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BIOLOGICALLY ACTIVE SUBSTANCES AND *IN VITRO* ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS FROM DANDELION (*TARAXACUM OFFICINALE*) ROOTS

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Abstract

Dandelion (Taraxacum officinale L. Weber ex F.H. Wigg.) roots were traditionally used in folk medicine worldwide due to its antidiabetic, choleretic, antirheumatic and diuretic properties. The aim of the current study was to determinate the biologically active substances in 95% ethanol and subsequent water extracts from dandelion roots and to evaluate their antioxidant activities. The carbohydrate composition was analyzed by the resorcinol assay, TLC and HPLC-RID methods. The total phenolic contents (TPC), total flavonoids (TF) and total dihydroxycinnamic derivatives contents were determined by Folin–Ciocalteau method, aluminium chloride colorimetric assay and Arnow's reagent, respectively. In vitro antioxidant activities of the extracts were estimated using ferric-reducing/antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assays. In spring harvested dandelion roots the total fructan content were established to be in range 17-19 % dw. The low molecular carbohydrate fraction presented by glucose, fructose, sucrose, 1-kestose and nystose dominated in 95 % ethanol extracts. The subsequent water extracts demonstrated the highest inulin content (12% dw), while TPC, TF and total dihydroxycinnamic derivatives contents were reported to be 9.2 mg GAE/g, 1.7 mg QE/g and 13.7 mg chlorogenic acid derivates/g dw, respectively. These extracts showed the highest antioxidant activity for both the DPPH (82.1 mM TE/g) and FRAP (52.9 mg mM TE/g) assays. Therefore, the subsequent water extracts possessed the highest antioxidant capacity, which positively correlated with the phenolic content. The results of current investigation demonstrate that dandelion root is a valuable source of dietary fibers and natural antioxidants and could be successfully used in foods with the potential to improve digestion and prevent from oxidative stress related diseases.

Key words: *dandelion roots, inulin, total phenolic content, antioxidant activity.*

INTRODUCTION

The interest in medicinal extracts constantly increased because of their improved healthy effect and protective properties against oxidative stress disorders (Aleksieva et al. 2013; Ivanov et al., 2014).

Dandelion *Taraxacum officinale* (L.) Weber ex F.H. Wigg is a medicinal plant member of Compositae family, subfamily Cichorioideae, tribe Lactuceae. It is widely distributed in the warmer temperate zones of the Northern Hemisphere as a perennial weed (Schütz et al., 2006 a). The plant reaches an average length of 15–30 cm and sometimes up to 70 cm (Nnamdi et al., 2012) with roots 60–100 cm in length (Schütz et al., 2006 a).

From century ago infusions and decoctions of the dandelion roots and herbs have been utilized for the treatment of various ailments such as kidney disease, dyspepsia, heartburn, spleen, liver complaints, hepatitis and anorexia (Sweeney et al., 2005; Schütz et al., 2006 a). In Bulgarian traditional herbal medicine, this plant is used for treatment of digestive diseases, prevention of renal gravel and loss of appetite (Pamukov and Ahtardjiev, 1990). The main suppliers of dandelion are Bulgaria, followed

by former Yugoslavia, Romania, Hungary and Poland (Bisset et al., 1994).

