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Factors affecting the amount of biologically active substances in extracts of Bulgarian medical plants typical of Western Rhodopes

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To obtain extracts maximally enriched with biologically active compounds (BAC), it is necessary to study and select suitable conditions for carrying out the extraction process. Five Bulgarian plants that thrive in the Western Rhodopes were selected: *Cirsium ligulare* Boiss. and *Crataegus monogyna* (flowers), *Hypericum perforatum* L. and *Thymus callieri* Borbás ex Velen. (stems), *Crataegus monogyna* and *Juniperus communis* L. (fruits). The effect of processing of fresh herbs (drying and freezing) on the content of BAC was investigated. Higher amounts of total phenols and flavonoids were contained in the dried materials. The highest amount of BAC from the dried herbs was found in the *Thymus callieri* Borbás ex Velen. and from the frozen ones – in the *Hypericum perforatum* L. The method of extraction (conventional and ultrasonic) was found to influence the amount of extracted BAC. In the conventional method of extraction, the yield of BAC was almost twice as high as in ultrasonic extraction. The concentration of ethanol (0%, 30%, 50%, 70%, or 95%) had a significant effect on the amount of BAC, as 70% ethyl alcohol showed the best results. Of the studied 5 medicinal plants, dried *Thymus callieri* Borbás ex Velen. and frozen *Hypericum perforatum* L. might be successfully used to prepare 70% ethanolic extracts by the conventional method.

Keywords: antioxidant activity; flavonoids; phenols; DPPH; FRAP

INTRODUCTION

The use of plant products for treating various diseases started with the beginning of human civilization. The earliest document shedding light on the use of medicinal plants was written between 4500 and 1600 BC [1].

Medicinal plants are usually perennial and their shoot system contains BAC that are of great interest due to their antioxidant and antibacterial properties. Synthetic antioxidants are mostly used in the food industry and cosmetics to prolong the stability of foods and cosmetic products. However, the use of these antioxidants has been questioned due to their potential health risks and toxicity [2]. Therefore, the search for antioxidants from natural sources, such as medical plants, attracts researchers' attention. The presence of phenols and terpenes in their composition allows their use as stabilizers in food. In cosmetics, essential oils derived from medicinal plants are used for flavoring and due to their antiseptic action, in the composition of lotions, eaux de toilette, and soaps. Dried herbs are used in medicine as tinctures and teas for colds, coughs, stomach and intestinal diseases. According to Naczk and Shahidi [3], approximately 10 to 20% of plants are used in a positive way in health care to treat harmful diseases.

Varied medicinal plants are known as a source of antioxidants that can protect organisms from oxidative stress and various chronic diseases [4]. The group of antioxidants includes water-soluble antioxidant metabolites (ascorbate and glutathione) and secondary metabolites, such as polyphenols, flavonoids, and terpenoids [5] which are present mainly in plants [6], and are distributed in different parts, mainly in flowers, leaves, and fruits. However, environmental factors can affect the production of antioxidants and secondary metabolites. Furthermore, different extraction techniques are used for the isolation of BAC to achieve maximum process efficiency. Conventional (classical) extraction (CE) is the most widespread technique for the extraction of antioxidant compounds from plant materials but this method consumes a great amount of energy, due to the heating process and is characterized by long duration. Non-conventional extraction methods include ultrasound-assisted extraction (UAE) which uses less energy and has a shorter duration, allows

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full extractions to be completed in minutes with high reproducibility, reduces the consumption of used solvents, simplifies manipulation and work-up.

The extraction efficiency of all methods mainly depends on the choice of using selective solvents. The solvent polarity, its environmental safety, and toxicity are the most important factors while selecting a solvent for the extraction of BAC. Ethanol is a solvent that is safe for human consumption due to its low toxicity [7].

Indisputably, fresh herbs have the highest quality but they can be used only in one season. Different preservation techniques can be exploited to ensure the quality, safety, and shelf-life extension of plants. Among these, freezing is recognized as one of the main processes for long-term preservation which has a low impact on the nutritional quality of food products [8, 9]. Air-dried herbs are also a good alternative to fresh ones, as the process itself is easy to perform and inexpensive. Drying is by far the most widely used treatment [10].

