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ORIGINAL PAPER

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EFFECT OF PRESSURE LIQUID EXTRACTION AND ULTRASONIC IRRADIATION FREQUENCY ON INULIN, PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY IN BURDOCK (*Arctium lappa* L.) ROOTS

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ABSTRACT

Burdock (*Arctium lappa* L.) roots are traditionally used in folk medicine as diuretic and curing rheumatism, gastritis, gout, throat pain, arthritis and rashes. These pharmacological properties are due to many bioactive compounds such as flavonoids and fructooligosaccharides. Nowadays, the the application of "green" methods for extraction of natural compounds gains more and more attention. The object of the current research was to determine inulin and sugars content, phenolic content and antioxidant potential in 70% ethanol and water extracts obtained by two "green" extraction methods, namely pressure-liquid extraction and ultrasound-assisted techniques. The content of total fructans, as well as inulin and sugars were analyzed by spectrophotometric resorcinol-thiourea method and high-performance liquid chromatography with refractive index detection. Total phenols and flavonoids were determined by Folin-Chiocalteu and $Al(NO_3)_3$ reagents. Antioxidant activity was evaluated by four reliable methods (DPPH, ABTS, FRAP and CUPRAC). The established inulin content varied from 0.27 to 4.0 g/100 g dw in prevalence of the ultrasound-assisted extract obtained with water. Additionally, the established phenolic compounds content (from 10.35 to 18.16 mg gallic acid equivalent (GAE)/g dw) and antioxidant activities demonstrated the burdock roots as potential source phytonutrients with health beneficial properties.

Key words: burdock roots, inulin, antioxidants, "green" extraction techniques

INTRODUCTION

Burdock (*Arctium lappa* L.) is a medicinal plant that belongs to Compositae (Asteraceae) family. Although it is mainly used in folk medicine for curing different diseases, in many Asian countries (Japan, Korea, and Thailand) burdock is also consumed as a vegetable [Jaiswal and Kuhnert 2011]. The health benefits of *Arctium lappa* L. could be aligned as antioxidant, anti-diabetic, anti-inflammatory, anti-carcinogenic, anti-allergic, anti-ulcer, anti-tubercular, anti-acne, anti-sterility, angiostrongyliasis, gastroprotective, hepatoprotective, anti-aging, anti-austeric and anti-cytotoxic effects [Machado et al. 2012, Fierascu et al. 2018]. The therapeutic effect of burdock has been attributed to the biologically active substances present in the roots. These are mainly phenolic acids, flavonoids and fructooligosaccharides [Ferracane et al. 2010, Fierascu et al. 2018]. The presence of phenolic compounds justifies the strong antioxidant activ-

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ity of *A. lappa* extracts. Besides phenolic compounds burdock could be used as a source of inulin. Various extraction methods were previously proposed for the extraction of inulin from burdock in order to obtain higher yield [Lou et al. 2009, Cao et al. 2018, Milani et al. 2012]. At the same time, the plant is still not well studied and more detailed research is needed, in particular in Bulgaria, where burdock is used as a medicinal plant.

Biologically active compounds usually occur in low concentrations in plants. In this point of view, the choice of extraction technique is critical in order to obtain high yield with minimal changes in the functional properties of the extract [Quispe Candori et al. 2008] and for evaluation of the biological activity of an object, as well. Nowadays, a priority is given to so-called "green extraction methods". Chemat et al. [2012] revised the principles of green extraction and gave the following definition: "discovery and design of extraction processes which will reduce energy consumption, the use of alternative solvents and renewable natural products, and ensuring a safe and high-quality extract/product". Supercritical fluid extraction, pressurized-liquid extraction, pressurized hot water extraction, as well as ultrasound-assisted and microwave-assisted extractions are techniques that meet the above mentioned requirements [Ameer et al. 2017]. However, the choice of extraction solvent is also critical. Water can be considered, in many cases, as the greenest solvent [Castro-Puyana et al. 2017], but most often the use of organic solvents (ethanol, methanol, acetone or hydroalcohlic mixtures) are needed for extraction of bioactive compounds, especially for phenolic compounds and antioxidants [Hayouni et al. 2017, Lou et al. 2009]. For food purposes water and ethanol, as well as mixture of them were the most preferred solvents for extraction of bioactive compounds.