Dandelion active ingredients are found in both the roots and leaves (Amin et al., 2013). Its roots contain sesquiterpenes, tetrahydroridentin B, taraxacolide-*O*-β-glucopyranoside, triterpenes, phytosterols (taraxasterols, their acetates and 16-hydroxy derivatives, arnidol, faradiol, α- and β-amyrin, β-sitosterol, β-sitosterol-D-d-glucopyranoside and stigmasterol), several phenolic compounds (chicoric acid, monocaffeoyltartaric, 4-caffeoylquinic, chlorogenic, caffeic, *p*-coumaric, ferulic, *p*-hydroxybenzoic, protocatechuic, vanillic, syringic and *p*-hydroxyphenylacetic acids), as well as three coumarins (umbelliferone, esculetin and scopoletin) (Williams et al., 1996; Schütz et al., 2006 a). Apart from above mentioned secondary metabolites, the dandelion roots are a rich source of polysaccharides, mainly inulin-type fructans and smaller amounts of pectin, resin, and mucilage (Schütz et al., 2006 a; Amin et al., 2013). Inulin and its short chains - fructooligosaccharides are fructans 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). Fructan content in dandelion roots ranges from 2% in spring to 40% in autumn (Bisset et al., 1994). Inulin and FOSs are soluble dietary fibers that stimulate growth of *Bifidobacteria*, low glucose blood level, improve mineral absorption and possess immunomodulation effects (Gibson and Roberfroid, 1995; Barcley et al., 2010). Trojanov´a et al., (2004) proved the prebiotic activity of dandelion oligofructans. They stimulate the growth of bifidobacterial cultures. Therefore, the presence of enormous variety of biologically active substances in dandelion roots enhances their nutritional and healthy effects. Leaves of this plant are often consumed as salads, their roots are a coffee substitute, while dandelion extracts have also been used as flavour enhancers in soft drinks and baked goods. In addition, dandelion is often marketed as a health food (Leung et al., 1996). Its roots (Taraxaci radix) are processed into pharmaceutical preparations such as teas, tinctures, capsules, tablets and juices (Schütz et al., 2006b).

Several health-promoting benefits, including diuretic, laxative, cholagogue, anti-rheumatic, antiinflammatory, choleretic, anti-carcinogenic, analgesic, anti-hyperglycemic activities, anti-coagulatory and prebiotic effects have been attributed to the use of dandelion extracts or the plant itself (Schütz et al., 2006 a; Kenny et al., 2014 a). Dandelion root has been reported to possess antioxidant activity that is linked to the presence of phenolic based compounds (Hagymasi et al., 2000, Cho et al, 2002). It was reported that water, ethanol and methanol extracts from the aerial art and root of dandelion possessed antimicrobial activity against *B. cereus, E. coli* and *S. aureus* as this effect was again linked to the phenolic content (Kenny et al., 2014).

Until now, there are uncompleted information about presence of inulin, total phenols, total dihydroxycinnamic derivatives and flavonoids in root of *Taraxacum officinale* L., growth in Bulgaria, especially during the spring season. Not detailed investigations have been reported regarding evaluation of radical-scavanging activities of dandelion roots. Only antioxidant potencial of water infusion from herb *Taraxacum officinale* L. evaluated by ABTS method (Ivanova et al., 2005) and leaves extracts measured by DPPH, FRAP and CuPRAC assays (Ivanov, 2014) were reported.

Therefore, the aim of the current research was to determinate the content of biologically active substances in 95% ethanol and subsequent water extracts from dandelion roots and to evaluate their *in vitro* antioxidant activities.

MATERIALS AND METHODS

All used reagents and solvents were of analytical grade scale. Carbohydrate standards fructose, sucrose, 1-kestose and nystose have been purchased from Sigma-Aldrich (Steinheim, Germany). Fructooligosacchrides Frutafit®CLR, and inulin Frutafit®TEX were supplied by Sensus (Roosendaal, the Netherlands).

The taproots of several randomly chosen dandelion plants were gathered from territory of South Bulgaria – Plovdiv (Plovdiv region), Parvomay (Krushevo village) and Chirpan (Stara Zagora region) during 13-20 April 2013. The underground parts were air-dried, finely

ground and passed through a 0.5 mm sieves. The root powder with approximately 12-14 % moisture content was stored in crew-capped containers for further use.

Extraction procedure

Dandelion roots (20 g) were extracted successively in a Soxhlet apparatus with hexane, $CHCl_3$ and ethyl acetate to remove lipophilic compounds (Olennikov et al., 2009) and the residue was dried. The extraction procedure was performed by previously described method (Petkova & Denev, 2013a). Dandelion dry roots (0.8 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 it was extracted successively with 40 mL, 40 mL and 20 mL boiling distilled water under reflux as the duration for each extraction procedure was 60 min. The obtained dandelion root extracts were analyzed for total fructans, total phenolic content, total dihydroxycinnamic derivative, total flavonoids and radical scavenging activity.

