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Evaluation of Biologically Active Substance and Antioxidant Potential of Medicinal Plants Extracts for Food and Cosmetic Purposes

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Abstract:

The aim of the current study was to determine the biologically active substance in different extracts from five medicinal plants collected from the Rhodopes mountain: thyme (*Thymus vulgaris* L), St John's wort (*Hypericum perforatym* L), cotton thistle (*Onopordum acanthium*), common hawthorn (*Crataegus monogyna*) and common juniper (*Juniperus communis*) and to evaluate their antioxidant potential. Three types of extracts were prepared with water, 50% ethanol and 70 % ethanol. The content of total phenols, total flavonoids, total chlorophylls, total carotenoids and total fructans were analyzed. The antioxidant activity of extracts were evaluated by two assays DPPH and FRAP. From the obtained results fructans were found only in flowering heads extracts of cotton thistle (*Onopordum acanthium*) –0.8 g/100 g dw plant material. The highest values of total chlorophylls and total carotenoids were detected in 70 % ethanol extracts from common hawthorn flowers (1897.2 and 266.3 µg/g dry extract, respectively). The 50 % ethanol extracts of St John's wort and thyme were characterized as rich sources of total phenols – above 200 mg GAE/g dry extract, while 50 % ethanol extract from flowering heads extracts of cotton thistle demonstrated the highest total flavonoids content - 100 mg QE/g dry extract. All extracts obtained by St John's wort and thyme showed the highest antioxidant potential (from 2000 to 2800 mM TE/g dry extracts). Extracts form thyme, St John's wort, cotton thistle and flowers of common hawthorn dominated significantly above all investigated plants. These four medicinal plants showed their application in food and cosmetic formulas with potential beneficial healthy effect.

Key words: medicinal plants, water and ethanol extracts, antioxidants, pigments, fructans

INTRODUCTION

The interest in crude extracts of herbs and spices, and other plant materials rich in phenolics increase in the food industry because of their preventive action against oxidative degradation of lipids and improvement of the quality and nutritional value of food [1]. In Bulgarian traditional medicine many herbs find significant application in curing different disorders [2-5]. In the current research five medicinal plants attracted our attention for production of extracts for food and cosmetic purposes. The information about their medicinal uses is summarized in Table 1.

Hypericum perforatum L. (Hypericaceae), commonly known as St. John's wort, is a perennial plant widely distributed in Europe, Asia and North Africa. It possessed antioxidant, cytotoxic, antimicrobial, anticonvulsant, anxiolytic and wound healing due to its main bioactive compounds as hypericin and hyperforin. *H. perforatum* dry hydroalcoholic (30/70) extract was reported to contain 0.3% hypericin [6]. In Bulgarian phytoteraphy it is mostly applied as infusion, but in some countries it was used in form of infusion or maceration and decoction [2]. Moreover, *Hypericum perforatum* and its derived products include complex phytopharmaceutical ingredients and food supplements in teas, tinctures, juices and oil macerates [11]. Additionally, infusions, alcoholic tinctures and fluid extracts of the plant are used in the flavouring industry to prepare liqueurs, especially digestive and tonic bitters [12].

Common hawthorn (*Crataegus monogyna* Jacq.) is a medicinal plant used in folk medicine and a common edible plant for the preparation of different foodstuff. Hawthorn is an endemic member of the Rosaceae family widely distributed in Europe, Africa, and Asia as a shrub or small tree 5–10 m tall [9]. Its small dark-red fruit (commonly called haw), which ripens in mid-autumn, is used for different culinary purposes, such as the preparation of jellies, jams, marmalade, canned fruit, wine and liquors syrups [13] or even consumed raw [8, 9]. Scientific evidence has demonstrated that hawthorn fruit possesses potent antioxidant and free radical scavenging activities, due to the presence of different bioactive compounds, such as epicatechin, hyperoside and chlorogenic acid [8, 13, 14]. These compounds are reported to have many pharmacological effects, including neuroprotective, hepatoprotective, cardioprotective, nephroprotective, etc. [9]. Moreover, young leaves of common hawthorn are eaten in salads, seasoned with olive oil, lemon or vinegar [8, 9].

