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Antioxidants and carbohydrate content in infusions and microwave extracts from eight medicinal plants

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ABSTRACT

The aims of the current research were to evaluate and to compare the antioxidant activity and the content of total phenolic, flavonoids and fructan content in infusions and microwave-assisted extracts from eight herbs: rosehip (*Rosa canina* L.), peppermint (*Mentha piperita* L.), thyme (*Thymus vulgaris* L.), coltsfoot (*Tussilago farfara* L.), dandelion (*Taraxacum officinale* Web.), elecampane (*Inula helenium* L.), great burdock (*Arctium lappa* L.) and echinacea (*Echinacea purpurea* Moench.). The total phenolic contents, total flavonoids and antioxidant activity were evaluated by Folin–Ciocalteau, $Al(NO_3)_3$ methods and DPPH assays. The total fructans and sugars were determined by resorcinol and HPLC-RID methods. Infusion and microwave-assisted extraction (MAE) were evaluated as efficient methods for preparation of herbal drinks. The highest values of biologically active substances and antioxidant activity were found in MAE herbal extracts. Coltsfoot, thyme and great burdock extracts demonstrated the highest content of total phenolic and flavonoids. Coltsfoot and thyme extracts showed the highest antioxidant activity (334 mM TEg^{-1} and 287 mM TEg^{-1}dw, respectively). Fructans were found in coltsfoot (4.6%) and echinacea (3.9 %) leaves, in great burdock, dandelion and elecampane roots – 14 %, 34% and 44% dw, respectively. Elecampane and dandelion roots were evaluated as sources of prebiotic inulin.

INTRODUCTION

Medicinal plants present a rich source of bioactive compounds and antioxidants with significant importance in human health. Herbs find application in many fields, including medicine, nutrition, flavouring, beverages, dyeing, repellents, fragrances, cosmetics (Djeridane *et al*., 2006). Crude extracts of herbs and spices, rich in phenolics are of increasing interest in the food industry because they improve the quality and nutritional value of food (Wojdyło *et al*., 2007). In addition, consumption of herbal infusions increased all over the world due to the beneficial and preventive effect over human body.

The quality of herbal drug is also depend on many factors like environment, collection method, cultivation, harvest, post-harvest processing, transport and storage practices (Kunle *et al*., 2012). Therefore, the great variety of herbs used for preparation of herbal infusions requires monitoring and analysis of final beverages. Rosehip, peppermint, thyme infusions are the mostly consumed herbal infusions. In Bulgarian folk medicine rosehip, peppermint, thyme, coltsfoot and elecampane are traditionally used for curing respiratory disorders (Pamukov and Ahtardjiev, 1990). Peppermint leavers, great burdock and dandelion roots enhance digestion and kidney function (Pamukov and Ahtardjiev, 1990, Pawlaczyk *et al*., 2009, Krachanova *et al*., 2010). The detailed information is summarized in Table 1. Many studies were dedicated to biological activity of rosehip fruits (Ognyanov *et al*., 2014, Mihaylova *et al*., 2015, Taneva *et al*., 2016), thyme (*Thymus vulgaris* L.) and peppermint (Ivanova *et al*., 2005, Kolaneva *et al*., 2016). Common thyme is an aromatic plant that belongs to *Lamiaceae* family. Thyme has been employed in medicine, food, agriculture, veterinary and pest control (Ghahfarokhi*et al*., 2016).

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Table 1: Application of medicinal plants in phytotherapy and traditional medicine (Pamukov and Ahtardjiev, 1990; Barnes *et al*., 2005, Pawlaczyk *et al*., 2009, Krachanova *et al*., 2010).

