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Antioxidant activity and fructan content in root extracts from elecampane (*Inula helenium* L.)

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ABSTRACT

The purpose of the present study was to investigate the antioxidant activity and fructans content in ethanol and water extracts of roots of elecampane *(Inula helenium* L.), a Bulgarian medicinal plant. The extraction procedure included 95% (v/v) ethanol extraction and subsequent water treatment. The antioxidant activity was evaluated by several reliable methods such as DPPH-, ABTS-, FRAP- and CUPRAC-assays, as well as the total phenolic content. In addition, the total fructans and sugar content were determinated by spectrophotomeric, TLC and HPLC-RID methods. The level of fructans in ethanol extracts was 14.1 g/100g dry weight, as nystose and 1-kestose were only 0.3g/100g dry weight, and 0.5g/100g dry weight, respectively. The absence of fructooligosacharides and sugars in water extracts after the ethanol pretreatment was established. Inulin content was evaluated to be 32g/100g dry weight. The metabolites profile of roots revealed their potential application as radical scavengers due to the presence of polyphenols. Therefore, the root extracts of elecampane could be assumed as a rich source of biologically active substance, in particular dietary fiber with potential prebiotic effect, due to the presence of polysaccharide inulin and fructooligosacharides.

Key words: *Inula helenium* L., inulin, total phenolic content, antioxidant activity

Introduction

Elecampane (*Inula helenium* L.) is a perennial herb from the *Compositae* family with thick aromatic roots, widely occurring in Europe, Asia and Africa (Spiridon et al., 2011, Wang et al., 2013). This medicinal plant is officially listed in some European pharmacopoeias (Stojakowska et al., 2006). The roots contain up to 5% of essential oil with eudesmane-type sesquiterpene lactones (mainly alantolactone and isoalantolactone shown on Figure 1), thymol derivatives, triterpenes, sterol (Blaschek et al., 1998; Stojakowska et al., 2006; Trendafilova et al., 2010), phenolic acids (caffeic, chlorogenic, dicaffeoyl quinic, hydroxibenzoic and ferulic acid-4-O-glucoside), different flavonoids (epicatechin, catechin gallate) (Spiridon et al., 2013), camphor, chamazulene, waxes, bitter substances, pectic polysaccharides and up to 44% inulin (Canadanović-Brunet et al., 2002). Due to the rich content of phytochemicals *Inula helenium* roots have been used in the folk medicine against a variety of ailments including asthma, cough, bronchitis, lung disorders, tuberculosis, indigestion, chronic enterogastritis, infectious, wounds healing and helminthic diseases (Konishi et al, 2002; Stojakowska et al., 2006). Recent studies have demonstrated that the root extracts possess diuretic, cholagogue, antihelminthic, anti-tumor, antimicrobial and insecticidal activities (Konishi et al, 2002; Huo et al., 2008). Alantolactone and isoalantolactone have hepatoprotective, antiproliferative, anticancer, antimicrobial activities, antiimflammatory and the potential to induce detoxifying enzymes (Blaschek et al., 1998; Lawrence et al., 2001; Konishi et al., 2002; Livermore, 2002).

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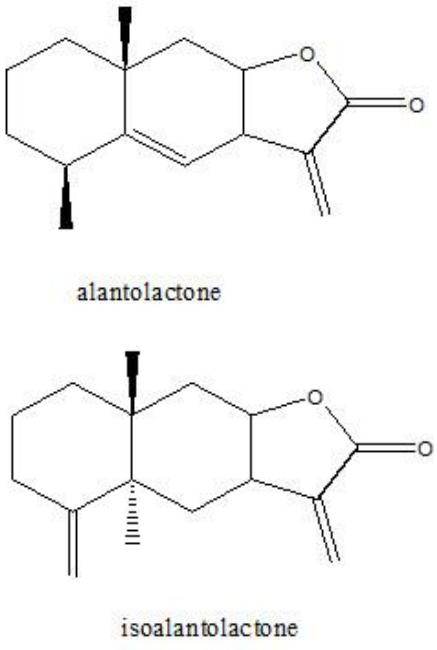

Figure 1. *Sesquiterpenes isolated from elecampane (Inula helenium L.) roots.*

The main carbohydrate in the roots of elecampane is inulin. It is fructan that consists mainly of β-(2→1) fructosyl fructose units (Fm), and usually, but not always, the chain terminates with α-glucopyranosyl unit (1→2) (GFn) (Figure 2). Its degree of polymerization (DP) varies from 2 to 70 and it depends on plant species, harvesting time and post-harvest conditions (De Leenheer & Hoebregs, 1994).