Thyme (*Thymus vulgaris* L.) is a medicinal herb with antispasmodic, expectorant, antitusive, antiseptic, antimicrobial and antioxidant properties used in cosmetic, culinary dishes and medical purposes [5, 7] It is used as expectorant, asthma, whooping-cough, insomnia, headache, anemia, gastritis, colics and diarrhea [6]. In Bulgaria it is applied as infusion [2, 5], but also in culinary as spice, especially for meat [2].

Table 1: Medicinal plants commonly used in traditional medicine

Medicinal plant	Family	Plant material	Medicinal use	Ref.
St. John's wort (*Hypericum perforatum*)	Hypericaceae	Aerial parts	Anti-inflammatory, astringent, antibacterial, diuretic, ulcer, colitis, gastritis, treatment of depressive disorders, antiviral (only against *Herpes simplex*)	2, 4, 6, 7
Hawthorn (*Crataegus monogyna)*	Rosaceae	Flowers, leaves Fruits	Promotes capillary formation, cardiovascular diseases, ischemia, atherosclerosis, arthritis, bronchitis, pneumonia, cellulite control, obesity and menopause disturbances, kidney stones	2,4,8, 9
Thyme (*Thymus vulgaris*)	Labiatae	Aerial parts	Expectorant, spasmolytic, antibacterial, antitusive, asthma, emphysema, whooping-cough, diseases of respiratory tract	2,4,5
Cotton thistle (*Onopordum acanthium* L.)	Compositae (Asteraceae)	Flowers	Cardiovascular diseases, diuretic, urogenital diseases, stimulant of gastric secretion, anticarcinoma	3, 10
Common juniper (*Juniperus communis* L.)	Cupressaceae	cones	Diuretic, balsamic,, antipyretic,antidiarrhoeic, muscular pains	2,4

Onopordum acanthium L. (known as cotton thistle or Scotch thistle) is a biannual herbaceous prickly plant, considered also as noxious weed, growing worldwide at wastelands, pastures, riverbanks and well drained sandy or gravelly soils. It is 100-300 cm in height, highly branched with broad leaves with longitudinal spines and red-purple flowers. It was reported that the flower basket, receptacle and roots contains inulin [10, 15-17]. In Bulgarian folk medicine cotton thistle extracts were used as restorative, increase excitability of nervous system. The plant has also antimicrobial and antibiotic action. Cotton thistle contains substances with antioxidant properties: tocopherols, flavonoids, phenolic acids [10]. *Onopordium acanthium* are edible plant in Italy [17]. However, not detail analysis of its inulin content was reported.

Common junipers (*Juniperus communis* L.) is a dioecious aromatic evergreen shrub belonging to the Cupressaceae family (genus *Juniperus*) with simple stiff green leaves arranged in whorls of three and berry-like fruits – cones. The fruits have straight peduncles and a globose to ovoid shape with elongated tubercles, which act as reservoirs of volatile oils. Common juniper berries take 2 years to ripen; thus, the green berries of the first year and the blue berries of the second year occur on the same plant. The berries and leaves are widely used in many countries as diuretics, antiseptics, and digestives in knowledgebased medicine systems and as a flavor to aromatize certain alcoholic beverages. The essential oils, infusions, decoctions, and alcoholic extracts exhibit diverse pharmacological features, including anti-inflammatory, hypoglycemic [18], antioxidant [19], antimicrobial, antifungal and memory-enhancing effects [20]. Juniperus cones are used as a raw material as a flavor to aromatize certain alcoholic beverages; in the perfumery, cosmetics, and pharmaceutical industries, because of their characteristic rich, fresh coniferous odor with a faint "fruitiness" and balsamic aroma; and as a spice, because of their slightly bitter taste [2, 21]. In Bulgaria common junipers cones were used as infusion, decoction, pseudofruits maceration in olive oil, while in Italy tincture in wine was prepare [2].

Moreover, some of them as thyme and common junipers find application for culinary purposes and preparation of herbal infusions and alcoholic beverage [2, 5].

The information about bioactive compounds extracted from Bulgarian medicinal plants is not detailed. Most of the extracts were obtained with acetone and methanol that are not applicable for food purposes. Therefore, solvent as hydroethanol and water attracted our attention for production of dry extracts with potential food application.

