





# Phytochemical Composition and Antimicrobial Properties of Burdock (*Arctium lappa* L.) Roots Extracts

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**Abstract:** Burdock (*Arctium lappa* L.) roots were used as a medicinal plant or vegetable worldwide. The research aimed to obtain different fractions by sequential extraction (hexane, chloroform, ethyl acetate water) of burdock roots and to evaluate phytochemical compounds in them. Antioxidant and antimicrobial properties of nonpolar fractions were evaluated. Ethyl acetate fraction contained the highest total phenolics, total flavonoids, and derivatives of caffeic acids. Phenolic acids (mainly chlorogenic, caffeic acid, and p-coumaric acids) were detected only in the ethyl acetate fraction, while in the hexane fraction was found only triterpenes. Due to the high polyphenol content, the ethyl acetate fraction demonstrated the highest antioxidant activity. Three fractions revealed antimicrobial activity against *Salmonella* sp., *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus cereus*, and *Candida albicans*. The lowest minimum inhibitory concentration (>1 mg/ml) showed an ethyl acetate fraction against *Pseudomonas aeruginosa* and *Bacillus cereus*. From the water fraction was isolated inulin-type fructan with a degree of polymerization 20-24 with promising functional properties. This study showed the potential application of burdock root fractions as a rich source of phytochemicals with antimicrobial, antioxidant activities, and potential prebiotic effect due to the 7 g/100 g inulin content in them.

**Keywords:** burdock, inulin, phenolic compounds, antimicrobial activity

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## 1. Introduction

*Arctium lappa* L. (*A. lappa* L.), commonly known as burdock or gobo (in Japan), bardane in (France and Italy), is a medicinal plant from Asteraceae family that originates from Asia and Europe and it is widespread and adapted for cultivation in different climates and continents [1–3]. The vegetation period varies from 8 to 12 months as its yield can reach from 8 to 40 t/ha. Moreover, it has been widely consumed as a vegetable in East Asia for centuries. It has a cylindrical shape (45-50 cm long and 3-6 cm in diameter), thin brown skin, and the inner part is from white to yellowish-white [1]. In European countries, burdock root is consumed as an herbal infusion, decoction, or tincture [4]. Extracts prepared from the different parts of the burdock plant possesses various biological activities and pharmacological

functions, including antioxidant, anti-inflammatory, anticancer, antidiabetic, antimicrobial, antiviral effects, anti-allergic, antiulcer, anti-tubercular, anti-sterility, angiostromyoliasis, gastroprotective, anti-esoteric, hepatoprotective, anti-cytotoxic effect and antiacne [4-14]. The water extract of *Arctium lappa* L. roots enhanced chondrogenic medium-induced chondrogenic differentiation [13], improved abdominal obesity and sex hormones in elderly women with metabolic syndrome [15], and demonstrated aphrodisiac effect in experimental rats [16]. The root extracts also demonstrated gastroprotective activity and neuroprotective effects due to several monocaffeoylquinic acids and dicaffeoylquinic acids [14]. The main biologically active substances in burdock roots comprise volatile compounds, tannin,  $\beta$ -eudesmol, caffeic acid, chlorogenic acid, inulin, trachelogenin, sitosterol- $\beta$ -D-glucopyranoside, lappaol, terpenes, polyynes, arctiin, arctigenin, and diartctigenin [14,17-22].

*A. lappa* L. roots have been received increasing interest, not only because of phenolic compounds but also for the content of polysaccharides with various health-beneficial properties [1,6]. Its roots contain pectic polysaccharides (993 mg uronic acid /100 g), as 53.9% of them characterized as low methoxyl pectin with a degree esterification 38% [23]. Anti-inflammatory activity of alkali-soluble polysaccharides from *Arctium lappa* L. was also demonstrated [24], while citric acid extracted pectin showed anti-constipation activity [25].