Combinations of different solvents with quick an efficient extraction technique are more efficient in recovering antioxidants and other bioactive compounds. Pressure liquid extraction (PLE) technique, known as pressured fluid extraction has some advantages in comparison with classical extraction procedure as elevated pressure and temperature, low volume of organic solvent and authomatization of process [Li et al. 2002]. Advantages of ultrasound-assisted extraction include operation at low temperature and reducion time, energy and expense [Petkova et al. 2017a]. However, the application of pressured-liquid extraction from burdock roots was not demonstrated until this moment. There some studies for extractions only of inulin [Milani et al. 2012] or only caffeic acid Liu et al. [2012] derivatives by ultrasound – assisted extraction, but the for combination of extraction of inulin as prebiotic compound and antioxidants are still absent.

The aim of the current study was to evaluate and compare the content of bioactive compounds, inulin and phenolic compounds in particular, and antioxidant potential of *Arctium lappa* root extracts obtained by two "green" methods of extraction (pressurized-liquid extraction and ultrasound-assisted extraction) at various conditions and thus to reveal the benefit potential of the plant.

MATERIALS AND METHODS

Chemicals, plant material and extracts preparation

All solvents and reagents were of analytical grade from Merck (Darmstadt, Germany) and were used without additional purification.

Dry burdock (*Arctium lappa* L.) roots were purchased from Decrassin Ltd. (Varna, Bulgaria) in 2016. The plant material was washed with tab water, dried at 40°C. Then burdock roots were finely ground in a laboratory homogenizer and different extraction procedures were conducted (Tab. 1).

Pressure liquid extraction (PLE) technique. Burdock roots (100 g) were randomly sampled from 200 g dry plant material. The PLE was carried out for 1.45 h in an automatic equipment (NM LAB/M Deputex 88, Limena, Padova, Italy) with 550 ml of ethanol/water solution (70 : 30, v/v), previously deoxygenated by flushing with nitrogen as reported by Rossetto et al. [2005]. The volume of each sample was measured to calculate the extract concentration (g/L). The extraction rate was 0.2249 g/mL. The extracts were subdivided in dark glass bottles and stored at –20°C [Rossetto et al. 2005] until the analyses. The obtained residues form roots were subsequently extracted with distilled water by ultrasonic irradiation at 35 kHz described below and used for further analysis.

Ultrasound-assisted extraction (UAE) technique. Burdock roots were extracted either with distilled H_2O

Table 1. List of samples obtained by "green" extraction methods of *A. lappa* roots

Sample	Extraction
A	pressure liquid extract obtained with 70% ethanol
B	residues after pressure liquid extraction, treated with water by ultrasonic extraction at 35 kHz
C	ultrasound – assisted extract obtained with water at 35 kHz
D	ultrasound – assisted extract obtained with water at 45 kHz
E	ultrasound – assisted extract obtained with 70% ethanol at 35 kHz
F	ultrasound – assisted extract obtained with 70% ethanol at 45 kHz

or 70% ethanol in a solid to liquid ratio of 1 : 10 (w/v). The extraction procedure was performed under two ultrasonic frequencies for both of the solvents. UAE at 35 kHz was carried out in an ultrasonic bath (SIEL, Gabrovo, Bulgaria, 35 kHz, and 300 W) for 20 min at 75°C. The parallel extraction was performed at 45 kHz at 45°C for 20 min (VWR, Malaysia). The extraction procedure was performed in duplicate. The obtained extracts were filtered and the combined extracts were used for further analysis.

Analysis of total fructans. The fructans content was determined by resorcinol-thiourea reagent [Petkova et al. 2017a]. The absorbance was measured at 480 nm against a blank sample. The results were expressed as fructose equivalent g/100 g dry weight (dw).