The aim of the current study was to determine the biologically active substance in water and hydroethanolic extracts from five medicinal plants collected from the Rhodopes mountain: thyme (*Thymus vulgaris* L), St John's wort (*Hypericum perforatym* L), cotton thistle (*Onopordum acanthium*), common hawthorn (*Crataegus monogyna*) and common juniper (*Juniperus communis* L.) and to evaluate their antioxidant potential.

MATERIALS AND METHODS

Materials

Five different medicinal plants were used for the current investigation: the aerial parts from thyme (*Thymus vulgaris* L), St John's wort (*Hypericum perforatym* L), flower heads of cotton thistle (*Onopordum acanthium*), flowers and fruits from common hawthorn (*Crataegus monogyna*), cones from common juniper (*Juniperus communis* L.). The plant material was collected from the region of Western Rhodope Mountains (Dospat, Bulgaria). The aerial parts and flowers of all plants were harvest during the flowering period May-September 2017. The fruits from common hawthorn and common juniper were collected in their mature stage during September-October 2017. The medicinal plants were dried at 25°C in dark and well ventilated places. The air dried plant materials were finely ground in the laboratory homogenizer.

Preparation of herbal extracts

Water extracts

Dry and ground plant materials (50 g) were poured with 1000 mL boiling water and after 30 min the solution was filtered through filtering paper. The obtained extract was stored at 4°C until further analysis.

Ethanol extracts

Two ethanol extracts (50 % v/v and 70 % v/v) were prepared in solid to solvent ratio (1:20). The prepared samples were stored at 20 °C in darkness for 14 days. After that obtained extracts were filtered and analyzed.

Total chlorophylls and carotenoids

Total chlorophylls and carotenoids were spectrophotometrically determinated in ethanol extracts and calculated according to Lichtenthaler and Wellburn [22].

Total phenolic contents

Total phenolic content was measured using a Folin-Ciocalteu reagent. Briefly, 1 mL Folin-Ciocalteu reagent diluted five times was mixed with 0.2 mL sample and 0.8 mL 7.5% Na_2CO_3. The reaction was performed for 20 min at room temperature in darkness. Then the absorbance was measured at 765 nm against blank. The results were expressed as mg equivalent of gallic acid (GAE) per g dry extract, according to calibration curve [23].

The total flavonoids content

The total flavonoids content was analyzed by $Al(NO_3)_3$ reagents [24]. The absorbance was measured at 415 nm against blank. The results were presented as mg equivalents quercetin (QE) per g dry extract according to the calibration curve with quercetin as a standard.

The DPPH radical scavenging ability

To conduct the assay, 0.15 mL from extract was mixed with 2.85 mL freshly prepared 0.1 mol solution of DPPH in methanol. The sample was incubated for 15 min at 37 °C in darkness. The reduction of absorbance was measured at 517 nm in comparison to the blank containing methanol and % inhibition were calculated [23].

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to Benzie and Strain [25] with slight modification. The FRAP reagent was freshly prepared by mixing 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 $FeCl_3$x6H2O in d. H_2O. The reaction was started by mixing 3.0 mL FRAP reagent 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 methanol. Antioxidant activity was expressed as mM Trolox® equivalents (TE) per g dry extract [23].

Fructan content

The fructans content in water extracts expressed as fructose equivalent was defined spectrophotometrically by resorcinol-thiourea reagent [5]. Hundred microliters extract were mixed with 100 µL resorcinol (1% ethanol solution), 100 µL thiourea (0.1% ethanol solution), 800 µL 95 % ethanol and 900 µL HCl and heated 8 min at 80°C, cooled and filled with water until 10 mL. Then the absorbance was measure at 480 nm against a blank sample [26].

RESULTS AND DISCUSSION

Yields of medicinal plant extract

The yields of medicinal plant extracts were summarized (Table 2).

The highest values of dry extracts were obtained from 50 % ethanol for most of the plants, with exception of water extract of common juniper (*Juniperus communis* L.) cones. The lowest yields were obtained from flower heads of cotton thistle 1.57 g/100 g dw plant (0.79 g/100 g dry extract).

Total phenols and flavonoids content

There is growing interest in natural antioxidants, namely phenols present in plants and herbs that can help prevent oxidative processes [27, 28]. According to Scalbert et al., [29] polyphenols are the most abundant antioxidants in the human diet, as their total dietary intake could be as high than 1 g/day, which is much higher than that of all other classes of phytochemicals and known dietary antioxidants.