Common name	Family	Vegetal part	Traditional use
Rosehip*Rosa canina* L.	Rosacea	fruits	In vitamin C- deficiency diseases, anti-flu, diuretic, cardiotonic
Peppermint *Mentha piperita* L.	Labiatae	leaves	Spasmolytic, antiseptic, gastric disorders, indigestion neuralgia, myalgia, antivomiting
Thyme *Thymus vulgaris*L.	Labiatae	Aerial parts	Expectorant, antitusive, asthma, whooping-cough, insomnia, headache, anemia, gastritis, colics, diarrhea
Coltsfoots *Tussilago farfara* L.	Asteraceae	leaves	Gastrointestinal and urinary ailments, asthma, bronchitis, blood purification, rheumatism and skin irritations
Dandelion *Taraxacum officinale* Web.	Asteraceae	roots	Cholagogue, diuretic, arthritis, antihepatic, antidiabetic, prevention of renal gravel and loss of appetite
Elecampane *Inula helenium* L.	Asteraceae	roots	Cholagogue, diuretic, antihelmintic, expectorant, wounds healing
Great burdock *Arctium lappa* L.	Asteraceae	roots	Diuretic, rheumatism, gastritis, gout, throat pain, arthritis, rashes
Echinacea *Echinacea purpurea* Moench	Asteraceae	leaves	Urinary diseases, curing syphilis and septic wounds, ‘‘anti-toxin’’ for snakebites

Its anti-MRSA activity was also demonstrated (Khadir *et al*., 2013). Herbal infusion of peppermint (*Mentha piperita*) leaves helps in curing indigestion, jaundice, liver diseases and gastric disorders. It was reported that the flavonoids and phenolic acids in *Mentha piperita* exhibited antioxidant, cholegogic and anti-allergic activities (Ivanova *et al.*, 2005, Krachanova *et al*., 2010).

Coltsfoot, dandelion, elecampane, great burdock and echinacea are medicinal plants from Asteraceae family. Coltsfoot has been used in folk remedies as herbal tea. The coltsfoot’s pharmacological properties includes also antibacterial, anti-inflammatory, antioxidant and also some neuroprotective activities (Nedelcheva *et al*., 2015).

In Bulgarian traditional herbal medicine dandelion is used mainly for treatment of digestive diseases. Its roots are a rich source of polysaccharides, mainly inulin-type fructans and smaller amounts of pectin, resin, and mucilage (Schütz *et al*., 2006, Petkova *et al*., 2015).

Arctium lappa L. possesses antioxidants and antidiabetic, anti-inflammatory effects, anti-cancers, anti-allergic effect, anti-ulcer effect, antitubercular activity, anti-acne, anti-sterility, anti-ulcerogenic, ulcerative colitis, angiostrongyliasis effect, gastroprotective activity, hepatoprotective effects, anti-aging effect, anti-austeric activity and cytotoxicity effect (Miraj and Keivani, 2016). Elecampane roots are used in folk medicine as infusions and tinctures for curing asthma, bronchitis, lung disorders, tuberculosis, indigestion, chronic enterogastritis, for wound healing, treatment of emesis and diarrhea. Its pharmacological properties include also anti-inflammatory, antioxidant, anticoagulant, anti-tumor, antimicrobial and insecticidal activities (Konishi *et al*., 2002, Stojakowska *et al*., 2006, Huo *et al*., 2008). *Echinacea purpurea* possesses important immuno-stimulatory and anti-inflammatory properties. The plant extracts have shown antioxidative, antibacterial, antiviral, and antifungal properties and are used for treating common cold and respiratory and urinary diseases (Barnes *et al*., 2005, Manayi *et al*., 2015). The aerial parts of echinacea contain alkamides, caffeic acid esters, polysaccharides and polyacetylenes (Barnes *et al*., 2005).

Most of the researches presented data about antioxidant activity and bioactive compounds obtained after 80 % methanol, ethanol or acetone extraction (Ivanova *et al*., 2005; Krachanova *et al*., 2010, Petkova *et al*., 2017). These values did not present correctly the content because of its toxicity or limited used as a tincture. The quality of herbal drug is also depended on many factors like environment, collection method, cultivation, harvest, post harvest processing,transport, storage practices,technique, solvents, time and extraction temperature.

The most commonly used herbal extracts for oral intake are some herbal infusions. Nowadays, people applied microwave irradiation for preparation of herbal extracts in domestic ovens. Therefore, the comparison of extraction of phytochemical compounds obtained by MAE and infusion presents interest for the consumers.