Literature deals mostly with the extraction methods, composition changes, structure identification, prebiotic effects, antitussive and anti-colic activity of fructan from burdock [7, 17, 18, 26]. Moreover, it was reported for isolation of linear  $\beta$ -1,2-glucofructan from *A. lappa* roots with a degree of polymerization 18–19 ( $M_w = 2.95$  kDa) with an antitussive effect and immunomodulatory activity, comparable with zymosan [18, 22]. Fuchigami et al. (1990) fractionated from the roots of Japanese edible burdock inulin with a molecular weight of about 5 kDa [23]. Lei [27] elucidated the fructan structure that comprised of D-Fruf and -D-Glcp in the molar ratio of 14:1. Liu et al. (2014) isolated inulin-type fructan from the roots of *Arctium lappa* with fructose and glucose in the ratio of 13.0:1.0, and with an average molecular weight of 4600 Da, possesses *in vitro* and *in vivo* antioxidant activity [7]. Prebiotic ingredients from burdock roots were isolated without organic solvents and wastes [18]. The accelerated method as ultrasonic and microwave irradiation and pressure-liquid extraction was performed to optimize fructan extraction from *A. lappa* roots, and inulin content was measured in the extracts [8,20,28]. Until now, the characteristics of inulin from burdock water fraction isolated by microwave irradiation were absent. Moreover, the fractional extraction of bioactive compounds using solvents with different polarities was not investigated in detail.

The aim of the current study was to obtain different fractions by sequential extraction of burdock roots and to evaluate phytochemical compounds in them. In addition, antioxidant and antimicrobial activities of nonpolar fractions were also of interest.

## 2. Materials and Methods

### 2.1 Materials.

The dried roots of burdock (*Radix Arctii Lappae*) produced by ALIN Company (Alino village, Samokov, Bulgaria) were bought from the local drugstore in Plovdiv, Bulgaria. The roots were finely ground in a laboratory homogenizer and sieved through 0.5 mm. All reagents were analytical grade (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany).

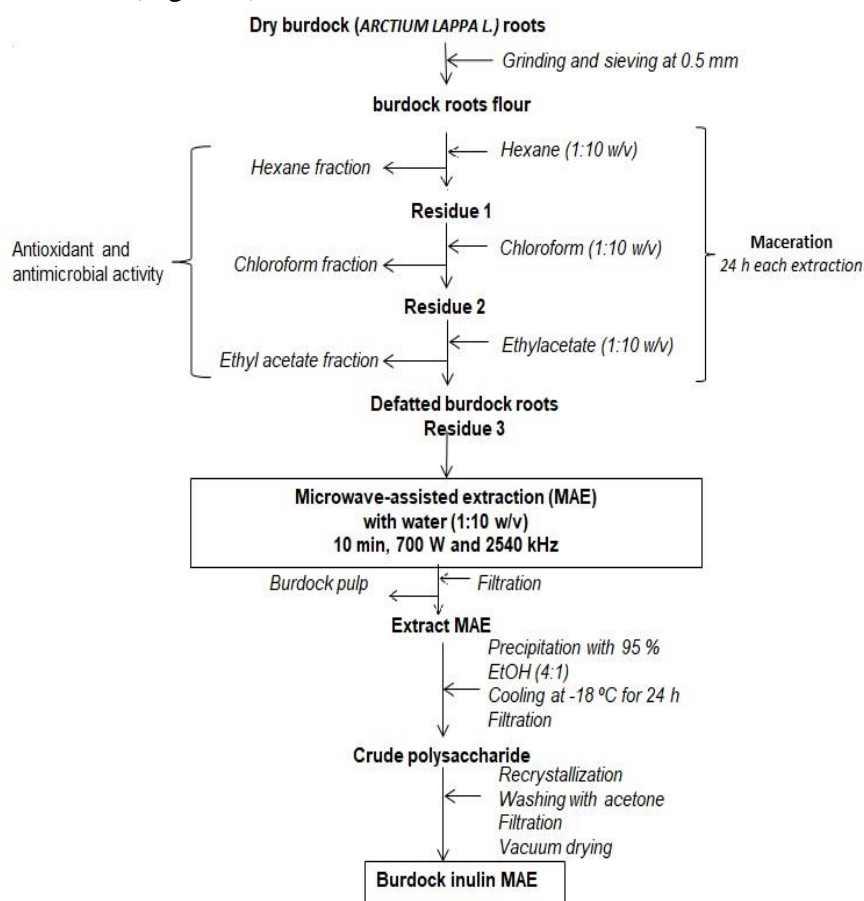
## 2.2. Moisture content.