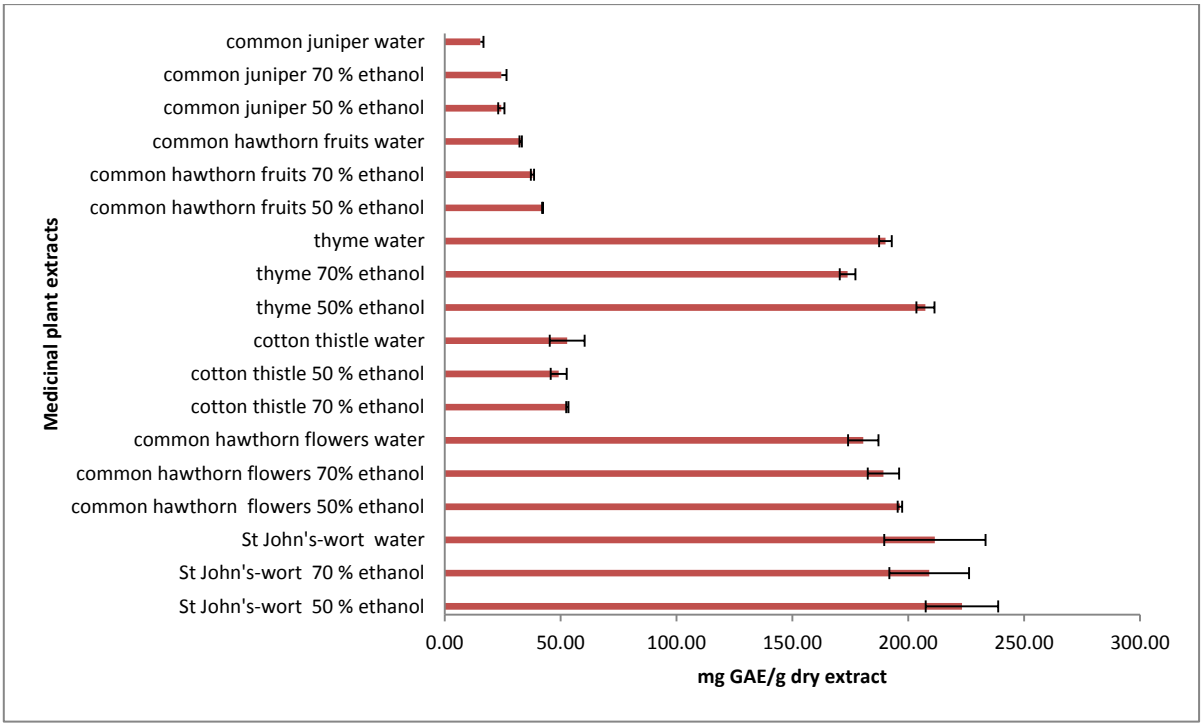

Figure 1: Total phenols content in medicinal plant extracts

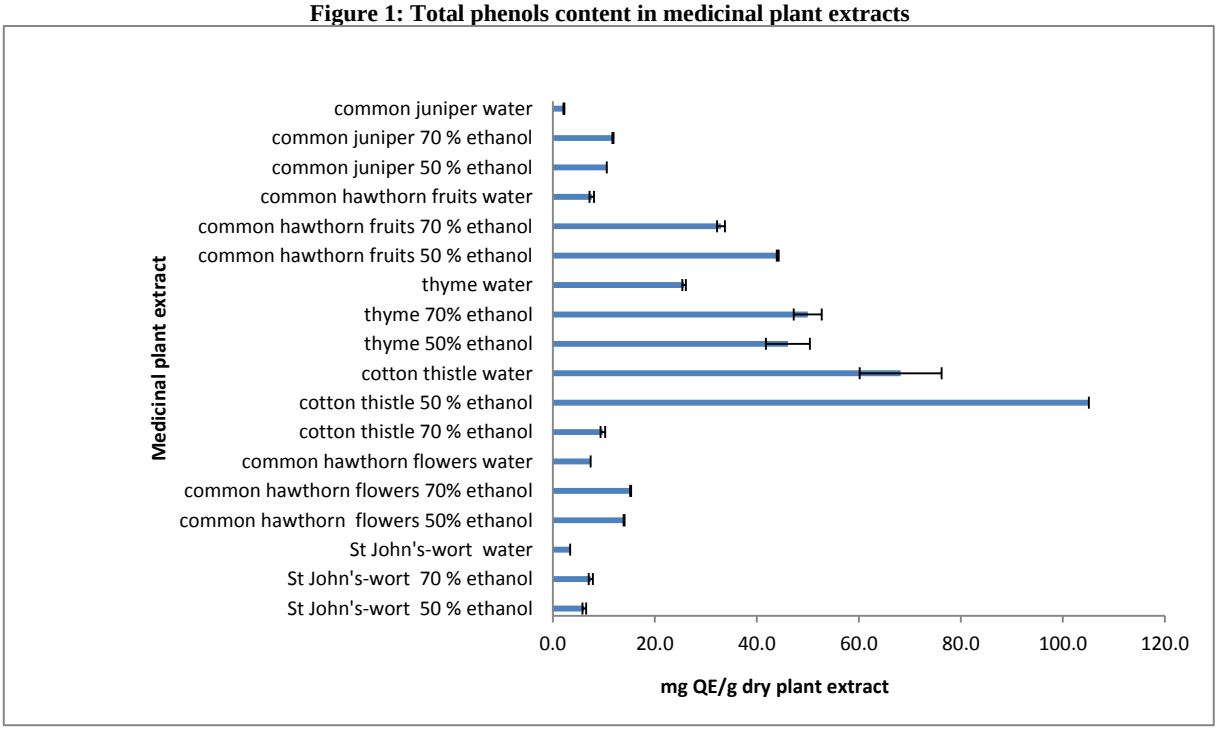

Figure 2: Total flavonoids content in dry medicinal plant extracts

Table 2: Yields of medicinal plant extracts

Sample	Plant material	Solvent	Yield, g/100 g extract	Yield of extract, g/100 g dry medicinal plant
St John's wort (*Hypericum perforatym* L.)	Aerial part	50% Ethanol	1.69	3.38
		70% Ethanol	1.57	3.14
		Water	1.52	6.07
Common hawthorn (*Crataegus monogyna*)	flowers	50% Ethanol	1.75	6.99
		70% Ethanol	1.58	6.30
		Water	1.20	4.78
	fruits	50% Ethanol	1.03	2.07
		70% Ethanol	0.81	1.62
		Water	1.05	4.21
Cotton thistle (*Onopordum acanthium*)	flower heads	50% Ethanol	0.83	1.67
		70% Ethanol	0.79	1.57
		Water	0.57	2.28
Thyme (*Thymus vulgaris* L.)	aerial part	50% Ethanol	1.16	2.31
		70% Ethanol	1.32	2.65
		Water	1.24	4.95
Common juniper (*Juniperus communis* L.)	cones	50% Ethanol	2.09	4.18
		70% Ethanol	2.23	4.46
		Water	1.75	7.01

Table 3: Concentrations of total chlorophylls (Chl a+b) and of total carotenoids in 70 % ethanol extracts from medicinal extracts µg/g dry extract

Sample	Chl a	Chl b	Total Chlorophylls	Total Carotenoids