The aim of the current study was to evaluate the content of biologically active compounds (phenolic, flavonoids and fructans) and antioxidant activity of the water extracts form eight medicinal plants rosehip fruits, elecampane roots, dandelion roots, great burdock roots, thyme, coltsfoot, peppermint leaves obtained by infusion and microwave-assisted extraction.

MATERIALS AND METHODS

Plant material

The plant material was purchased from the local drugstore in Plovdiv, Bulgaria.The dried and coarsely ground roots of burdock (Radix Arctii Lappae), dandelion(Radix Traxaci), elecampane (Radix Inulae), echinacea tea and peppermint were produced by ALIN Company, Alino village, Samokov, Bulgaria. Coltsfoots leaves (Farfara folium) were produced by Thalloderma Ltd., Varna, Bulgaria. Rose hip fruits and thyme leaves were produced by Bioprogramme Ltd, Bulgaria. The plant material was finely ground, kept in closely tight containers and used for further analysis.

Preparation of the extract

The infusions were prepared as it was described by producer of herbal products. In brief a paper bag (1 g) of rosehip, thyme, mint, echinacea (2 g) was poured with 250 ml boiling water and was left for 3-5 min. Coltsfoots leaves (2 g) was pour with 200 ml boiling water and was left for 1 hour. Dandelion and burdock roots were infused with 500 ml water for 10 min, while 7 g elecampane roots were left with boiling water for 5 min. Microwave-assisted extraction (MAE) was performed in a microwave oven CROWN with 700 W power and frequency 2450

MHz for 5 min at maximum power 700 W. The extraction was performed in the same water to solid ratio as described above.

Determination of total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu's reagent according to Stintzing *et al*. (2005) with some modification. Basically, 0.2 ml herbal extract was mixed with 1 ml Folin–Ciocalteu reagent diluted five times and 0.8 mL 7.5% $Na_2CO_3$was added. After 20 min, the absorption was measured at 765 nm against a blank sample. The results were expressed in mg equivalent of gallic acid (GAE) g^{-1} dw, according to a calibration curve; built in range of 0.02 - 0.10 mg.

Determination of total flavonoids content

The total flavonoids content was determinated by $Al(NO_3)_3$ reagent. The absorbance was measured at 415 nm. The results were presented as mg equivalents quercetin (QE) per g dw according to the calibration curve with quercetin as a standard (Ivanov *et al*., 2014).

The DPPH radical-scavenging ability

Each herbal extract (0.15 ml) was mixed with 2.85 ml freshly prepared 0.1mM solution of DPPH in methanol. The reduction of absorbance at 517 nm was measured by spectrophotometer in comparison to the blank containing methanol. A standard curve was built with Trolox. The results were expressed in mM Trolox® equivalents (TE) g^{-1} dw (Ivanov *et al*., 2014).

Spectrophotometric determination of total fructans

The fructans content in water extracts expressed as fructose equivalent was defined spectrophotometrically by resorcinol-thiourea reagent (Petkova and Denev, 2012). 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.

HPLC-RID analysis of sugars, fructooligosacharides and inulin

The sugars, FOSs and inulin content extracts were analyzed by HPLC-RID method. Chromatographic separationwas performed on HPLC Elite Chrome Hitachi, coupled refractive index detector Chromaster 5450 and a column Shodex® Sugar SP0810(300 mm × 8.0 mm i.d.) with $Pb^{2+}$and a guard column Shodex SP - G (5 µm, 6 × 50 mm) operating at 85°C. The mobile phase was distilled water with a flow rate 1.0 mL min^{-1} and the injection volume was 20 µL (Petkova *et al*., 2014).

Statistical analysis

The presented results were average from two independent experiments carried out in triplicates. The data were expressed as mean ± SD and statistically analyzed using MS Excel software.

RESULTS AND DISCUSSION

In this study the infusion and microwave water extracts from eight medicinal plants from Asteraceae, Rosacea and Labiatae family were investigated for content of total phenolic, total flavonoids, total fructans and antioxidant activity.