The moisture content of roots was determined according to the AOAC method [29].

## 2.3. Sequential solvent extraction and fructan isolation by microwave extraction.

Extraction using solvents with different polarities was performed. Powdered burdock roots (100 g) were extracted successively with solvents in the following order: hexane, chloroform, and ethyl acetate by maceration for 24 h at 25°C. The collected three extracts were filtered and evaporated to dryness by removing the solvents under a vacuum on a rotary evaporator (Figure 1). Then these three extracts were dissolved in methanol, and their antioxidant and antimicrobial activities were tested.

For fructan extraction, the residual plant material after drying was extracted with distilled water (at a solid to solvent ratio of 1:10 w/v) in duplicate using a microwave device (Daewoo KOR, with microwave power 700 W and frequency of 2450 MHz) for 5 mins duration of each extraction. The cooled filtrate was precipitated with 95 % ethanol (1:4 v/v). The precipitate was separated by centrifugation and then purified by recrystallization of boiling water and precipitated with acetone (1:5 v/v). The resulting polysaccharide was filtrated and dried under a vacuum (Figure 1).



**Figure 1.** Sequential solvent extraction of *Arctium lappa* L. roots.

## 2.4. Total phenols.

The total phenolic content (TPC) was evaluated by the Folin–Ciocalteu’s reagent [30]. Burdock root extract (200 µL) was mixed with 1 ml Folin–Ciocalteu reagent diluted five times and then 800 µL 7.5% Na<sub>2</sub>CO<sub>3</sub> was added to the mixture. The sample absorbance was read at

765 nm after 20 min against a blank. The data were shown in mg equivalent of gallic acid (GAE) g/dry extract.

#### 2.5. Total dihydroxycinnamic derivative (DCA).

The total dihydroxycinnamic acid (including caffeoyl derivatives) content was expressed as chlorogenic acid derivatives per g extract [31].

#### 2.6. Total flavonoids.

Al(NO<sub>3</sub>)<sub>3</sub> reagent were used for estimation of the total flavonoids. The sample was read at 415 nm. The data were expressed as mg equivalents quercetin (QE) per g extract according to the quercetin calibration curve [32].

#### 2.7. HPLC analysis of terpenes and phenolic acids.

Burdock extracts were dissolved in methanol. Analysis of triterpene was done on a Hitachi LaChrom Elite®HPLC System (Hitachi High Technologies America, Inc., Schaumburg, Illinois, USA), with diode-array detector (DAD, L-2455) and EZChrom Elite™ software. The separation of oleanolic was performed on a reverse-phase column Supelco, Discovery®HS C18 (5µm, 25 cm×4.6 mm) at 26°C with mobile phase methanol:0.1% HCOOH = 92:8 (v/v) at a flow rate of 0.4 mL/min in an isocratic operation mode [33]. HPLC analysis of phenolic acids was performed as previously described [34] using the same apparatus and column, operated at 30°C with gradient mode with mobile phase consist of 2% (v/v) acetic acid (solvent A) and acetonitrile (solvent B) and the flow rate was 0.8 mL/min. The detection was performed at 280 and 320 nm,. The results were presented as mg of the respective phenolic acid per g extract [34].

#### 2.8. Antioxidant activity.

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method and ferric reducing antioxidant power (FRAP) method were used to evaluate antioxidant activity.

##### 2.8.1. DPPH method.

For DPPH method, burdock extract (150 µL) was added to 2.85 ml 0.1 mM DPPH dissolved in methanol. The absorption was measured after 15 min at 37 °C at 517 nm in comparison to the blank containing methanol, and % inhibition was calculated [32].

##### 2.8.2. FRAP method.

The FRAP method was performed with some modifications [35]. The sample extract (100 µL) was added to 3.0 ml FRAP reagent. After 10 min at 37 °C in darkness, the absorbance was measured at 593 nm against a blank. Antioxidant activity was expressed as mM Trolox® equivalents (TE) per g extract [32].