HPLC-RID analysis of inulin and sugars. The chromatographic determination of inulin and sugars were performed on an HPLC instrument Elite Chrome (Hitachi, Japan), coupled with refractive index detector (RID) Chromaster 5450 operating at 35°C. The separation was done with mobile phase consisted of distilled water on a column Shodex® Sugar SP0810 (300 × 8.0 mm i.d.) with Pb^{2+} and a guard column Shodex SP-G (5 µm, 6 × 50 mm) at 85°C, flow rate 1.0 mL/min and the injection volume 20 µL [Petkova et al. 2017b].

Determination of total polyphenolic content (TPC). Total phenolic content was determined by a Folin-Ciocalteu reagent [Stintzing et al. 2005]. Five times diluted Folin-Ciocalteu reagent (1 mL) was mixed with 0.2 Ml of the extract and 0.8 mL of 7.5% Na_2CO_3. The reaction was performed for 20 min. Then the absorbance was measured at 765 nm against blank. The results were expressed as mg equivalent of gallic acid (GAE)/g dw.

Total flavonoid content. Total flavonoid content was evaluated according to the method described by Kivrak et al. [2009]. Burdock sample (0.5 mL) was added to 0.1 mL of 10% $Al(NO_3)_3$, 0.1 mL of 1 M CH_3COOK and 3.8 mL of 95% ethanol. After 40 min at room temperature, the absorbance was measured at 415 nm against blank sample prepared without addition of 0.1 mL of 10% $Al(NO_3)_3$. The results were expressed as mg quercetin equivalents (QE)/g dw.

Determination of antioxidant activity

DPPH$^{\bullet}$ radical scavenging assay. The ability of the extracts to donate an electron and scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by the slightly modified method of Brand-Williams et al. [1995] as described by Mihaylova et al. [2015]. Freshly prepared 4×10^{-4} M of DPPH was mixed with the samples in a ratio of 2 : 0.5 (v/v). The absorbance was measured at 517 nM after 30 min incubation. The DPPH radical scavenging activity was presented as a function of the concentration of Trolox equivalent antioxidant capacity (TEAC) and expressed as the µM/g dw (µM TE/g dw).

ABTS$^{\bullet+}$ radical scavenging assay. The radical scavenging activity of the extracts against 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) was determined according to Re et

al. [1999]. Afterward, the $ABTS^{\bullet+}$ solution was diluted with ethanol to the absorbance of 0.70 ±0.02 at 734 nm and equilibrated at 30°C. After the addition of 1.0 mL of diluted $ABTS^{\bullet+}$ to 0.01 mL of samples, the absorbance was read at 30°C after 6 min. The results were expressed as TEAC value (μM TE/g dw).

Ferric-reducing antioxidant power (FRAP) assay. The FRAP assay was carried out according to Benzie and Strain [1999]. Burdock root extracts (0.15 mL) reacted with 2.85 mL of the FRAP reagent for 4 min at 37°C, and the absorbance was measured at 593 nm. The results were expressed as μM TE/g dw.

Cupric ion reducing antioxidant capacity (CUPRAC) assay. The CUPRAC assay was performed according to Apak et al. [2004]. 1 mL of $CuCl_2$ solution (1.0×10^{-2} M) was mixed with 1 mL of Neocuproine methanolic solution (7.5×10^{-3} M), 1 mL NH_4Ac buffer solution (pH 7.0), and 0.1 mL of burdock extract, followed by addition of 1 mL water (total volume = 4.1 mL) and mixed well. Absorbance against a reagent blank was measured at 450 nM after 30 min. The results were expressed as μM TE/g dw.

Statistical analysis

Statistical analysis of the data was performed by analysis of variance using STATISTICA 5.5 (Stat Soft Inc, Tulsa, OK, and USA) software and a probability value of $p \leq 0.05$ was considered to denote a statistical significance difference. Extraction experiments were performed in duplicated and all other analyses were replicated three times.