The higher plants that grow in Bulgaria are very diverse. The vegetation in the municipality of Dospat, which is located in the sub-region of the Western Rhodopes, is characterized by rich biodiversity [11]. The relief is typical mountainous and significant variations in altitude (560 – 1653 m), and specific microclimatic conditions are the prerequisites for the rich floristic diversity [12]. Typical for the Western Rhodopes and the municipality of Dospat and with long-term traditional use (widely used in folk medicine, as medicines and less often in drinks and food) are the medicinal plants like thyme, St. John's wort, cirsium, hawthorn , juniper, etc.

Thymus callieri Borbás ex Velen. is a new species in the Balkans floristic regions [13] and *Thymus* extracts obtained with polar solvents are an attractive target for the screening of BAC for possible industrial applications in distinct fields, including food, cosmetics or pharmaceutical industries [14, 15]. St. John's Wort (*Hypericum perforatum* L.) is presently one of the most consumed medicinal plants in the world [16, 17]. So far, data on *Cirsium ligulare* Boiss. in literature are scarce. However, plants from the *Cirsium* genus are rich in phenolic compounds [18]. Hawthorn (*Crataegus monogyna*) is a medicinal plant widely used in phytotherapy for the treatment of many cardiovascular diseases [19], as from the plant are most often used its flowers, leaves and fruits. *Juniperus communis* L. is an evergreen aromatic shrub with high therapeutic potential for the treatment of diseases in human and animals [20]. These five medicinal plants are typical for the Bulgarian flora but little is known about their antioxidant activity.

This study aimed to evaluate the influence of the method of herbs processing (drying or freezing), extraction approach (conventional or ultrasound), and the concentration of the extracting agent (ethanol) on the antioxidant activity of herbal extracts prepared from different morphological parts of five different Bulgarian medical plants collected from Dospat (Western Rhodopes, Bulgaria).

MATERIALS AND METHODS

Plant materials and treatments (drying and freezing)

The different morphological parts of the five Bulgarian medicinal plants grown in the Western Rhodopes, Dospat municipality were selected: TC – thyme (*Thymus callieri* Borbás ex Velen.) stem, HP – St. John's wort (*Hypericum perforatym* L.) stem, CL – cirsium (*Cirsium ligulare* Boiss.) flower, CM-flower – Hawthorn (*Crataegus monogyna*) flower, CM-fruit – Hawthorn (*Crataegus monogyna*) fruit, JC – juniper (*Juniperus communis* L.) fruit.

A portion of fresh plant material was inspected, cut into small pieces, dried in a thin layer in the shade at 22 – 25°C and stored in tightly closed bags in a dry place until the time of analysis (dried herb). A second portion of fresh plant material was inspected, cut into small pieces, placed in plastic bags, and frozen in a refrigerator at –18 °C until the time of analysis (frozen herb).

Preparation of plant extracts

1. Conventional (classical) method of extraction. An aqueous extract (0%) and 30%, 50%, 70% or 95% ethanolic extracts from dried and frozen plant mass were obtained according to [21] with small modifications: 15 g (20 g) of the dried (frozen) plant were mixed with 300 mL of H_2O, 30%, 50%, 70% or 95% ethanol and kept for 1 h at 60ºC, then were left for 24 h at room temperature under constant stirring. The obtained mixtures were filtered through nylon cloth (250 mesh), and insoluble residues were extracted with an additional 200 mL of the same extractant at the same conditions. The two filtrates were combined and homogenized well.

2. Ultrasound-assisted extraction. The extraction process of BAC from the used experimental plants was carried out with the appropriate concentration of ethanol (solid to liquid ratio 1:20) in an ultrasonic bath (VWR, Malaysia;

45 kHz, 30 W) at 45 °C for 15 min, according to [22] with same modifications. The extracts were centrifuged at 1800×g for 15 min (MPW-251, Med. Instruments, Poland) and used for further analysis.

Chemical analyses

1. *Total flavonoid content* was evaluated using $Al(NO_3)_3$ reagent and measuring the absorbance at 415 nm as described by Kivrak *et al*. [23]. The calculation was made by using a standard curve prepared with quercetin.

2. *Total phenols* were determined according to [24]. The calculation was made by using a standard curve prepared with gallic acid.

Determination of antioxidant activity

The antioxidant activity of the extracts was evaluated by two methods: FRAP (ferric reducing antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method.