#### 2.9. Antimicrobial activity.

The agar well diffusion method was used for the antimicrobial activity [36]. The selected microorganisms from the collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria, were used: Gram-positive (*Listeria*

*monocytogenes*, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* 52/G11), Gram-negative (*Salmonella* sp., *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Proteus vulgaris*), and yeast *Candida albicans*. The test bacteria (except of *Bacillus cereus* 52/G11) and yeasts were cultured on Luria-Bertani agar medium supplemented with glucose (LBG agar) at 37 °C for 24 hours as previously described [37]. *B. cereus* 52/G11 was cultured on LBG agar medium at 30 °C for 24 hours.

Minimal inhibitory concentration (MIC) of hexane, chloroform, and ethyl acetate burdock extracts was determined by a series of twofold dilutions of the extract, ranging from 10 to 0.079 mg/mL were prepared. The samples were pipetted in the quantity of 50 µL per well in a preliminary inoculated with the test microorganisms LBG agar media. The Petri dishes were incubated at the above-mentioned conditions. The MIC values were evaluated as the lowest concentration of the extract that completely inhibited the growth of tested microorganisms around the agar well [36].

The MIC values evaluated as mg/mL were recalculated and expressed as mg/50 µL (quantity of the extract in the agar wells). Ampicillin (10 µg/ml), Kanamycin (6 mg/ml), Streptomycin (6 mg/ml), and Penicillin (1.2 mg/ml) were used as standards, while only for *Candida albicans* Nystatin (40 µg/ml) was applied.

#### 2.10. Total fructans and HPLC-RID analysis of inulin and sugars in water extract.

The water extraction was performed as previously described procedure [20]. The fructans content in water burdock extracts was determined by spectrophotometer using resorcinol-thiourea reagent at 480 nm [38]. Inulin and sugars were analyzed on an HPLC instrument Elite Chrome Hitachi (Japan), coupled with refractive index detector (RID) Chromaster 5450. The separation was performed with mobile phase distilled H<sub>2</sub>O on a column Shodex® Sugar SP0810 (300 mm × 8.0 mm i.d.) with a guard column operating at 85°C with a flow rate 1.0 ml/min.

#### 2.11. Characterization of isolated fructan.

##### 2.11.1. Physicochemical characterization.

The melting point of isolated inulin was measured with a melting point apparatus Kofler apparatus, coupled with a camera. The reducing groups were determined by the PAHBAH method at 410 nm [39], while total fructose content by resorcinol-thiourea reagent at 480 nm [38]. The purity of burdock inulin was analyzed by HPLC instrument Elite Chrome Hitachi with a Shodex® Sugar SP0810 (300mm × 8.0mm i.d.) at 85°C, a flow rate 1.0 ml/min and the injection volume 20 µl [40].

##### 2.11.2. Homogeneity and molecular weight .

High-performance size-exclusion chromatography (HPLC-SEC) was used for the determination of number average molecular weight (M<sub>n</sub>), and weight average molecular weight (M<sub>w</sub>) was used. Analysis of burdock inulin was performed on HPLC chromatograph ELITE LaChrome (VWR Hitachi, Japan) on a column Shodex OH-pack 806 M (ID 8mm and length 300 mm), (Shodex Co., Tokyo, Japan) at 30°C and a RI detector (VWR Hitachi Chromaster, 5450, Japan) with 0.1M NaNO<sub>3</sub> [41]. Polydispersity index (X) of inulin was calculated as the ratio of the two molecular weights (M<sub>w</sub>/M<sub>n</sub>).

#### 2.11.3. Fourier transformation infrared spectroscopy (FT-IR) spectroscopy.

Burdock inulin (2 mg) was pressed into KBr tablets. The FTIR spectrum was collected on a Nicolet FTIR Avatar Nicolet (Thermo Scientific, USA) spectrometer in the wavelength range of 4000–400  $\text{cm}^{-1}$  after 132 scans at a resolution of 2  $\text{cm}^{-1}$ .

#### 2.11.4. NMR spectroscopy.

$^{13}\text{C}$  NMR spectra were recorded using a Bruker AVIII 500 MHz spectrometer operating at a frequency of 126 MHz, respectively. Inulin sample was dissolved in 99.95 %  $\text{D}_2\text{O}$  (25 mg/0.6 ml).