RESULTS AND DISCUSSION

Phenolic compounds and antioxidant activity. The investigated extracts of burdock roots revealed the presence of both flavonoids and total phenolic compounds (Tab. 2). Among the six studied samples, the total phenolic content ranged between 2.20 ±0.03 and 18.16 ±0.09 mg GAE/g dw in prevalence for the ultrasound-assisted extract obtained with water at 45 kHz. It has to be noticed that when UAE was conducted with ethanol the increase of the frequency from 35 to 45 kHz did not seem to be reasonable in respect of the biologically active substances yield. Despite of this, the extraction with water showed better results when the frequency of 45 kHz was applied. However, among the conducted extraction techniques the ultrasound-assisted procedure was more effective in order to achieve a better yield of phenolic compounds in comparison with the pressure liquid extraction technique. This was possibly due to the method – ultrasound-assisted solvent extraction is a process that uses high intensity and high frequency sound waves, and solvents to obtain targeted compounds from various matrices. Physical and chemical properties of the materials subjected to ultrasound are altered due to the propagation and interaction of sound waves as they disrupt the plant cell walls, thereby, facilitating the release of extractable compounds and enhancing mass transport of solvent from the continuous phase into plant cells. However, reduced extraction time and solvent consumption are the main advantages of the

Table 2. Total phenolic content (mg GAE/g dw), total flavonoids (mg QE/g dw) and antioxidant activity (μM TE/g dw) of *A. lappa* extracts

Sample	TPC	TF	DPPH	ABTS	FRAP	CUPRAC
A	12.13 ±0.34a	6.31 ±0.25a	390.86 ±2.97e	212.77 ±6.71f	320.39 ±1.04a,b	494.62 ±3.05d
B	2.20 ±0.03b	4.31 ±0.11b	85.67 ±0.92b	47.00 ±1.13b	72.53 ±0.56a	112.25 ±1.58a,b
C	16.70 ±0.15b	5.51 ±0.12b	179.99 ±1.75c	166.33 ±2.87d	181.48 ±1.35b	268.63 ±2.12c
D	18.16 ±0.09b	7.74 ±0.15b	136.14 ±0.95b	69.71 ±1.18b	106.26 ±3.20d	186.13 ±1.16b
E	17.74 ±0.91c	6.78 ±0.23a	106.47 ±1.87c	172.47 ±2.32c	376.20 ±1.30b	484.53 ±7.98e
F	10.35 ±0.54d	6.33 ±0.20a	187.22 ±1.43a	86.13 ±1.45a	211.33 ±1.70c	298.72 ±9.35f

Values are mean ±standard deviation of three separate experiments. Different alphabetic lettersindicate significant differences ($p < 0.05$) among extraction methods

method. The highest yield in the case of ultrasound-assisted solvent extraction may be explained in terms of the cavitation effects caused by high intensity ultrasound [Li et al. 2004].

In comparison with our results, Horng et al. [2013] reported that the content of total polyphenols in burdock extract obtained by reflux extraction for three hours reached to 48.4 ±5.6 mg/g (mg gallic acid/g burdock extract). It was demonstrated that the content of total phenols in the burdock roots was lower in comparison with the seeds and leaves content [Cai et al. 2004, Ferracane et al. 2010]. However, our results were higher compared to the results of Ferracane et al. [2010], who reported the phenolic content as 2.87 g/100 g for UAE obtained with methanol/water (70 : 30, v/v) at room temperature.

The content of flavonoids was established to be from 4.31 to 7.74 mg QE/g dw (Tab. 2). Total maximum flavonoid content was evaluated in the UAE obtained with water at 45 kHz (7.74 ±0.15 mg QE/g dw), which was followed by UAE with ethanol at 35 kHz (6.78 mg QE/g dw). A similar trend as in the case of the total phenolic compounds extraction prepared using UAE with water at 45 kHz was observed. Obviously, the water extracts or water containing extracts were rich in bioactive components. A similar observation was reported by Lee and Kim [2017] when comparing water and methanol extractions of burdock roots.