1. *FRAP method* is based only on a single electron transfer mechanism and was measured according to [25] with some modification. Three ml of freshly prepared FRAP reagent (10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3.6H_2O$ in d. H_2O) were mixed with 0.1 ml of investigated extract. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm against blank prepared with the same solvent. A standard curve was built with $FeSO_4.7H_2O$. The results of FRAP analysis were expressed as μmol Fe^{2+} equivalents per gram plant.

2. *DPPH method* is based on mixed hydrogen atom transfer and single electron transfer mechanisms and was estimated according to [25] with some modification. Briefly, 0.15 ml of extract was mixed with 2.85 ml 0.06 mM DPPH fresh solution in 96% ethanol. The mixture was left for 30 min (kept in the dark at room temperature) so that a reaction could take place, and then the absorbance at 517 nm was read by a spectrophotometer against a blank containing the same solvent. The results of DPPH analysis were expressed as mmol Trolox equivalents (TE) per gram plant.

Statistical analyses

Analyses were performed in triplicate. Results are presented as means ± standard deviation (SD). Data were analysed by one-way analysis of variance (ANOVA) using Statgraphics Centurion statistical program. Mean differences were established by Fisher's least significant difference test for paired comparison with a significance level $p \leq 0.05$.

RESULTS AND DISCUSSION

Influence of herbs processing (drying or freezing) on the amount of biologically active substances

According to our data (Table 1), the difference in plants processing had a significant effect on the amount of phenols and flavonoids. The results showed that the amount of BAC in the dried herbs was higher than in the frozen ones. Cell breakages during freezing can lead to the decompartmentalization of antioxidants such as anthocyanins and other phenolic compounds, and their degradation due to the interaction with oxidative enzymes [26]. The values of total flavonoids and phenols varied in a wide range (Table 1) - from 2.27 to 26.43 mg QE/g weight for flavonoids and from 7.55 to 86.19 mg GAE/g weight for phenols, respectively. The highest content of total flavonoids and phenols from dried herbs showed TC (26.43 mg QE/g weight and 86.19 mg GAE/g weight) and from frozen plants – HP (12.05 mg QE/g weight and 29.29 mg GAE/g weight). On the other hand, the 70% ethanolic extracts from CM – fruit and CL were found to be least rich in total phenols and flavonoids in both dried and frozen herbs. The content of BAC in different parts of the plant (flower and fruit) in CM showed variations, as significantly more noticeable was this difference in the dried samples. However, in both studied materials (dried and frozen), the amounts of both total phenols (81.36 mg QE/g weight in dried flowers and 26.88 mg QE/g weight in dried fruits) and flavonoids (14.96 mg QE/g weight in dried flowers and 2.89 mg QE/g weight in dried fruits) were higher in flowers. The established results were in agreement with Abdulkadir *et al.* [27] who investigated total phenolic and flavonoid contents of the ethanolic extracts from fruit, stem, and leaf of *Solanum torvum*. Their data showed the highest level of phenolic content in the stem (43.92 mg GAE/g), lower in the leaf (37.48 mg GAE/g) and the lowest in the fruit (16.15 mg GAE/g). Similar to phenols, the flavonoid content of 2.89 mg QE/g weight in dried CM – fruit was found to be significantly lower than that of the dried flower – 14.96 mg QE/g weight.

Table 1. Quantities of total flavonoids and phenols of 70% ethanolic extracts obtained by conventional extraction of dried and frozen plant material

70 % C_2H_5OH extracts	Total flavonoids, mg QE/g weight		Total phenols, mg GAE/g weight	
	dried	frozen	dried	frozen
TC	26.43±0.09^{a,A}	7.47±0.18^{b,B}	86.19±0.36^{a,A}	20.32±0.61^{b,B}
HP	8.32±0.22^{c,B}	12.05±0.42^{a,A}	36.83±0.42^{c,A}	29.29±0.44^{a,B}
CL	4.95±0.19^{e,A}	2.49±0.08^{e,B}	19.07±0.31^{f,A}	7.55±0.29^{f,B}
CM – flower	14.96±0.37^{b,A}	3.79±0.11^{d,B}	81.36±0.35^{b,A}	14.73±0.43^{d,B}
CM– fruit	2.89±0.07^{f,A}	2.27±0.05^{e,B}	26.88±0.41^{e,A}	13.19±0.42^{e,B}
JC	6.92±0.17^{d,A}	6.49±0.20^{c,B}	29.85±0.21^{d,A}	18.02±0.32^{c,B}

$^{a-f}$: Means in a column without a common letter differ significantly ($p \leq 0.05$); $^{A-B}$: Means in a row for a dried and a frozen plant (for a particular method) without a common letter differ significantly ($p \leq 0.05$).