Cotton thistle	209.2	143.2	353.0	192.2
Common hawthorn flowers	1331.2	566.4	1897.2	266.3
Common hawthorn fruits	92.3	94.2	186.5	263.2
Thyme	1115.1	466.4	1581.9	n.d.
Common juniper	n.d.	n.d.	n.d.	88

n.d. – not detected

Table 4: Antioxidant activity of medicinal plant extract, mM Trolox equivalents per g dry plant extract

Sample	Plant material	Solvent	DPPH	FRAP
St John's wort (*Hypericum perforatym* L.)	aerial part	50% Ethanol	2137.1±1.7	1574.9±2.9
		70% Ethanol	2135.9±0.1	1251.5±1.2
		Water	2196.4±3.3	1683.6±0.8
Common hawthorn (*Crataegus monogyna*)	flowers	50% Ethanol	1955.9±2.8	1989.8±1.1
		70% Ethanol	1786.6±0.1	1879.3±0.5
		Water	1707.7±0.2	1333.9±0.2
	fruits	50% Ethanol	384.3±8.2	365.2±0.5
		70% Ethanol	381.6±1.7	332.9±0.6
		Water	161.6±0.9	315.5±0.6
Cotton thistle (*Onopordum acanthium*)	flower heads	50% Ethanol	245.0±1.9	374.5±0.1
		70% Ethanol	298.3±0.1	353.1±0.1
		Water	267.3±2.3	774.7±0.3
Thyme (*Thymus vulgaris* L.)	aerial part	50% Ethanol	2001.7±0.6	1931.7±0.2
		70% Ethanol	1037.9±3.7	2225.4±0.5
		Water	2604.4±0.8	2830.9±0.7
Common juniper (*Juniperus communis* L.)	cones	50% Ethanol	183.8±1.6	241.1±0.7
		70% Ethanol	222.2±1.0	229.7±0.2
		Water	102.7±0.8	246.3±0.4