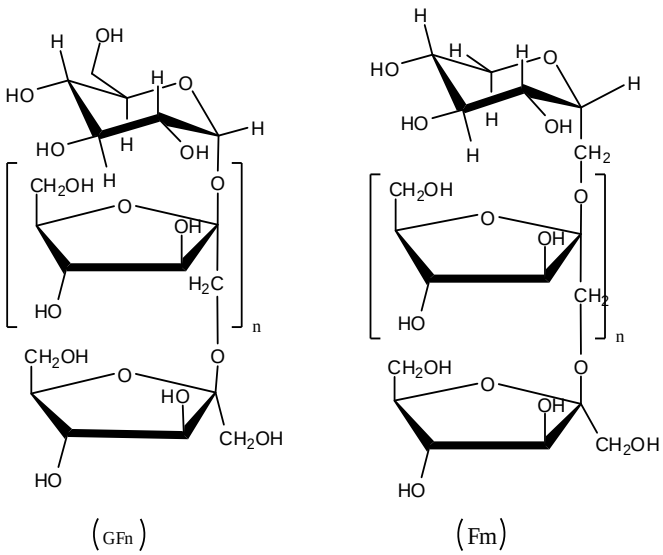

Figure 2. *Chemical structure of inulin.*

Molecules with DP<10 are called fructooligosacharides (FOSs) and form a subgroup of inulin (Niness, 1999). Inulin and FOSs are soluble dietary fibers that stimulate growth of *Bifidobacteria*, low glucose blood level, improve mineral absorption and possess immunomodulation effects (Gibson & Roberfroid, 1995; Barcley et al., 2010).

During the last decade, the studies about the biologically active substances in elecampane root extracts constantly increased (Canadanović-Brunet et al., 2002; Wojcikowski et al., 2007, Wojdyło et al., 2007; Spiridon et al., 2013). Many solvents and extraction techniques (maceration, heat reflux, ultrasonic- and microwave-assisted extraction) with various solvent were applied for evaluation of radical scavenging activity of *Inula helenium* roots (Wojdyło et al., 2007; Trendafilova et al., 2010; Vrancheva et al., 2012; Wang et al., 2013). The radical scavenging effect and total phenols were studied in extracts obtained with acetone (Denev et al., 2013), chlorophorm, ethyl acetate (Spiridon et al., 2013), methanol (Wojdyło et al., 2007) or water-ethanol mixture (Vrancheva et al., 2012; Wang et al., 2013). Antioxidant activities in extracts were analyzed by different method ORAC (Wojcikowski et al., 2007; Denev et al., 2013), DPPH (Canadanović-Brunet et al., 2002; Wojdyło et al., 2007; Spiridon et al., 2012; 2013), ABTS, FRAP (Wojdyło et al., 2007). The expanded application is due to their protective properties against oxidative stress disorders, as well as oxidative damage in food products (Ivanov et al., 2014).

Until now not relevant studies on antioxidant activity and carbohydrate composition of ethanol and water extracts from *Inula helenium* root has been reported in literature. Incomplete investigations have been reported regarding evaluation of antioxidant potency of elecampane roots. No detailed information about sugars and inulin content in the roots of *Inula helenium*, growth in Bulgaria were found.

Therefore, the objective of the present study was to evaluate the total phenolic content, carbohydrate composition and antioxidant potential of *Inula helenium* roots extracts and to enrich the knowledge about this medicinal plant.

Materials and Methods

All chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany) and Sigma (St. Louis, USA). Fructooligosacharides Frutafit®CLR with the average chain length of 7-9 monomers and inulin Frutafit®TEX with DP 22 were supplied by Sensus (Roosendaal, Netherlands).

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Dried roots of elecampane were purchased from the local herbal drugstore. The plant material was ground and passed through a 0.5 mm sieve. The moisture content analyzed by AOAC 945.32 (AOAC, 2007) was established to be 11.7%. The ground roots were kept in a screwed capped container at room temperature for further analysis.