#### 2.12. Functional properties of burdock inulin.

Swelling properties of isolated inulin were evaluated as previously described [42]. The water-holding and oil-holding capacities of burdock inulin were evaluated [42,43]. Briefly, inulin (100 mg) was added to the preweighed 50 ml polypropylene centrifuge tubes and 10 ml deionized water or sunflower oil was added, respectively. After 24 h at 20°C the samples were centrifuged at 3500 rpm for 15 min. The excess of water and oil was removed. The tubes were then weighed and dried at 105°C to constant weight.

#### 2.13. Statistical analysis.

All analyses were performed in triplicate ( $n = 3$ ) and replicated at least twice. Statistical analysis 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.

### 3. Results and Discussion

#### 3.1. Bioactive compounds and antioxidant activity in burdock fractions.

In our study, the initial moisture content in dry burdock roots was  $12.9 \pm 0.2\%$ . The results for yield and phytochemical composition of three burdock root extracts were summarized in Table 1. The highest yield was detected in hexane fraction  $3.5 \pm 0.2\%$ . Only in this extract were triterpenes found, especially oleanolic acid – 268.2 mg/g dry extract. As a nonpolar solvent, it dissolves mainly lipophilic molecules. However, the hexane extract demonstrated the lowest levels of total phenolic compounds and the radical scavenging activity evaluated by the DPPH method due to the lowest phenols. The lowest extraction yield had chloroform extract 1%, which also contained not only total phenols but also and total flavonoids (Table 1), as well. However, any phenolic acids were not detected in it. This could explain the highest antioxidant activity of this fraction comparing to the hexane fraction. In general, the highest value of bioactive phenolic compounds was detected in the third subsequent fraction obtained by ethyl acetate.

Ethyl acetate fraction did not contain triterpenes (oleanolic and ursolic acids), but it was rich in phenolic compounds. The level of total phenols and total flavonoids were the highest among this analyzed burdock root fraction -  $45.5 \pm 0.5$  mg GAE/g extract and 7.9 QE/g dry extract, respectively. Similar results for total phenols in burdock extract were reported by Horng *et al.* (2013), who found the content of  $48.4 \pm 5.6$  mg/g (mg gallic acid/g extract) [44].

The content of flavonoids in burdock roots varied in the range from 4.31 to 7.74 mg QE/g dw [20].

**Table 1.** Terpenes, phenolic compounds, phenolic acids, and antioxidant activity per g dry burdock extracts.

Phytocomponents	Extracts		
	Hexane	Chloroform	Ethyl acetate
Yield, %	3.5±0.2 <sup>b</sup>	1.0±0.1 <sup>a</sup>	1.5±0.2 <sup>b</sup>
Terpenes, mg/g			
Oleanolic acid	268.2±0.5 <sup>a</sup>	absent	absent
Phenolic acids, mg/g			
Chlorogenic acid	absent	absent	5.0±0.2
Caffeic acid	absent	absent	0.4±0.1
p-Coumaric acid	absent	absent	2.1±0.2
Cinnamic acid	absent	absent	traces
Gallic acid	absent	absent	traces
Total phenolic acids	absent	absent	7.5±0.2
Total phenols, mg GAE/g extract	5.1±0.1 <sup>a</sup>	9.4±0.2 <sup>b</sup>	45.5±0.5 <sup>c</sup>
TDCA, mg CE/g	absent	absent	34.9±0.5
Total flavonoids, mg QE/g extract	absent	3.5±0.1	7.9±0.3
Antioxidant activity, mM Trolox/g extract			
DPPH	8.9±0.1 <sup>a</sup>	41.1±0.3 <sup>b</sup>	308.3±6.1 <sup>c</sup>
FRAP	absent	absent	169.5±0.5

Values are mean ± standard deviation. Different letters within each column indicate significant differences according to Tukey’s test at  $p < 0.05$ .

It was reported that burdock roots contain chlorogenic acids, ester of caffeic acid, and quinic acid [6]. In our case, five phenolic acids (chlorogenic, caffeic, p-coumaric, cinnamic, and gallic) were detected, as chlorogenic acid was in the highest amount - 5 mg/g extract. Due to the high content of chlorogenic acids, roots possessed a bitter and astringent taste [1]. Phenolic constituents as caffeic acid and chlorogenic acid possess a strong inhibitory effect on herpes virus (HSV-1, HSV-2) and adenovirus (ADV-3, ADV-11) [3]. It was published that among phenolic compounds, the chlorogenic acids were with the highest nutritional value in the burdock roots [17]. Literature data also showed that burdock roots contain caffeic acid derivatives (chlorogenic acid, caffeic acid, cynarin), quercetin [6].