Total phenolic content

The total phenolic content varied in infusion and microwave water extracts from 5.3 to 47.1 mg GAEg^{-1}dw (Table 2). The highest content was found in thyme extracts, while the lowest TPC was detected in dandelion roots infusions (5.3 mg GAE g^{-1}dw). Coltsfoots (42.7 mg GAE g^{-1}dw), peppermint (37.7 mg GAE g^{-1} dw) and roots of great burdock (34.8 mg GAE g^{-1} dw) also demonstrated higher levels of phenolic compounds in comparison with other investigated herbal extracts. Two other medicinal plants with significant amount of TFC were rosehip fruits and Echinacea leaves extracts. The total phenolic content in infusions and microwave extracts decreased in the following order: thyme>coltsfoots>peppermint>great burdock>rosehip> Echinacea > elecampane>dandelion.

Table 2: Total phenolic (mg GAE g^{-1} dw) and total flavonoid content (mg QE g^{-1} dw) in medicinal plant.

Plant	Infusion		MAE	
	TPC	TF	TPC	TF
Rosehip (*Rosa canina* L.)	26.8±0.1	2.9±0.4	30.1±0.3	3.0±0.2
Thyme (*Thymus vulgaris* L.)	41.4±0.6	6.3±0.3	47.1±0.2	9.5±0.4
Peppermint (*Mentha piperita* L.)	35.1±1.2	12.5±0.4	37.7±0.5	12.8±0.5
Coltsfoots (*Tussilago farfara* L.)	38.5±1.1	13.1±0.6	42.7±0.3	15.0±1.1
Dandelion (*Taraxacum officinale* Web.)	5.3±1.0	2.0±0.5	6.0±0.6	2.2±0.4
Elecampane (*Inula helenium* L.)	9.4±0.1	3.7±0.3	10.7±0.1	4.2±0.2
Great burdock (*Arctium lappa* L.)	34.8±0.2	13.1±0.4	32.4±1.4	15.6±1.4
Echinacea (*Echinacea purpurea*)	26.7±0.4	4.1±0.7	24.1±1.1	7.4±0.6

Total flavonoids content

Total flavonoids present in the range from 2.0 to 15.6 mg QEg^{-1} dw. The highest content of flavonoids was found in infusions and extracts of great burdock roots: 13.1 and 15.6 mg QEg^{-1} dw, respectively. Coltsfoots and peppermint leaves extracts demonstrated also high content of the total flavonoids content. In contrast, the extracts from dandelion and elecampane roots contained the lowest levels -4.2 and 2.2 mg QEg^{-1} dw. Our results were in an agreement with previous reports that the most common flavonoids were mainly distributed in representatives from Labiatae and Compositae (Asteracea) (Wojdyło *et al*., 2007, Kolaneva *et al*., 2016, Petkova *et al*., 2017) The results from our study were near or less than some previous reports for total phenolic content in *Rosa canina* L. fruits from Turkish and Bulgarian origins (Demir and Ozcan, 2001; Pirone*et al*., 2007; Ghazghazi *et al*., 2010, Ognyanov *et al*., 2014; Mihaylova *et al*., 2015) that could be explained by extraction procedure and used solvent. In general, the highest total phenolics and flavonoids content were established in water extracts obtained after microwave irradiation for 5 min. The difference between infusion and microwave-assisted extraction was not so significant. Similar results were obtained for total phenolic and flavonoid content in both extracts for each medicinal plant (Table 2). *Echinacea purpurea* water extracts showed significantly high content of total phenolic and flavonoids (Table 2) and the antioxidant activity was also high 193.2 mMTE g^{-1} dw for MAE and 164.1 $mMTEg^{-1}$ dw, respectively (Fig. 1). This activity could be explained with presence in the aerial parts of *E. purpurea* of flavonoids (0.48%) (quercetin, kaempferol, isorhamnetin and their glycosides and also anthocyanin, but also and free phenolic acids (chicoric, *p*-coumaric, *p*-hydroxybenzoic and protocatechnic acids) (Barnes *et al*., 2005). Our results showed that the total phenols and AOA was higher than the reported previous for 80 % methanol extracts from the same plant (Pietta *et al*., 1998, Wojdyło *et al*., 2007). Therefore, the content of bioactive compounds in extracts strongly depends on extracting solvent and technique. Dandelion and elecampane root, as well as thyme water extracts showed higher results for total phenolic content and antioxidant activity in comparison with previous reports for 80% methanol extracts (Wojdyło *et al*., 2007). The total flavonoids content of MAE extracts coincided with our previous study with conventional six hours extraction with ethanol and water (Petkova *et al*., 2015a, Petkova *et al*., 2015b). Therefore, the application of accelerated extraction procedure reduces significantly the extraction time with 5 hours and 55 sec.