Table 2. Antioxidant activity of 70% ethanol extracts obtained by conventional extraction of dried and frozen plant materials

70 % C_2H_5OH extracts	DPPH method, mM TE/g weight		FRAP method, µmol Fe^{2+}/g weight	
	dried	frozen	dried	frozen
TC	218.97±0.28^{a,A}	121.30±0.23^{b,B}	1110.77±0.85^{a,A}	218.96±0.94^{b,B}
HP	216.47±0.28^{b,A}	184.59±0.39^{a,B}	296.76±0.56^{c,B}	339.23±0.52^{a,A}
CL	90.57±0.48^{e,A}	26.74±0.68^{e,B}	155.85±0.28^{f,A}	66.74±0.81^{f,B}
CM- flower	217.06±0.27^{b,A}	97.02±0.38^{c,B}	966.01±0.31^{b,A}	171.95±0.87^{d,B}
CM- fruit	176.23±0.72^{c,A}	85.43±0.21^{d,B}	278.24±0.63^{d,A}	130.91±0.88^{e,B}
JM	126.13±0.75^{d,A}	120.95±0.56^{b,B}	223.76±0.86^{e,A}	188.28±0.42^{c,B}

$^{a-f}$: Means in a column without a common letter differ significantly ($p \leq 0.05$); $^{A-B}$: Means in a row for a dried and a frozen plant (for a particular method) without a common letter differ significantly ($p \leq 0.05$).

Table 3. Influence of the solvent concentration on the amount of total flavonoids and phenols

Bioactive compounds	Herb	Ethanolic extracts				
		0 %	30 %	50 %	70 %	95 %
Total flavonoids, mg QE/g weight	Dried TC	12.22±0.03^{d,A}	18.09±0.08^{c,A}	24.29±0.05^{b,A}	26.43±0.09^{a,A}	10.63±0.04^{e,B}
	Frozen HP	4.23±0.04^{d,B}	9.59±0.17^{c,B}	10.72±0.30^{b,B}	12.05±0.42^{a,B}	11.06±0.22^{b,A}
Total phenols, mg GAE/g weight	Dried TC	57.47±0.43^{d,A}	59.11±0.42^{c,A}	69.19±0.42^{b,A}	86.19±0.36^{a,A}	33.96±0.36^{e,A}
	Frozen HP	18.54±0.29^{e,B}	21.32±0.17^{d,B}	24.38±0.33^{c,B}	29.29±0.44^{a,B}	27.35±0.60^{b,B}

$^{a-e}$: Means in a row without a common letter differ significantly ($p \leq 0.05$); $^{A-B}$: Means in a column for dried and frozen plants (for a particular method) without a common letter differ significantly ($p \leq 0.05$).

Alam *et al.* [28] reported a decrease in phenols content in the order leaf>fruit>stem and in flavonoid content in the order leaf>stem>fruit in *Solanum nigrum*. Our study indicates that the different parts of a plant species might accumulate various levels of polyphenols and flavonoids.

Antioxidant properties of 70% ethanolic extracts obtained by conventional extraction of dried and frozen plant materials were determined by two different methods - DPPH and FRAP. Our study revealed that the extracts with higher phenolic and flavonoid contents presented higher antioxidant activities (Table 2). Concerning the antioxidant activity quantified by DPPH in each of the study species, the lowest value was obtained for CL (90.57 mM TE/g weight for dried one and 26.74 mM TE/g weight for frozen one). The species with the highest values were dried TC (218.97 mM TE/g weight) and frozen HP (184.59 mM TE/g weight). Regarding the antioxidant activity quantified by FRAP, the results for highest activity were the same but this method established a difference of around

3.3 times higher antioxidant activity for dried TC compared to frozen HP.

In all the methods used, except for the total flavonoids and FRAP method for HP, the dried plants gave higher results for BAC. For this reason, in the next series of experiments, one dried (TC) and one frozen (HP) herb, showing the best results, were used in the next phase of the study.

