxidant activity determined by the DPPH method is higher in the extracts obtained by static extraction, while by the ABTS method the values are comparable.

The difference in the content of total phenols in the extracts (Table 3) can be explained by the origin of the samples. The values of antioxidant activity differ from the data in the literature, for example, by the DPPH method, the percentage of radical scavenging activity was 72.3% and by ABTS – 86.3% [10], by DPPH method (IC50 25.1 mg/mL by maceration and IC50 14.1 mg/mL by ultrasonic extraction) [15], also explainable by the origin, type of raw material and technological conditions of the extraction. The antioxidant activity of thyme extracts can be explained by the content of polyphenol acids, as well as by that of thymol, which as a phenol has a pronounced effect [14]. The antioxidant activity values are much lower than the essential oil data obtained from the same thyme samples [12]. This can be explained by the chemical composition of both products – essential oils and extracts, found in other plants. It is known that oxygenated derivatives, such as phenols thymol and crvacrol, have strong antioxidant activity, while hydrocarbons, such as p-cymene, α- and y-terpinene, and β-caryophyllene have a weaker. On the other hand, polyphenolic acids, such as rosmarinic acid [5], also have a pronounced antioxidant activity. However, their amount in the extracts studied varies, which is why it is difficult to have clarity on which of these components is responsible for the activity. This, in our opinion, is the subject of future additional research.

Table 3. Antioxidant activity of garden thyme extracts.

Indicators	Static extraction			Ultrasound assisted extraction		
	Sample 1	***Sample 2***	***Sample 3***	***Sample 1***	***Sample 2***	***Sample 3***
Total phenols, mg GAE/mL	14.65±0.10[a]	8.56±0.07 [b]	6.50±0.06 [c]	13.73±0.12[d]	5.93±0.05 [c]	3.24±0.03 [e]
DPPH, mM TE/mL	13.59±0.10[a]	12.89±0.12[b]	12.47±0.11[b]	12.43±0.11[b]	12.38±0.11[b]	12.24±0.11[b]
ABTS, mM TE/mL	11.87±0.10[a]	11.71±0.10[a]	11.27±0.10[a]	11.78±0.10[a]	11.68±0.10[a]	10.94±0.09[b]

[1] Values with different letters in the same row indicate significant differences ($p < 0.05$).

4 Conclusion

The chemical composition and antioxidant activity of ethanol extracts from cultivated and wild thyme growing in different regions of Bulgaria are influenced by the extraction method (conventional or ultrasonic). The main component in all extracts is thymol, and its amount varies depending on the type of thyme (cultivated or wild) and the extraction method.

Extracts obtained from wild thyme by static extraction are highest in thymol. Ultrasonic extracts more phenyl propanoid hydrocarbons and less oxygenated phenyl propanoids compared to extraction without ultrasound assisted extraction. In the extracts, the polyphenolic acids were dominated by rosmarinic acid, and among the flavonoids, rutin, followed by quercetin. Extracts obtained by static extraction are richer in polyphenols than those obtained using ultrasound assisted extraction. The content of total phenols was also higher in the extracts obtained by static extraction.

Despite the established differences in the composition of the extracts, all exhibited comparable antioxidant activity, determined by DPPH and ABTS methods.

The rich chemical composition and antioxidant activity of ethanol extracts of cultivated and wild thyme from Bulgaria is a reason to use them in the development of functional foods.

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