Preparation of plant extracts for determination of antioxidant properties, sugars, inulin and total polyphenol content

The extraction procedure was performed by previously described method by Olennikov et al. (2008) with slight modification (Petkova & Denev, 2013a). Elecampane dry roots (0.5 g) were placed into a round-bottom flask. A total of 40 mL of 95% ethanol was added and the sample was boiled under reflux for 60 min. The extraction process was repeated twice with 40 mL and 20 mL solvents, respectively. The residue was dried and then it was extracted successively with 40 mL, 40 mL and 20 mL distilled water under reflux for 60 min. The obtained extracts were analyzed in terms of antioxidant activity, total phenolic and carbohydrate content. Each sample was extracted in triplicate.

Evaluation of antioxidant activities (AOA)

Determination of total phenolic content (TPC)

The total polyphenol content was analyzed using the Folin-Ciocalteu method of Kujala et al. (2000) with some modifications. Each sample extract (0.1 mL) was mixed with 0.5 mL of Folin-Ciocalteu phenol reagent and 0.4 mL of 7.5% Na_2CO_3. The mixture was vortexed well and left for 5 min at 50°C. After incubation, the absorbance was measured at 765 nm against blank sample. The TPC in the extracts was expressed as mg gallic acid equivalent (GAE) per g dry weight (dw).

DPPH radical scavenging activity

The ability of the extracts to donate an electron and scavenge DPPH radical was determined by the slightly modified method of Brand-Williams et al. (1995). Freshly prepared 4×10^{-4} mol methanol solution of DPPH was mixed with the samples in a ratio of 2:0.5 (v/v). The light absorption was measured at 517 nm. The DPPH radical scavenging activity was presented as a function of the concentration of Trolox® - Trolox® equivalent antioxidant capacity (TEAC) and it was defined as the concentration of Trolox® having equivalent AOA expressed as μmol TE/g dw.

ABTS radical scavenging assay

The radicals scavenging activity of the investigated extracts against radical cation ($ABTS^{\bullet+}$) was estimated according to the previously reported procedure with slight modifications (Re et al. 1999). The results were expressed as TEAC value (μmol TE/g dw).

Ferric-reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to the procedure of Benzie and Strain (1996). FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe (II)-tripyridyltriazine compound from colorless oxidized Fe (III) form by the action of electron donating antioxidants. The results were expressed as μmol TE/g dw.

Cupric ion reducing antioxidant capacity assay (CUPRAC)

To a test tube, the solutions were added as follow: 1 mL of $CuCl_2$ solution (1.0×10^{-2} M), 1 mL of neocuproine methanol solution (7.5×10^{-3} mol), and 1 mL NH_4Ac buffer solution (pH 7.0), and mixed; 0.1 mL of sample followed by 1 mL of water was added (total volume = 4.1 mL), and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min (Ak & Gulcin, 2008). Trolox was used as standard and total antioxidant capacity of herbal extracts was measured as μmol TE/g dw.

TLC analysis

The sugars and fructan quantitative analysis of obtained extracts were done by TLC chromatography. Five microliters of each sample were performed on silica gel 60 F_{254} plates (Merck, Germany) with mobile phase n-BuOH:i-Pro:H_2O:CH_3COOH (7:5:4:2) (v/v/v/v). The TLC plates were dipped in the detecting reagent diphenylamine-aniline-H_3PO_4–acetone (Lingyun et al., 2007), heated and scanned as previously described by Petkova and Denev (2013 a).

Spectrophotometric determination of total fructans

The total carbohydrate content in ethanol and water extracts expressed as fructose equivalent was defined spectrophotometrically at wavelength 480 nm by resorcinol-thiourea reagent (Petkova & Denev, 2012). Hundred microliters extract were place in glass tube of 10 mL, and 100 μL resorcinol (1% ethanol solution), 100 μL thiourea (0.1% ethanol solution), 800 μL 95% ethanol and 900 μL HCl were added to them. The sample was heated 8 min at 80°C, cooled and filled with water until 10 mL.

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Then the absorbance was measure against blank sample prepared at the same procedure with distilled water.