Caffeoylquinic acid derivatives were one of the main active ingredients of burdock roots, such as 1,3-, 1,4-, 1,5-dicaffeoylquinic acids [45] and possessed antiulcer activity [46] and neuroprotective effects [14]. In this study, the level of TDCA was 34.9 mg/g extract, which is 76% of the total phenolic content detected in the burdock ethyl acetate fraction. The highest antioxidant activity by DPPH method was evaluated in ethyl acetate burdock fraction - 309 mM TE/g extract. Moreover, this extract also demonstrated metal-reducing activity evaluated by FRAP method.

The better antioxidant potential of ethyl acetate fraction could be explained by the high content of phenolic compounds in it. An increase in the antioxidant capacity was observed with the increase in the number of caffeoyl units [17], especially on the quinic ring [14].

### 3.2. Antimicrobial activity.

Three sequential fractions (hexane, chloroform, and ethyl acetate) were tested for antimicrobial activity against *Salmonella* sp., *Escherichia coli* ATCC 8739, *Listeria monocytogenes*, *Pseudomonas aeruginosa* ATCC 9027, *Proteus vulgaris*, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 52/GI1 and *Candida albicans* (Table 2).

**Table 2.** Antimicrobial properties of root extracts from *Arcticum lappa* L. (MIC, mg/ml).

Microorganisms	Extract			Amp	Kan	Str	Pen	Nys
	Hexane	Chloroform	Ethyl acetate					
<i>Salmonella</i> sp.	>10	>10	5	28	20	15	25	na
<i>E. coli</i>	>10	>10	5	30	20	20	24	na
<i>L. monocytogenes</i>	>10	>10	>10	32	27	27	30	na
<i>P. aeruginosa</i>	>10	>10	0.16	-	17	32	-	na
<i>P. vulgaris</i>	>10	>10	1.25	-	22	22	13	na
<i>S. aureus</i>	>10	>10	>10	32	25	23	28	na
<i>B. cereus</i>	>10	>10	0.31	28	30	30	20	na
<i>C. albicans</i> *	>10	>10	2.5	na	na	na	na	13

Legend: Extracts from *Arcticum lappa* L. where: controls: Amp – Ampicillin (10 µg/ml); Kan - Kanamycin (6 mg/ml); Str – Streptomycin (6 mg/ml); Pen - Penicillin (1.2 mg/ml), \*Nystatin (40 µg/ml) – only for *Candida albicans*. na – not applied.

In general, the tested burdock fractions showed better antimicrobial activity in comparison to the used antibiotic controls Ampicillin (10 µg/ml), Kanamycin (6 mg/ml), Streptomycin (6 mg/ml), and Penicillin (1.2 mg/ml). Hexane and ethyl acetate fractions showed antimicrobial activity against all eight microorganisms in the concentrations of the extract below 10 mg/ml. However, the ethyl acetate burdock fraction demonstrated the highest activity in comparison to the other two fractions. The lowest minimum inhibitory concentration below 1 mg/ml demonstrated ethyl acetate fraction from burdock against *Ps. aeruginosa* and *Bacillus cereus* 52/G11. The lower MIC of ethyl acetate and better antimicrobial activity could be explained with detected phenolic acids - chlorogenic and caffeic acids.