Antioxidant activity

The radical scavenging activity of different medicinal water extracts was tested using methanol solution of the stable free radical DPPH. The results were summarized in Fig. 1. Great difference in antioxidant activity was observed between species. The results for scavenging of DPPH radical were in ther range from 18.4 ± 0.6 to 334.1 ± 0.3 mM TE g^{-1} dw. The highest antioxidant activity possessed great burdock 245.12mM TE g^{-1} dw, followed by coltsfoots and peppermint extracts (Fig. 1). The high antioxidant activity of *Arctiticum lappa* L. could be explained with the presence of caffeic and chlorogenic acid that possesses antioxidant, strong inhibitory effect on herpesvirus (HSV-1, HSV-2) and adenovirus (Chan*et al*., 2011).

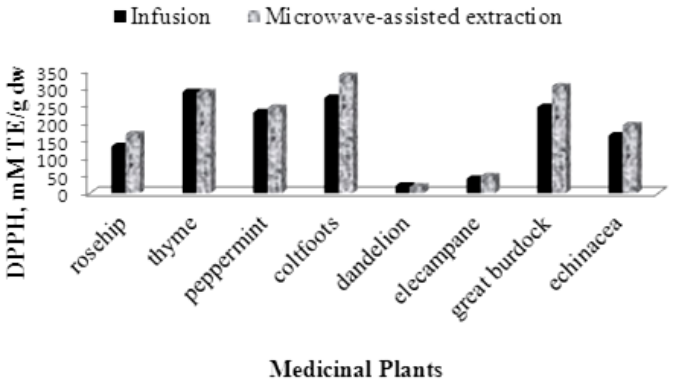

Fig. 1: Antioxidant activity of infusion and water microwave-assisted extracts from selected medicinal plants.

The lowest antioxidant activity possessed dandelion extracts 18.43mM TE g^{-1} dw. DPPH was chosen as an assay for evaluation of antioxidant activity because is quick and simple to be performed. The strongest antioxidant activity was established in the herbal water extracts from microwave extraction. Two representatives from Compositae (Asteracea) (coltfoots and great burdock) and two from Labiate (peppermint and thyme) herbs showed significantly high antioxidant potentials. The high antioxidant activity of coltsfoot could be explained with presence of terpens: arnidol, faradiol, sterols, flavonoids (kaempferol, quercetin and its glycosides, rutin, hiperoside), phenolic acids (ferulic, caffeic, chlorogenic, galic and *p*-hydroxybenzoic acids) (Ivancheva and Stancheva, 2000; Nedelcheva *et al*., 2015).

Correlation between antioxidant capacity and total phenolic content

The correlation between total antioxidant capacities obtained by DPPH and total phenolic contents of medicinal plant extracts were presented (Fig. 2A), as well as correlation between DPPH and total flavonoids content (Fig. 2B). The results showed positive linear correlations between total antioxidant activities and total phenolic contents (coefficient of correlation $r^2 = 0.95$ (Table 3).

Table 3: Correlation between total phenolic content (TFC) and antioxidant activities.