Total fructan content

The fructan content in the obtained extracts expressed as fructose equivalent were analyzed spectrophotometrically at wavelength 480 nm by resorcinol-thiourea reagent (Petkova and 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. Then the absorbance was measure against distilled water.

Identification of mono-, di-, fructooligosaccharides (FOS) and inulin by Thin layer chromatography

TLC analysis were used to elucidate the presence of mono-, di-, fructooligosaccharides (FOS) and inulin in the ethanol and water extracts obtained from dandelion roots. 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 (Petkova and Denev, 2013a).

Carbohydrate analysis by HPLC-RID method

HPLC-RID methods were used for quantification of sugars (glucose, fructose, sucrose), 1-kestose, nystose and inulin in dandelion root extracts. Chromatographic separation was performed on HPLC Shimadzu, coupled with LC-20AD pump, refractive index detector Shimadzu RID-10A and software program LC solution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan). The analysis were performed on an analytical column Shodex® Sugar SP0810 with Pb^{2+} (300 mm × 8.0 mm i.d.) coupled with a guard column (50 × 9.2 mm i.d.) at 85 °C. The mobile phase used for separation was distilled water with flow rate 1.0 ml/min. The injection volume of the samples was 20 μL (Petkova et al., 2014).

Total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent according to the described procedure (Stintzing et al., 2005) with some modifications. Basically, 0.2 ml dandelion root extract was mixed with 1 ml Folin–Ciocalteu reagent diluted five times 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 sample developed the same way but without extract. The results were expressed in mg equivalent of gallic acid (GAE) per g dry weight (dw), according to calibration curve; build in range of 0.02 - 0.10 mg (Ivanov et al., 2014). All determinations were performed in triplicate (n = 3).

Determination of total dihydroxycinnamic derivative

The content of total dihydroxycinnamic acid (including caffeoyl derivatives) was expressed as chlorogenic acid as previously described in the European Pharmacopoeia (6th ed. 2008). The dandelion root extract (1 ml) was added to 2 ml 0.5 M HCl, 2 ml Arnow's reagent (10 g

sodium nitrite and 10 g sodium molybdate made up to 100 ml with distilled water), 2 ml 2.125 M NaOH and 3 ml water. Each sample was compared with the same mixture without Arnow's reagent. Absorbance was read at 525 nm. The results were calculated and expressed as mg chlorogenic acid derivates per g dw (Fraisse et al., 2011).

The total flavonoids content

The total flavonoids content was analysed by $Al(NO_3)_3$ reagents. The absorbance was measured at 415 nm. The results were presented as mg equivalents quercetin (QE) per g dry weight (DW) (Kivrak et al., 2009) according to the calibration curve, linear in range of 10-100 µg/mL quercetin as a standard.

Antioxidant activity (AOA):

The antioxidant activity of ethanol and subsequent water extracts from dandelion roots (*Taraxacum officinale L.* Weber ex F.H. Wigg) was evaluated by two methods: DPPH (1,1-diphenyl-2-picrylhydrazyl radical based on mixed hydrogen atom transfer (HAT) and single electron transfer mechanisms and FRAP (ferric reducing antioxidant power) based only on single electron transfer mechanism.

DPPH radical scavenging activity

Dandelion root extract (150 µl) was added to 2.85 ml freshly prepared DPPH solution (0.1 mM in methanol). The sample was incubated for 15 min at 37 °C in darkness. The reduction of absorbance at 517 nm was measured by spectrophotometer in comparison to the blank containing methanol and % inhibition were calculated (Ivanov et al., 2014). A standard curve was built with Trolox in concentration between 0.005 and 1.0 mM. The results are expressed in mM Trolox® equivalents (TE) per g dry weight (dw).

FRAP assay: 0.1 ml of investigated extracts were added to 3 ml FRAP reagent (0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), 20 mM $FeCl_3 \times 6H_2O$ (10:1:1; v/v/v) and allowed to stand for 10 min at 37 °C in darkness. The absorbance of the formed coloured product was measured at 593 nm (Benzie and Strain, 1996). The results from both antioxidant methods were expressed as mM Trolox® equivalents (TE) per g dry weight (dw).