From all investigated medicinal plant extracts 50 % ethanol contained the highest total phenols (Figure 1). Total phenols dominated in St John's wort, thyme extracts and hawthorn flower extracts (180 - 220 mg GAE/g dry extract). Total phenols in thyme extracts were in range from 190-173 mg GAE/g extract are comparable with reports of Turkish authors for water and ethanol extracts - 256 and 158 µg/mg dry extract [30]. The fruits of common hawthorn, cones of common juniper and flowering heads of common thistles contained more flavonoids than total phenols in their extracts (Figure 1 and Figure 2), while St John's wort extracts demonstrated higher total phenols than flavonoids. It was reported that *Hypericum perforatum* extracts contain several classes of plant phenolic with biological activity, including antidepressant phloroglucinols (hyperforin and its derivative adhyperforin), antiviral, antibacterial and photosensitizing naphthodianthrones (hypericin and pseudohypericin, as well as their precursors - protohypericin and protopseudohypericin), antioxidant flavonoids (mostly quercetin and kaempferol glycosides and aglycones, as well as bioflavonoids), and phenolic acids (mostly isomeric caffeoylquinic acids) [31]. Common juniper fruits extracts demonstrated the lowest values of total phenols (from 15 to 24 mg GAE/g dry extract). The total phenols

in fruits of common hawthorn extracts were in range from 43 to 32 mg GAE/g dry extract. Our data were in good agreement with Tadić et al., [32] who found 35.4 mg GAE/g in hawthorn berries ethanolic extracts (according to European Pharmacopoeia 6.0, hawthorn berries consist of the dried pomes of *Crataegus monogyna* Jacq. and *Crataegus oxyacantha* L. or their mixture) and lower than values for ripen fruits - 274 mg GAE/ g extract [8]. Our findings for higher total phenolic content in *Crataegus monogyna* confirmed observation that phenolics were the major antioxidant components in common hawthorn (247.03–701.65 mg GAE/g of extract) [8]. In our study, total phenols found in flowers of common hawthorn were 180-190 mg GAE/g dry extract that is in good agreement with findings of Borros et al. [8]. The high values of total phenols could be explained with presence of chlorogenic acid, caffeoylquinic acid and 3- and 4-O-caffeoyl derivatives [33]. Contrary to some report [3] in our study water extract of cotton thistle demonstrated higher phenolic compounds than common hawthorn.

From the obtained extracts 50 % ethanol from cotton thistle showed the highest total flavonoids content in dry medicinal plant extracts 105 mg QE/g. In general, hydroalcoholic extracts from investigated medicinal plants contained more total flavonoids content. The content of these bioactive substances lower in the following order cotton thistle>thyme>common hawthorn fruits> common hawthorn flowers>common juniper>, St John's wort. The high flavonoids content in infusions of cotton thistle was reported previously 121 μg/mL water extract [3]. In addition, Angelov et al. [10] also reported that cotton thistle contained substances with antioxidant properties: tocopherols, flavonoids and phenolic acids.

Among flavonoids quercetin isomarnetine and apigenins dominated in cotton thistle [34]. The levels of total flavonoids in thyme (25 -50 mg QE/g dry extract) were also high and were near to a report [30]. The flavonoids content in water and ethanol extracts (44-37 μg/mg dry extract) were near to the concentrations described by Spiridon et al., [7] 40 % methanol extracts (30 mg rutin/g). In our study, thyme and St. John's wort extracts showed higher results than Romanian sample extracts [7], only St. John's wort showed lowers flavonoids content (Figure 2). It was demonstrated that *H. perforatum* ethanolic extracts contain many phenolic compounds, namely flavonoids and phenolic acids, suggesting that they could have important antioxidant properties [31].