HPLC-RID analysis of sugars, fructooligosacharides and inulin

The sugars, FOSs and inulin content in elecampane root extracts were analyzed by HPLC method. Chromatographic separations were performed on HPLC Shimadzu, coupled with LC-20 AD pump, refractive index detector Shimadzu RID-10A. The control of the system, data acquisition, and data analysis were under the control of the software program LC solution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan). The separation of inulin and sugars in extracts were performed on a Shodex® Sugar SP0810 with Pb^{2+} a guard column (50 × 9.2 mm i.d.) and an analytical column (300 mm × 8.0 mm i.d.) at 85°C. The mobile phase was distilled water with flow rate 1.0 mL/min. The injection volume of the samples 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

Until now, the antioxidant activity of elecampane root gathered from territory of Bulgaria was not evaluated in details. In literature, there were scantly data about foreign species *Inula helenium* L. concerning the radical scavenging activities (Canadanović-Brunet et al., 2002; Wojdyło et al., 2007; Spiridon et al., 2013). No data available about application of CUPRAC method for evaluation of radical scavenging activity. However, in the present research for the first time the antioxidant potential of 95% ethanol (v/v) and subsequent water extracts obtained after the conventional extraction method was evaluated by four reliable methods such as DPPH, ABTS, FRAP and CUPRAC. The water extracts showed higher antioxidant activities (26.0±0.2, 30.2±3.4, 76.4±0.9, 78.9±1.1 µM TE/g dw according DPPH-, ABTS-, FRAP- and CUPRAC- method, respectively) compared to the ethanol ones (Table 1). The reported by us results were much higher than the antioxidant activity of 80% methanol extracts evaluated by three methods: DPPH, FRAP and ABTS (Wojdyło et al., 2007). In addition, the water extracts also showed higher total phenolic content 3.1±0.04 mg GAE/ g dw (Table 1), which correspond well with the other reported results in this study. However, the phenol content in 95% ethanol extracts (1.5±0.03 mg GAE/g dw) was lower in comparison with the results obtained by ultrasonic extraction with 70% ethanol - 7.8 mg GAE/g dw (Vrancheva et al., 2012) and with 30% ethanol - 6.13 mg/g dw (Wang et al., 2013). The results of the water extracts from conventional extraction were similar to the values in water extracts obtained by ultrasonic irradiation (Wang et al., 2013). Nevertheless, values of the total phenol content in water extracts (3.1 mg GAE/g dw) were higher than the values of the acetone extract - 1.7 mg/g GAE dw reported by Denev et al. (2013), as well as of the absolute methanol (Afemei et al., 2012) and 80% methanol (Wojdyło et al., 2007). The differences between the reported in the present study and in other researcher papers results probably are due to the different origin of the plant samples, solvent used and the applied extraction technique.

Higher antioxidant activity in water extracts from elecampane roots corresponds to the higher total phenolic content. According to Spiridon et al. (2013) the antioxidant activity was probably based on the ability of the phenolics and also other biomolecules (tannins, terpenes) presented in elecampane to act as donors of hydrogen atom.

TLC chromatograms of the sequentially obtained ethanol and water extracts from elecampane (*Inula helenium* L.) root showed that a large number of carbohydrates were successively extracted (Figure 3). The presence of fructose (R_f = 0.50), sucrose (R_f = 0.44), FOSs including 1-kestose (R_f = 0.37), nystose R_f = (0.32) and 7-8 oligomers, equivalent with used inulin standard Frutafit CLR, was established in 95 % (v/v) ethanol extracts. The carbohydrates profile of root extracts was presented by HPLC chromatograms (Figure 4 and Figure 5).

Table 1. *Total phenolic content (TPC) and antioxidant activities (µM TE/ g dw) in root extracts of Inula helenium L.*

Extract	TPC mg GAE/ g dw	FRAP	CUPRAC	ABTS	DPPH
95% EtOH	1.5 ± 0.03	26.0 ± 0.68	55.6 ± 1.28	19.7 ± 3.16	19.8 ± 0.26
water	3.1 ± 0.04	76.4 ± 0.92	78.9 ± 1.09	30.21 ± 3.40	26.0 ± 0.20

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In ethanol soluble fraction dominated sugars (glucose, fructose, sucrose) and oligosaccharides (1-kestose and nystose), while in water fraction only inulin was presented. The carbohydrate composition shown in HPLC chromatograms of 95% ethanol extracts were quite similar to the same extracts obtained from tubers of *Helianthus tuberosus* L. (Petkova et al., 2013b). Our study revealed that the high molecular fraction of inulin dominated in the root of elecampane – 31.6 ± 0.7 g/100 g (Table 2).