All determinations were performed in triplicate(n = 3) and the data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using MS Excel 2010. A difference was considered statistically significant, when $P < 0.05$.

RESULTS AND DISCUSSIONS

Dandelion roots were evaluated as a rich source of inulin-type fructans with different chain length. TLC analysis showed that extracts from spring plants were characterized with high levels of fructo-oligosaccharides and sugars (Figure 1). The presence of fructose (R_f = 0.50), sucrose (R_f= 0.44), 1-kestose (R_f= 0.37), nystose (R_f=0.32) and FOSs until 8 monomer units (from GF3 to GF7) equivalent to inulin standard Frutafit CLR was detected in 95 % (v/v) ethanol extracts (Figure 1 a). Water extracts obtain after 95 % ethanol treatment contained small amount of residual sucrose, 1-kestose, nystose and also FOSs up to GF8 dominated together with high molecular inulin with DP 22 similar to the used standard (Figure 1 b).

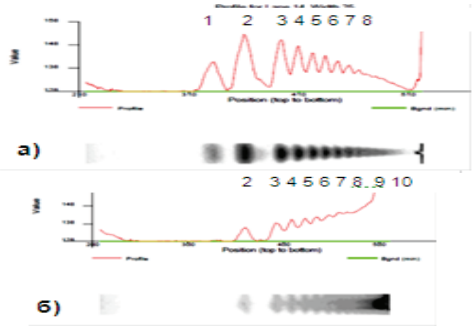


Figure 1. Thin-layer chromatograms of 5 µl a) 95 % ethanol and b) water extracts from root of *Taraxacum officinale L.* Weber ex F.H. Wigg where 1. fructose, 2. sucrose, 3. 1-kestose (GF2), 4. nystose (GF3), 5-8. fructooligosaccharides (respectively GF4, GF5, GF6, GF7, GF8) and 10. inulin

The total fructan content in dandelion roots expressed as fructose equivalents was in range from 17.7 to 19.7 g/100 g dw (Table 1). The high molecular fraction dominated above the ethanol soluble low molecular fraction (sugars and FOSs). Low-molecular fraction (Fru, Suc and FOSs) were around 8 g/100 g dw, while inulin fraction was in range from 8 to12 g/100

g dw. The results obtained were in accordance with previously reported by us data for spring harvested dandelion roots collected from Northern and South Bulgaria locations - 7 and 5 g/100 g dw, respectively (Petkova et al., 2013b). At the same time, the total fructan content in dandelion root harvested in April were lower than the reported content in summer and autumn plants, 21% and 34 %, respectively (Bagaoutdinova et al., 2001; Petkova et al., 2012; Petkova et al., 2013b). Dandelion roots contained more sugars and FOSs in 95 % ethanol extracts than the same extract from the autumn plants (Petkova et al., 2013b).

The detailed analysis of carbohydrate content in 95 % ethanol and the subsequent water extracts was performed by HPLC method (Table 2). The separation of the present compounds was shown on HPLC chromatograms (Figure 2).

Generally, it was considered that glucose levels in dandelion roots seemed to be much lower than fructose and sucrose levels, reaching the maximum level in April and May (Schütz et al., 2006b). Nevertheless, in our case the presence of glucose was not found. Its content could be too small to be detected. The level of fructose reached up to 5 g/100 g dw, while sucrose was in range from 1.8 to 2.2 g/100 g dw (Table 2). In accordance with Wilson et al., (2001) the highest fructose content was defined as dandelion plants initiated new growth. It was found that 1-kestose was the predominant fructooligosaccharide, followed by nystose. The content of 1-kestose and nystose, both considered as best probiotics (Van Loo et al. 1995), reached about 2 % dw. This was consistent with previous reports for common chicory roots and *Helianthus tuberosus* L. tubers (Petkova et al., 2013c; Denev et al., 2014). In contrast, 1-kestose and nystose total amount in dandelion roots exceeded that of artichoke (Schütz et al., 2006b) and elecampane (Petkova et al., 2015), reflecting the short chain characteristic of the inulin. The detected amounts of fructose, sucrose, 1-kestose and nystose expressed on a dry weight basis (Table 2) coincided with those reported previously for dandelion roots harvested in May (Schütz et al., 2006b). The inulin content in the investigated spring dandelion roots was relatively high – 8-11 g/100 g dw (Table 2).