In our study, four reliable methods – DPPH, ABTS, FRAP, and CUPRAC assays were used for the determination of the antioxidant activity of the different extracts prepared using PLE and UAE. The antioxidant capacities of the burdock extracts were expressed as μM TE/g dw (Tab. 2).

The results varied among the assays conducted from 47.00 ±1.13 to 494.62 ±3.05 μM TE/g dw. Despite the highest yield in respect of flavonoids and total phenolic compounds in the ultrasound-extracts according to DPPH, ABTS and CUPRAC assays, the highest potential was established for PLE of burdock roots, followed by ultrasound-assisted extract with 70% ethanol at 35 kHz (Tab. 2). This revealed possible contribution of other constituents of the extract to the antioxidant capacity. Furthermore, Duh [1998] established higher antioxidant activity in a linoleic acid system for the water extract (96.3 ±0.047%) when comparing with ethanol extract (92.8 ±0.368%) and Petkova et al. [2017b] reported 245.12 mM TE/g dw of water microwave extract of burdock toward DPPH radical. In contrary, according to the FRAP assay the highest results for the ultrasound-assisted extract obtained with 70% ethanol at 35 kHz was recorded – 376.20 ±1.30 μM TE/g dw, revealing the influence of the extracted phenolic compounds. In this regard, Ferracane et al. [2010] reported that burdock roots contain chlorogenic acids, ester of caffeic acid, and quinic acid. Liu et al. [2012] demonstrated that caffeoylquinic acids and lignans are responsible for the antioxidant activity of burdock roots. Therefore, phenolic compounds inflated the antioxidant activity of obtained burdock extracts.

Inulin and sugars content

The results of carbohydrates content in burdock roots were summarized in Table 3, and the detected carbohydrates (inulin and sugars) were shown in Figure 1.

All extracts were established to consist of inulin, fructooligosacharides (nystose and 1-kestose), sugars (sucrose, glucose, and fructose) (Tab. 3). In the residues after pressure liquid extraction 1-kestose and fructose were not found (sample B). The total fructans content in burdock root extracts ranged from 0.6 ±0.11 to 9.27 ±0.10 g/100 g dw. However, regarding the yield of inulin and fructans, the ultrasound-assisted water extraction revealed as the preferred method for extraction. This could be explained with the efficiency of inulin extraction due to the cavitation process [Lou et al. 2009]. UAE at high temperature and frequency (35 kHz) gave better results for inulin extraction, than this with high frequency and low extraction temperature (sample D). In water extracts (samples C and D) the amount of inulin was higher than in the ethanol extracts (samples A, E, and F). It could be explained with better solubility of inulin. On the other hand, fructooligodsacchrides (nystose and 1-kestose) presented in both ethanol and water extracts. Moreover, the total amount of detected fructooligosacchrides was 20–25% of total fructan content. The higher amount of fructose in water extracts (samples C and D) could be due to partial hydrolysis of inulin during the ultrasonic extraction. Inulin content in ultrasound-assisted water extract (sample C) was comparable with our previous data regarding microwave-assisted water extraction