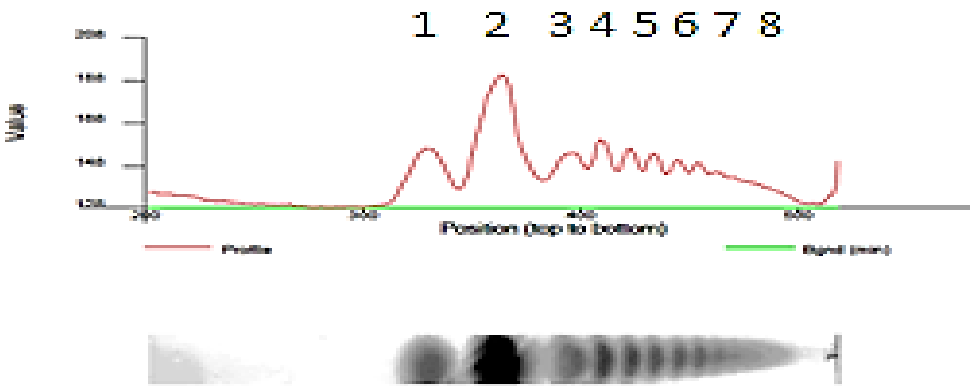

Figure 3. *TLC analysis of 95% ethanol extracts from roots of Inula helenium L, where 1. fructose, 2. sucrose, 3. 1-kestose, 4. nystose, 5 to 8. fructooligosaccharides (respectively GF4, GF5, GF6, GF7).*

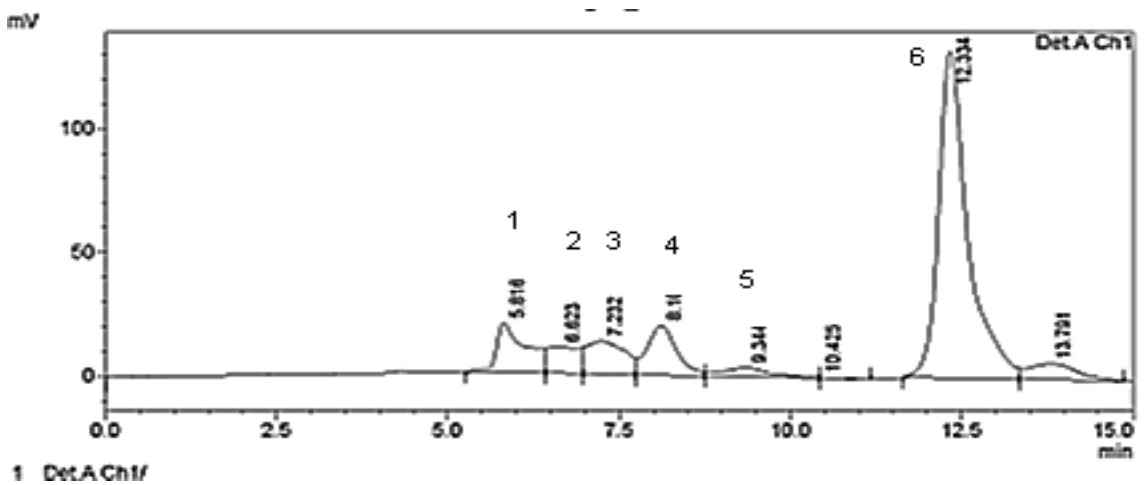

Figure 4. *HPLC-RID chromatogram of 95 % (v/v) ethanol extracts obtained from Inula helenium roots, where: 1. inulin, 2. nystose, 3. 1-kestose, 4. sucrose, 5. glucose, 6 fructose.*

The total carbohydrate content expressed as fructose equivalent was higher in water root extract than the ethanol one – 33.4±0.6 g/100g dw against 14.0±1.3 g/100g dw. Ethanol treatment procedure extracted the low molecular fraction in which fructose and fructooligosacharides (nystose and 1-kestose) were the main components. In our case the ethanol soluble carbohydrate in elecampane roots 14.0±1.3 g/100g dw was higher than the results reported by Bagaoutdinova et al., (2001).The water extracts obtained after ethanol pretreatment contained only high molecular fructan from inulin-type 33.4±0.6 g/100 g dw. The total carbohydrates content express as fructose equivalent in both extracts (47.4±1.9) from *Inula helenium* L. were in accordance to these reported by Bagaoutdinova et al. (2001), Olennikov et al. (2008), Varancheva et al. (2012) and Petkova & Denev (2012). In comparison of our early reported results for this plant the main advantage of elecampane together with chicory was the constant level of inulin in their

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roots during seasons (Petkova & Denev, 2012; Petkova et al., 2013c).