Influence of ethanol concentration of biologically active substances

The extractability of antioxidants from two herbs – dried TC and frozen HP was studied at 0%, 30%, 50%, 70%, and 95% ethanol (Tables 3 and 4). As different concentrations of ethanol affect the physical properties of the solvent [29], this is likely to change the extraction yield of the various BAC in both studied herbs. Also, antioxidant compounds in a plant have different polarity and solubility [30] and extraction solvent properties may affect the extraction yield. The ethanol with the highest concentration (95%) had a negative effect on the extractability of BAC in the same studied methods. Generally, the extractability increased with increasing alcohol concentration, reaching a maximum at 70% ethanol. The amount of total phenolics in the ethanolic extracts ranged from 33.96 to 86.19 mg GAE/g weight for dried TC and from 18.54 to 29.29 mg GAE/g weight for frozen HP, as shown in Table 3.

Flavonoids (including flavones, flavanones, isoflavones, flavonols, and anthocyanidins), which are most commonly found and widely distributed in plant polyphenol compounds, were in the range of 10.63 to 26.43 mg QE/g weight in ethanolic extracts from dried TC and from 4.23 to 12.05 mg QE/g weight for ethanolic extracts from frozen HP, respectively, in this study. The highest total flavonoids and phenols values were determined in 70% ethanolic extracts of the two herbs but in dried TC the lowest content was in 95% ethanolic extract, while in frozen HP the lowest content was in a water solvent. Our results were consistent with the previous studies. For example, Sun *et al.* [31] who evaluated the effect of different ethanol/water solvents on the total phenols and flavonoids, observed their highest contents in 75% ethanol. According to those authors, water and 25% ethanol seemed to be less effective in extracting phenolics than ethanol/water extraction solvents with high concentrations. Kim *et al.* [32] suggested that a natural antioxidant extracted by a diluted ethanol solution has higher extraction yield compared to that extracted by pure ethanol.

DPPH radical scavenging activity and ferric reducing antioxidant power of ethanolic extracts from the two plants are shown in Table 4.

Table 4. Influence of the solvent concentration on the amount of bioactive compounds, determined by DPPH and FRAP methods.

Method	Herb	Ethanolic extracts				
		0 %	30 %	50 %	70 %	95 %
DPPH, mM TE/g weight	Dried TC	198.84±0.82^{d,A}	206.20±0.53^{c,A}	212.92±0.79^{b,A}	218.97±0.28^{a,A}	191.36±0.30^{e,A}
	Frozen HP	100.10±0.72^{e,B}	163.73±0.73^{d,B}	173.35±0.48^{c,B}	184.59±0.39^{a,B}	177.90±0.72^{b,B}
FRAP, µmol Fe^{2+}/g weight	Dried TC	697.88±1.32^{d,A}	889.07±2.47^{c,A}	1083.20±1.11^{b,A}	1110.77±0.85^{a,A}	438.98±1.96^{e,A}
	Frozen HP	102.56±1.30^{e,B}	254.83±0.52^{d,B}	266.27±1.12^{c,B}	339.23±0.52^{a,B}	289.73±0.89^{b,B}

$^{a-e}$: Means in a row without a common letter differ significantly ($p \leq 0.05$); $^{A-B}$: Means in a column for dried and frozen plants (for a particular method) without a common letter differ significantly ($p \leq 0.05$).

Table 5. Influence of the type of extraction on the amount of total flavonoids and phenols.

70 % C_2H_5OH extracts	Total flavonoids, mg QE/g weight		Total phenols, mg GAE/g weight	
	Conventional extraction	Ultrasonic extraction	Conventional extraction	Ultrasonic extraction
Dried TC	26.43±0.09^{a,A}	15.01±0.33^{b,A}	86.19±0.36^{a,A}	42.20±0.38^{b,A}
Frozen HP	12.05±0.42^{a,B}	6.97±0.31^{b,B}	29.29±0.44^{a,B}	13.89±0.30^{b,B}

$^{a-b}$: Means in a row for a particular method for determination of bioactive compounds and a herb without a common letter differ significantly ($p \leq 0.05$); $^{A-B}$: Means in a column for a particular method of extraction without a common letter differ significantly ($p \leq 0.05$).