Natural pigments

The natural pigments responsible for green and yellow color of extracts were presented in Table 3.

The highest content of chlorophylls and carotenoids was detected in common hawthorn flowers -1897 μg/g and 266 μg/g dry extracts. Both common hawthorn flowers and fruits were evaluated as rich source of carotenoids. In addition, Borros et al., [8] reported that β-carotene levels in fruits and flowers were 54 mg/100 g and 16 mg/100g respectively. In thyme extracts total carotenoids were not detected, while in common juniper chlorophylls were not found.

In thyme herb El-Qudah [35] showed that the content of chlorophyll a is 2.82 mg/g dry weight and chlorophyll b is 1.31 mg/g dry weight. In our case also chlorophyll a dominated over chlorophyll b. Similar results were reported [35, 36] for loss of some important dietary components, as carotenoids were reported such as studies.

Antioxidant activity

The highest antioxidant activity demonstrated all extracts obtained from St John's wort, followed by thyme and common hawthorn flowers (Table 4).

It is probably due to high phenolic and flavonoids content [31]. The antioxidant activity of *H. perforatum* extracts is well known and is to be expected due to a high content of phenolic compounds [33]. In our study the radical scavenging properties of hawthorn extracts from fruits and flowers evaluated by DPPH method showed highest results than previous report of Borros et al., [8]. It was also demonstrated that ethanolic extract of Crataegus monogyna Jacq., showed promising antioxidant activity by ABTS and CUPRAC methods [37]. Therefore, these three medicinal plants can be considered as the best sources of antioxidants for future application in food formula.

The correlation between total antioxidant capacities obtained from DPPH and FRAP methods and total phenolic contents, total flavonoids, total carotenoids in extracts were presented in Table 5.

The results showed positive linear correlations between total antioxidant activities, total phenolic contents, total flavonoids and total carotenoids content (coefficient of correlation r = 0.90 and 0.92 for FRAP and DPPH values, respectively). Therefore, mainly total phenols and total carotenoids content in medicinal plant extracts provided antioxidant activity. According to the current study, phenolic and flavonoid compounds have antioxidant, radical scavenging, and metal chelating properties. Several studies have indicated a positive correlation between phenolic contents and the ferric reducing powers of plant extracts [3, 5, 38].

Table 5: Correlation coefficient (r) between total phenolic content, total flavonoids, total carotenoids and antioxidant activities (DPPH and FRAP)

	DPPH	FRAP
Total phenols	0.9189	0.9026
Total flavonoids	0.8074	0.6744
Total carotenoids	0.9444	0.9287

Table 6: Total fructans in extracts from flower heads of *Onopordum acanthium*

Extract	Total fructans, μg/g dry extract	Total fructans, g/100 g dw plant material
70% Ethanol	55±9	0.68±0.11
50% Ethanol	68±9	0.83±0.11
Water	71±15	0.84±0.17

Fructan content

The results for total fructan content in extracts obtained from flower heads of *Onopordum acanthium* were summarized (Table 6). Water extracts showed the highest fructan content 71 μg/mL fructose equivalents. The presence of inulin in receptacles (flowering heads) of *Onopordum acanthium* was reported [16, 17], but without any quantities. Petkova and Mihaylova [15] reported the fructan content in *Onopordum tauricum* 7.90±0.34 g/100 g and inulin 4.5 g/100 g. However, in current research 0.84±0.17 g/100 g total fructan was observed in water infusions. The lower level of fructans in could be explained with extraction procedure, vegetative stage and time of harvesting, especially during flowering. This is the first report for total fructan content in extracts of flower heads of *Onopordum acanthium.*

CONCLUSION

The current research enriched the information for application of tinctures and infusions of Bulgarian medicinal plants. From all investigated plants extracts from thyme, St John's wort, cotton thistle and flowers of common hawthorn were evaluated as rich sources of polyphenols and carotenoids, as in addition they demonstrated high antioxidant potentials. In addition, the water extracts from the flowering heads of cotton thistle were evaluated as source of dietary fiber (fructans) with potential prebiotic activity. Therefore, this extracts can be successfully used in food and cosmetic products for improvement of consumer health status.

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Conflicts of Interests

All authors declare no conflicts of interests.

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