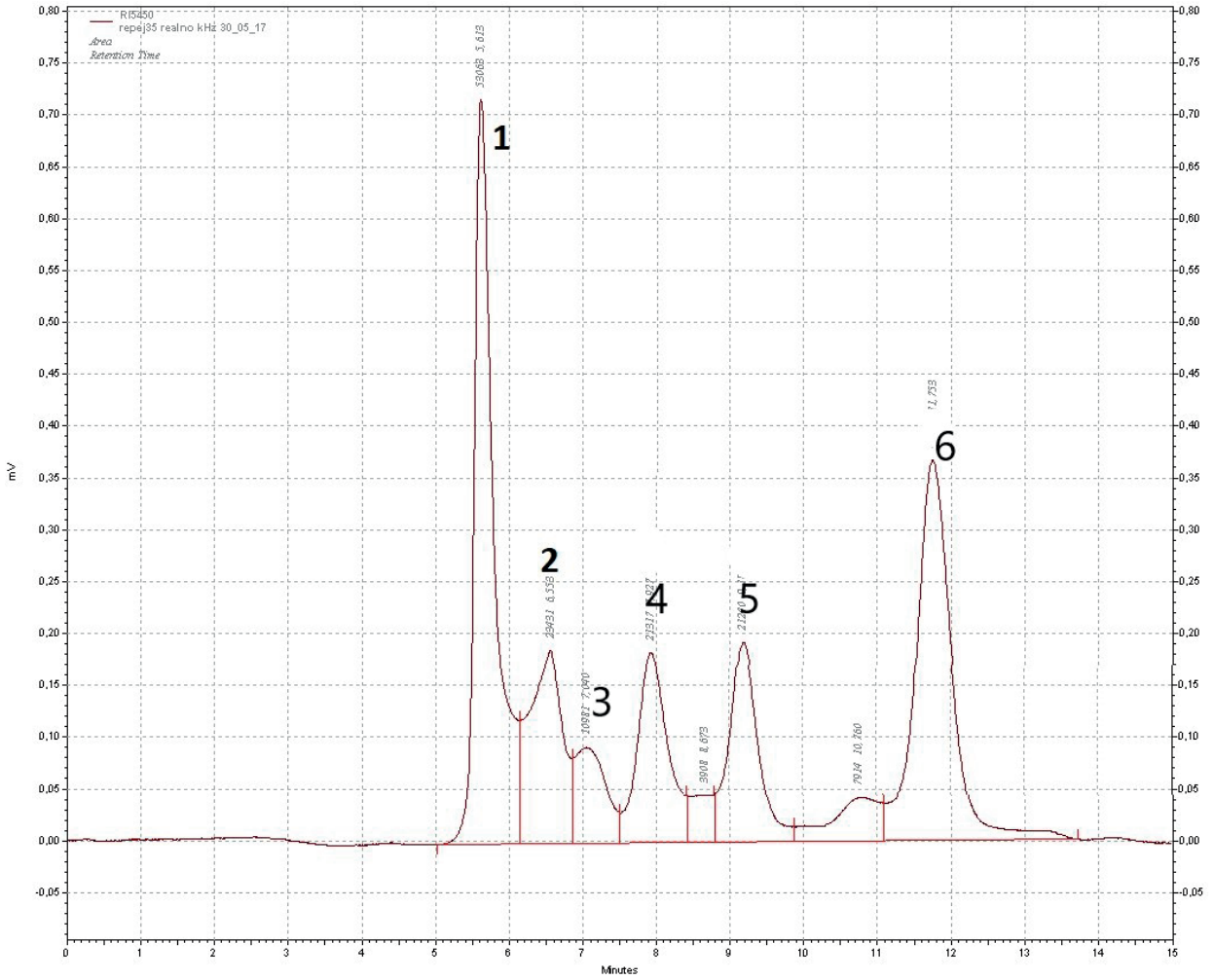

Fig. 1. HPLC-RID chromatogram of water burdock root extract obtained by UAE 35 kHz (sample D), where 1 – inulin, 2 – nystose, 3 – 1-kestose, 4 – sucrose, 5 – glucose and 6 – fructose

(4.7 g/100 dw) [Petkova et al. 2017b]. The established in the present study inulin content of *A. lappa* extracts was in correspondence with other authors reports (5.2–4.7% dw) [Olennikov and Tankhaeva 2011, Petkova et al. 2017b]. However, inulin content in the burdock roots collected from Bulgaria was lower compared to Russian representatives (inulin content up to 30%) [Bagaoutdinova et al. 2001] and to Korean origin plants (9% dw) [Cao et al. 2018].