	TPC	Flavonoids
DPPH	0.9596	0.8000

These results suggested that the total phenolic compounds contributed significantly to the antioxidant activity of the investigated infusions and microwave plant extracts. In addition, similar observations about linear correlation between the content TPC and their antioxidant activity were reported by other researchers (Wojdyło *et al*., 2007; Krachanova *et al*., 2010).

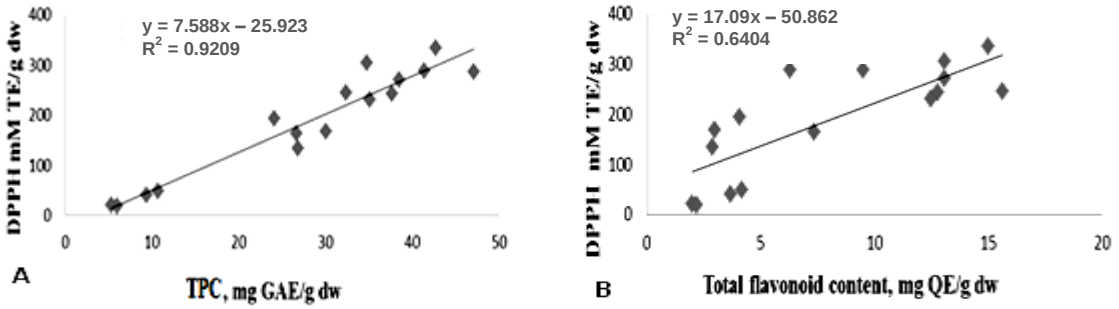

Fig. 2: Linear correlations between DPPH values and total phenolic content (A) values; Coefficient of determination (R^2=0.9209), as well as DPPH values and total flavonoid content (B) for medicinal plant extracts; Coefficient of determination (R^2=0.6404).

Carbohydrate content

Fructose, sucrose and fructans were found in detectable content in all extracts from medicinal plants except thyme (Table 4). In general, the carbohydrate content in microwave water extracts was higher than the infusions. Therefore, MAE accelerates extraction of monosaccharides and other carbohydrates in solvents. The highest values were found in elecampane and dandelion roots, where the total fructans dominated significantly. The data obtained for *Rosa canina* L. fruits were in range from 6.7 to 8.2g100 g^{-1} dw. These data were in accordance with previous reports that mature rosehip fruits contained fructose (6.5%) and sucrose (0.5%) (Pirone *et al*., 2007; Ognyanov *et al*., 2014).

Table 4: Carbohydrates content express as fructose equivalent in medicinal plant extracts, g 100 g^{-1} dw, means (n=3).

Plant	Plant material	Infusion	MAE
Rosehip	fruits	6.7±0.1[a]	8.2±0.2[a]
Thyme	aerial parts	n.d[a]	n.d[a]
Peppermint	aerial parts	1.7±0.1[a]	1.9±0.1[a]
Coltsfoots	leaves	2.0±0.2[b]	4.7±0.2[b]
Dandelion	roots	27.6±0.4[b]	34.6±0.6[b]
Elecampane	roots	41.9±0.6[b]	44.4±0.4[b]
Burdock	roots	12.0±0.2[b]	14.5±0.2[b]
Echinacea	roots	2.9±0.1[b]	4.7±0.2[b]

[a]fructose and sucrose; [b]fructose, sucrose and total fructans (inulin and FOS), n.d –not detected.

More detailed investigation was done for evaluation of fructose, sucrose, inulin and the total fructans. From the obtained results the highest values of inulin (33 g 100 g^{-1} dw) and FOS content were found in elecampane rootsextracts. It is well-known that inulin form chicory and dandelion roots possesses immunomodulation activity and bifidogenic properties (Trojanova *et al*., 2004).Therefore, its content in herbal extracts is important for human digestion and health. Significant amount of inulin presents in dandelion, elecampane and burdock roots (Fig. 3). Kardošová *et al*. (2003) and Olennikov and Tankhaeva (2011) reported earlier isolated linear *β*-1,2glucofructan from *A. lappa*roots with a degree of polymerization 18–19 (Mw =2.95 kDa) that demonstrated an antitussive effect and a pronounced immunomodulatory activity. Inulin content(4.7 g 100 g^{-1} dw) in extracts of *Arcticum lappa* was in agreement with a previous report of Olennikov and Tankhaeva (2011). Moreover, in Bulgarian burdock the levels of inulin and total fructans were lower than Russian representatives, where the content of high molecular inulin can reach up to 30 % (Bagaoutdinova *et al*., 2001). This could be explained with cold climate conditions, which stimulates accumulation of fructans as storage carbohydrates.