Alongside with tubers of Jerusalem artichoke (*Helianthus tuberosus* L.), 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. 2013b). Because of this, the elecampane roots could be successively applied in nutrition formula for stimulation function of gastrointestinal tract and immune system.

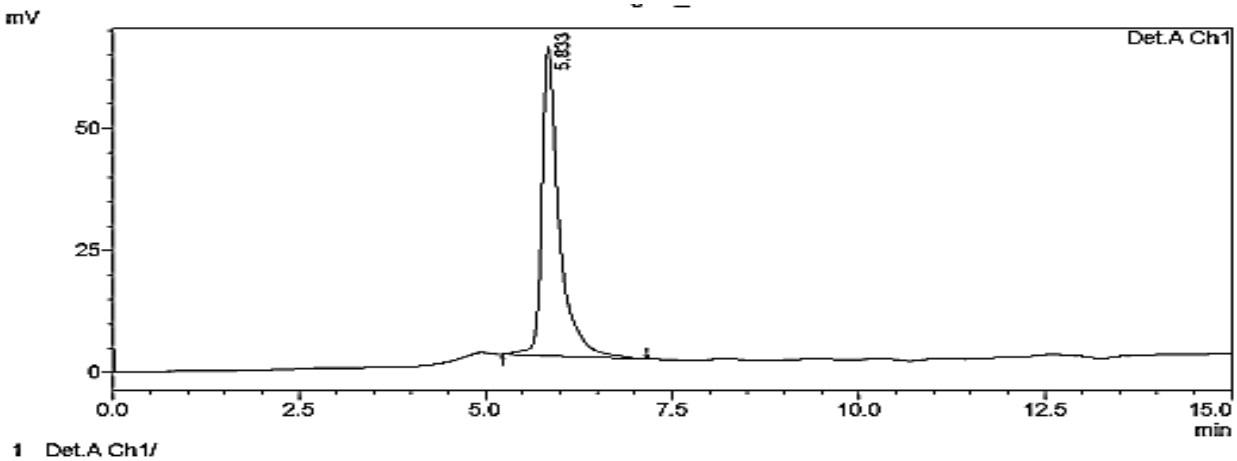

Figure 5. *HPLC-RID chromatogram of inulin in water extract obtained from Inula helenium roots.*

Table 2. *Carbohydrate composition in extracts from roots of Inula helenium L. g/100 g dw[1] (mean ± SD[2], n=4)*

Extract	Total carbohydrates[3]	Inulin	Nys	1-Kes	Suc	Glc	Fru
95%(v/v) EtOH	14.0 ± 1.3	0.4±0.1	0.3±0.1	0.5±0.1	0.6±0.1	0.1±0.1	2.6±0.3
water	33.4 ± 0.6	31.2±1.2	n.d	n.d	n.d	n.d	n.d

[1]dw – dry weight, [2]SD – standard deviation, [3]expressed as fructose equivalent, n.d – not defined

Conclusion

The conducted quantitative and qualitative analysis of the ethanol and water extracts of *Inula helenium* roots showed that this Bulgarian medicinal plant is a valuable source of biologically active compounds. The current investigation demonstrated that polysaccharide inulin is presented in high quantities in the water extracts (32 g/100 g dw) obtained from elecampane roots. However, the ethanol extracts could be a valuable source of fructooligosacharides as well. The water extracts after ethanol treatment showed significant antioxidant activity and higher phenolic content. A positive correlation between total antioxidant activity and total phenolic content was observed, revealing that elecampane root could possessed considerable benefits when applied in human nutrition and for production of functional food with well-pronounced healthy effect. This complex of biologically active substance as well as the high molecular inulin in *Inula helenium* L. roots reveals many future applications not only in field of herbal medicine and nutrition, but also in pharmacy for potential drug carrier and vaccine adjuvant.

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