In addition, there are also some contradictory reports about the quantity of inulin in burdock (*A. lappa*) roots. According to some authors, inulin content varied between 12 and 17% in fresh root (or 50–70% dried weight) or even of 3.6% in fresh plant material [Van Loo et al. 1995]. It was also demonstrated that burdock roots are a valuable source of fructooligosacchride (17%) with a degree of polymerization 13 [Zhang et al. 2018]. Milani et al. [2012] demonstrated the efficiency of the extraction process of inulin from burdock roots using high intensity ultrasound irradiation and reported 12% yield of inulin after optimization of conventional extraction. However, in our study, the highest yield of inulin by UAE did not exceed 4 g/100 g dw. The variation of inulin content could be explained with cold climate conditions, geographical origin of burdock and storage conditions [Ishiguro

Table 3. Inulin and sugars content in different extracts of *A. lappa* roots (g/100 dw)

Sample	Total fructans	Inulin	Nystose	1-Kestose	Sucrose	Glucose	Fructose
A	5.56 ±0.12b	1.10 ±0.05b	0.30 ±0.05b	0.83 ±0.11b	1.33 ±0.25c	0.49 ±0.11b	2.30 ±0.25a
B	0.60 ±0.11a,b	0.27 ±0.02b	0.03 ±0.01b	n.d.	0.07 ±0.01b	0.08 ±0.25c	n.d.
C	7.24 ±0.10b	4.00 ±0.34a	1.90 ±0.34a	0.70 ±0.15c	1.45 ±0.15d	1.45 ±0.15b	3.14 ±0.11b
D	9.27 ±0.10b	2.78 ±0.13c	1.44 ±0.25c	0.70 ±0.13a	0.91 ±0.12a	1.27 ±0.05b	3.44 ±0.89d
E	5.43 ±0.10a	1.10 ±0.07b	0.30 ±0.06b	0.70 ±0.12b	1.23 ±0.08b	1.31 ±0.30a	2.10 ±0.25a
F	6.87 ±0.12b	0.99 ±0.03b	0.18 ±0.05b	0.50 ±0.10b	1.55 ±0.05b	1.16 ±0.15b	1.99 ±0.34c

Values are mean ±standard deviation of three separate experiments. Different alphabetic lettersindicate significant differences ($p < 0.05$) among extraction protocols; n.d. – not detected

et al. 2010, Petkova et al. 2017b]. The carbohydrate metabolism in the stored burdock depends partly on temperature and other physiological factors [Ishiguro et al. 2010].

In a previous study, UAE was evaluated as a promising approach for the simultaneous extraction of bioactive compounds (dietary fibers and antioxidants) from elecampane roots in comparison with microwave irradiation [Petkova et al. 2017b]. However, microwave-assisted extraction was also successfully performed for extraction of fructans [Petkova et al. 2017a], inulin and ten sugars from burdock roots [Li et al. 2013]. It has to be noted that despite of the low values of some bioactive compounds, the present research is the first report demonstrating the application of pressure liquid extraction of phenolic compounds and fructans (including fructooligosacharides and inulin) from burdock roots. The PLE and UAE techniques showed promising results for preparation of 70% ethanol burdock extracts with high antioxidant potential. However, regarding the extraction of soluble dietary fiber with prebiotic effect (including inulin and fructooligosaccharides) ultrasound-assisted water extraction seems to be the better approach.

CONCLUSION

The present study aimed to demonstrate different "green" extraction techniques in order to obtain biologically active substances of *A. lappa* (burdock) roots. In this point of view, PLE and UAE were conducted and the extracts were analyzed for the total polyphenolic and flavonoid contents, total fructans, inulin and sugars contents, and antioxidant activity. The investigated samples consisted of bioactive compounds in moderate amounts, which could be attributed to the influence of the extraction process itself. The results revealed that ultrasound-assisted technique was more effective in respect of total polyphenolic and flavonoid contents, total fructans, inulin and sugars contents in contrary to the better result in respect of antioxidant activity established for the pressure liquid extract. The reported data provided valuable information about the most suitable extraction approach of *A. lappa* as a source of beneficial health effects in respect of fructooligosaccharides, phenolic compounds, and antioxidant activities.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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