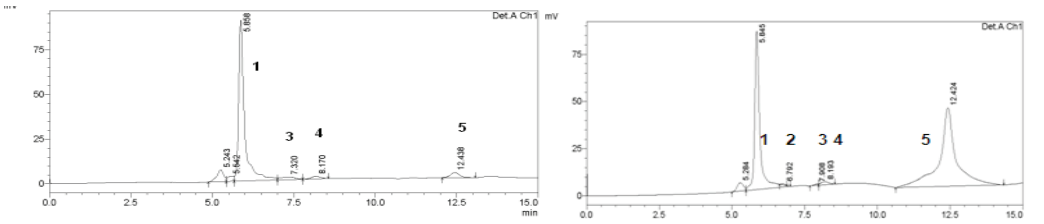
Table 1. Fructan content expressed as fructose equivalents in dandelion roots, g/100 g dw[1] (mean ± SD[2], n=3)

Location	Low-molecular fraction (Fru[3], Suc[4] and FOSs)	High-molecular fraction (inulin)	Total
Chirpan	8.8 ± 0.5	8.8 ± 0.1	17.7 ± 0.6
Plovdiv	7.0 ± 0.6	11.8 ± 0.9	18.8 ± 1.6
Parvomay	7.8 ± 0.1	11.9± 0.3	19.7± 0.3

[1]dry weight, [2]SD – standard deviation, [3]Fru – fructose, 4 - sucrose

Table 2. Carbohydrates content in root extracts of *Taraxacum officinale L. Weber ex F.H. Wigg,* g/100 g dw

Location	Fructose	Sucrose	1-Kestose	Nystose	Inulin
	mean ± SD, n=3				
Chirpan	5.3± 0.1	2.2± 0.2	1.0± 0.1	0.8± 0.1	8.4± 0.1
Plovdiv	4.2± 0.2	1.8± 0.1	0.9± 0.1	0.7± 0.3	10.9± 0.2
Parvomay	4.3± 0.1	2.1± 0.2	1.2± 0.2	1.0± 0.1	11.2± 0.3



a) b)

Figure 2. HPLC chromatograms of extracts obtained from dandelion taproots from different locations: a) Plovdiv and b) Chirpan, where 1. inulin; 2. nystose, 3. 1-kestose 4. sucrose, 5 fructose

Table 3. Total phenolic contents, total dihydroxycinnamic derivatives, total flavonoids contents and antioxidant activity of the extracts obtained from dandelion root extracts

Location	Extracts	TPC, mg GAE[1]/g dw	Total dihydroxycinnamic derivatives, mg CAE[2]/g dw	Total flavonoids, mg EQ[3]/g dw	DPPH	FRAP
		Mean±SD[3]				
Chirpan	95% EtOH	4.5 ± 0.1	1.9 ± 0.1	0.5 ± 0.1	11.4±0.3	17.4±0.2
	water	6.4 ± 0.2	8.4 ± 0.6	1.7± 0.4	37.0±1.6	40.3±0.5
Plovdiv	95% EtOH	6.1 ± 0.4	7.0 ± 0.9	0.7 ± 0.1	20.8±0.9	25.7±2.9
	water	7.1 ± 0.4	13.8 ± 0.1	1.5 ± 0.1	38.7±1.3	52.9±0.3
Parvomay	95% EtOH	7.4± 0.3	3.6± 04	0.5± 0.1	51.2±0.8	26.6±1.8
	water	9.2± 0.3	9.8± 0.9	1.4± 0.1	83.1±3.2	46.9±1.3

[1]Expressed as milligram of gallic acid per gram dry material,[2]cinnamic acid equivalent, [3]Expressed as milligram of quiercetin per gram dry extract, [3]SD – standard deviation (n=6)

During our study the tendency of increasing the concentration of higher polymerized fructan and sucrose was observed similarly to early reported (Van den Ende et al., 2000; Wilson et al., 2001. This fructans served as a starter for selective bifidogenic fermentation in the colon, whereas the long-chain inulin is fermented twice as slowly than the low polymerized inulin, maintained the metabolic activity of the improved flora in more distal parts of the colon (Roberfroid et al., 1998). Therefore, dandelion roots harvested in April can be applied as a rich source of prebiotics in preparation of healthy food and nutrition formula.