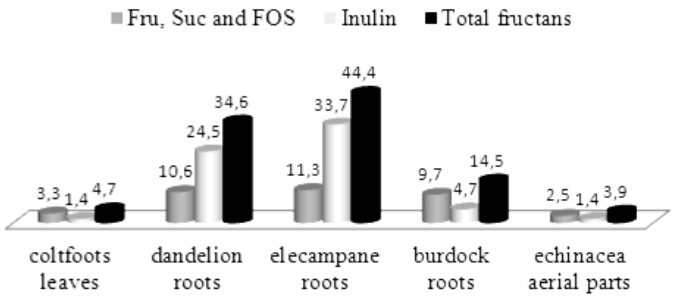

Fig. 3: Total fructans content (g 100 g^{-1} dw) in microwave-assisted extracts from medicinal plants.

Echinacea and coltsfoots leaves contained fructose, sucrose and FOS (1-kestose and nystose), as inulin in them did not increase 1.4 g 100 g^{-1} dw.The total fructans in coltsfoot leaves were in small amount in its roots where the content reached up to 17% (Stoyanov, 1974). Our results for total fructan content 4.7 g 100 g^{-1} dw in *Tussilago farfara* L. leaves coincided with mentioned data by Holligan *et al*.(1973).

The roots of medicinal plant *Inula helenium* L. present a valuable source of fructans from inulin-type, which are classified as soluble dietary fibers (Bagaoutdinova *et al*., 2001; Petkova *et al*., 2015b). Contrary to the low total phenolic, total flavonoids and antioxidant activity, root extracts from dandelion and elecampane contained high amount of inulin 24.5 and 33.7 g 100 g^{-1}dw. The efficiency of microwave-assisted extraction was demonstrated in our earlier research with elecampane roots. The total fructans content in dandelion and elecampane roots extracts coincided with the reported content typical for autumn collected plants 34 % and 44 %, respectively (Bagaoutdinova *et al*., 2001; Petkova *et al*.,

2012; Petkova *et al*., 2015a,b). Therefore, the dandelion and elecampane roots are properly harvested when high molecular inulin is accumulated. This is important feature for biological activity, nutritional value and healthy effect of these plants, as potential prebiotics. The prebiotic activity of dandelion oligofructans stimulated the growth of *Bifidobacterium* cultures (Trojanova *et al*., 2004).

According to the results from the present work, every cup of microwave extracts prepared from Asteracea representatives contains inulin-type fructans, as follows: *Tussilago farfara* -14 mg, *Taraxacum officinale* - 245 mg, and *Inula helenium* 337mg, *Arcticum lappa* -47 mg and Echinacea14 mg. The obtained results enrich the information about presence of inulin in herbal infusions and extracts from medicinal plants. Therefore, herbal infusions as well as microwave extracts from these plants present essential source of soluble dietary fiber.

CONCLUSION

The prepared herbal extracts were evaluated as rich sources of phenolic compounds with high antioxidant activities. Positive correlation between the total phenolic content and antioxidant activity of extracts was established. The infusions and microwave extracts from some of medicinal herbs were evaluated as significant source of inulin with potential prebiotic effect, especially great burdock, elecampane and dandelion roots. One cup of tea from great burdock extract is enough to supply not only the needed content of antioxidants, but also and soluble dietary fiber in human diet. This study provides useful information for consumers and encourages them to consume herbal teas, because of the antioxidant and prebiotic potential of hot drink products and additives with appropriate healthy properties.

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