Table 6. Influence of the type of extraction on the amount of bioactive compounds determined by DPPH and FRAP methods.

70 % C_2H_5OH extracts	DPPH method, mM TE/g weight		FRAP method, µmol Fe^{2+}/g weight	
	Conventional extraction	Ultrasonic extraction	Conventional extraction	Ultrasonic extraction
Dried TC	218.97±0.28^{a,A}	216.40±0.59^{b,A}	1110.77±0.85^{a,A}	604.83±0.29^{b,A}
Frozen HP	184.59±0.39^{a,B}	152.56±0.40^{b,B}	339.23±0.52^{a,B}	138.48±0.39^{b,B}

$^{a-b}$: Means in a row for a particular method for determination of bioactive compounds and a herb without a common letter differ significantly ($p \leq 0.05$); $^{A-B}$: Means in a column for a particular method of extraction without a common letter differ significantly ($p \leq 0.05$).

In extracts from dried TC, as ethanol concentration increased, DPPH and FRAP increased too. The 70% ethanolic extracts showed the highest results but with the next tested concentration (95%) the values decreased. The results obtained by us agreed with those in the literature [33], where an ethanol/water solvent was more efficient for extracting antioxidant compounds compared to pure solvents. The extracts from frozen HP showed different extractability in water/ethanolic solutions. The best results were achieved with 70% ethanol followed by 95% concentration. Lowering the concentration of ethanol in the range from 0 to 50% led to a reduction in the number of participants in the reaction BAC, determined by both DPPH and FRAP methods. Thus, the addition of 70% ethanolic extracts from the two herbs as natural antioxidants might be the most effective.

Influence of the method of extraction - conventional or ultrasound-assisted (CE or UAE) on the amount of biologically active substances

The total phenols and flavonoids contents were affected significantly ($p<0.05$) by the type of extraction process. As shown in Table 5, the CE gave higher results, while dried TC revealed significantly higher activity (86.19 mg GAE/ g weight and 26.43 mg QE/g weight) than frozen HP (29.29 mg GAE/ g weight and 12.05 mg QE/g weight). In general, the yield of BAC was about two times higher during CE. These results were not in agreement with those of a study by Um *et al.* [34]. They found that the yield using UAE was significantly higher than that obtained using CE. Probably the higher results obtained by the CE method were due to the longer extraction time and the higher temperature used in this method. The UAE was done at a lower temperature for a shorter time. According to [34], at a temperature of 40 – 50 °C the yield of total phenols in the extract decreased with increasing reaction time because of the oxidative degradation of phenolic compounds. Albu *et al.* [35] investigated the difference in the application of CE and UAE on the concentration of BAC in sage and they concluded that the content of BAC was by approximately 60% higher under the influence of ultrasound. The higher values of polyphenols extracted with UAE were also reported by Dent *et al.* [36]. Under optimal conditions (output power of 400 W, 11 min) using 30% ethanol these authors achieved a 20% higher yield of BAC than with CE (60°C, 30 min). To achieve better results in ultrasonic extraction, the conditions of the process (frequency (kHz), amplitude (%), applied cycle (%), nominal output power (W), and geometrical parameters of the sonotrode (length and diameter – mm) itself must be optimized in details in our further research.

DPPH and FRAP were also affected by the method of extraction (Table 6). Regardless of statistical differences, the quantity of BAC in 70% ethanolic extract obtained by the CE method was only by 1.17% (for dried TC) and by 17.35% (for frozen HP) higher than that achieved by the UAE method. The lower ultrasonic extraction results may be a consequence of the shorter extraction time (15 min). According to [37], the best time of sonication was 40 min with an ethanol concentration of 35%. To achieve better results, the time of sonication in our research must be also optimized.

CONCLUSIONS

The herbs processing (drying or freezing) had a significant effect on the amount of BAC. The different concentrations of ethanol affected the antioxidant extractability as 70% ethanolic solution showed the highest results. The yield using ultrasound-assisted extraction was significantly lower than that obtained using a conventional method of extraction. Of the studied five Bulgarian medical plants, *Thymus callieri* Borbás ex Velen. and *Hypericum perforatum* L. were the richest sources of secondary metabolites. Hence, their 70%

ethanolic extracts (as antioxidants) might be of interest for application in the food industry.

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