The results obtained for total phenolic content, total dihydroxycinnamic derivatives, total flavonoids, anti-radical scavenging activity measured by DPPH and FRAP assays were shown in Table 3. In all cases the subsequent water extracts showed the higher value of antioxidant capacities and phenolic contents in comparison to their 95 % ethanol extract. The amount of total phenolics ranged from 4.5 to 9.2 mg GAE/g dw. The highest total polyphenol content, total dihidrixycinnamic derivatives and total flavonoids were registered by subsequent water extraction (9.2 ± 0.3 mg GAE/ g DW, 13.8 ± 0.1 mg CAE/ g DW and 1.7 mg EQ/g dw, respectively) (Table 3). The highest level of total phenolic content was found in water extracts from dandelion roots gathered from Parvomay location, while the lowest was in ethanol extracts from Chirpan location. In our study the content of total dihidrixycinnamic derivatives in both extracts were higher than reported in teas obtained from the roots 1.2 mg/g cinnamic acids (Williams et al., 1996). The results obtained for total phenolic content and total dihidrixycinnamic derivatives from roots extracted with 95 % ethanol were higher than the reported by Ivanov (2014) for dandelion leaves.

The highest level of total flavonoids was observed in water extracts obtained after ethanol pretreatment. The reported results were lower than their content in roots of common chicory (2.8 ± 0.2, mg EQ/g dw) (Denev et al., 2014).

Taraxacum officinale L. subsequent water roots extracts from Parvomay location demonstrated the highest antioxidant activity (DPPH, 83.1± 3.2 mg TE/g dw; FRAP, 46.9 ± 1.3 mg TE/g dw), while water extracts from Plovdiv location showed high activity defined only by FRAP assay: 52.9±0.3 mg TE/g dw. The reported results for antioxidant activity of dandelion roots were near to previously published data for dandelion leaves (Ivanov, 2014). In the current study the TE values reported for both the DPPH and FRAP assays and phenolic content for roots were higher than those for crude methanol extract (80% v/v) from dandelion described by Wojdylo et al. (2007) - DPPH (53.312 ± 1.191 mg TE/g dw), FRAP (3.979 ± 0.776 mg TE/g dw and the phenolic content 0.126 ± 0.03 mg GAE/g dw, respectively. Our results for radical scavenging activity were near to the data for DPPH and FRAP assays of water extracts from dandelion root reported by Liu et al., (2008), Amin et al., (2013) and higher than the results published by Kenny et al., (2014b) for water extract and dialysates. The reason for the last can be explained with that the results were reported according to the extract, not on the dry weight of the plant, as was in the case of our study. In contrast, the

findings of the present study have shown that water dandelion root extracts had considerably higher total phenolic content and antioxidant activity from the same extract obtained by same extraction procedure from elecampane and common chicory (Denev et al, 2014; Petkova et al., 2015).

According to Schütz et al., (2006b) the healthy-effect of dandelion root were attributed to their high phenolic content, mainly hydroxycinnamic acid derivatives and flavonoids. From the results obtained a strong relationship between antioxidant activity and phenolic content in the extracts for dandelion roots was observed. The presence of inulin and FOS in dandelion roots additionally increase their biological activity and will improve the health benefits for human nutrition.

CONCLUSIONS

The current study represented the first comprehensive report for evaluation of biologically active substances: total fructan, individual sugars, inulin, as well as total phenolic content, total dihydroxycinnamic derivatives, total flavonoids and the radical scavenging activity of dandelion root harvested in spring. The wide distribution of this edible, medicinal plant and its roots reveals the enormous potential for their application as a commercial crop and a cheap natural source of inulin and antioxidants. The carried research has shown the efficacy of extracts from its root to be considered as food additive and natural antioxidant preservatives for functional food production with the potential to delay oxidative stress and to stimulate biffidobacteria growth.

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