In other research, it was reported that crude 70% ethanol extract of *A. lappa* showed *in vitro* antimicrobial activity against some Gram-positive and Gram-negative pathogenic bacteria, such as *P. aeruginosa*, *S. aureus*, *Salmonella typhimurium*, *E.coli*, *Listeria monocytogenes*, *Streptococcus pyogenes*, *Brucella abortus*, and *Bacillus anthracis* in concentration (50-100 mg/ml) higher than that in our study [47]. It was also reported that the volatile constituents of roots exhibited moderate antimicrobial activity against bacteria (*Escherichia coli* and *Bacillus subtilis*) and significant antifungal activity (*Candida albicans* and *Aspergillus niger* ferm-Bam C-21) [48]. The antimicrobial activity hydroalcoholic extract of burdock roots was tested by the serial dilution method against bacterial strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Salmonella abony* NCTC 6017) [49]. Moreover, Gentil *et al.* (2006) demonstrated that ethyl acetate fraction *A. appa* was used effectively as an intracanal medication for 5 days in teeth infected with *C. albicans*, *E. coli*, *L. acidophilus*, *P. aeruginosa* [50]. Therefore, the current research enriched the information about the antimicrobial potential of ethyl acetate, as parallel with it revealed the antimicrobial activity of the other two fractions.

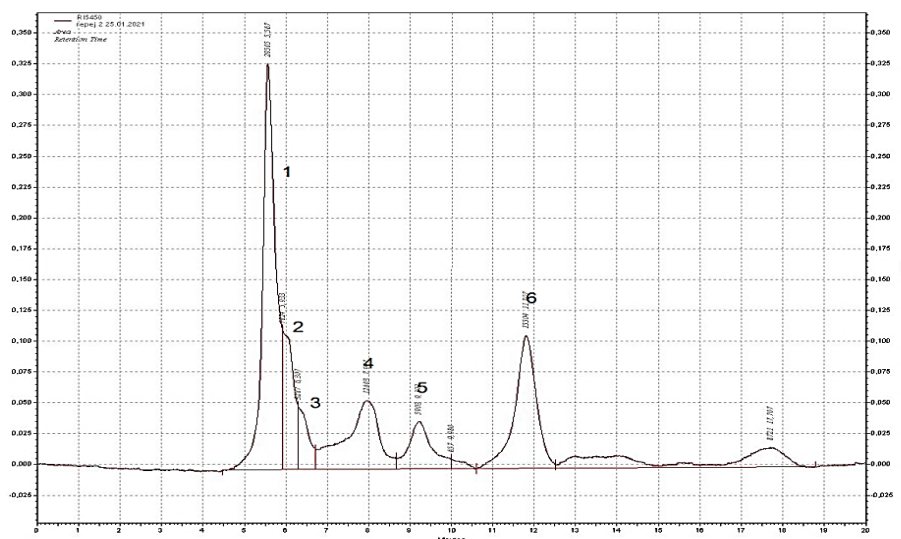
### 3.3. Carbohydrate composition of water fraction.

Carbohydrate content detected in water extract from burdock roots was shown in Table 3. The carbohydrates - inulin, fructooligosaccharides (nystose and 1-kestose), sugars (sucrose, glucose, and fructose) were found (Figure 2).

**Table 3.** Fructan and sugar content in *Arcticum lappa* L roots, g/100 g dry weight.

Compounds	Total fructans	Inulin	Nystose	1-Kestose	Sucrose	Glucose	Fructose
Value	9.0±2.2	7.3±0.1	0.2±0.1	0.1±0.0	0.5±0.1	0.7±0.1	0.8±0.2





**Figure 2.** HPLC-RID chromatograms of water extract from *Arcticum lappa* L. after sequential solvent extraction where: 1 – inulin, 2 – nystose, 3 – 1-kestose, 4 – sucrose, 5 – glucose, and 6 – fructose

The total fructan content was  $9.0 \pm 2.2$  g/100 g dry weight, as 80% of the fructan content was represented by inulin - 7.3% (Table 3). 1-kestose and nystose content in this extract did not exceed 0.2 g/100 g. The detected results were 1-kestose and nystose, which are degraded compounds of fructan, were reported at concentrations of  $8.82 \pm 0.99$  mg/g (0.88%, w/w) and  $14.07 \pm 0.32$  mg/g (1.41%, w/w) [51]. Similar results were reported for nystose and 1-kestose in other research using different extraction approaches [20].

Among fructooligosaccharide only nystose and 1-kestose was detected in a small amount. In most of the studies, inulin content in the burdock roots collected from Bulgaria (2-5%) [4,20] was lower compared to Russian representatives (inulin content up to 30%) [52] and the results from Iranian burdock 24-12% [28]. Inulin content in burdock roots varied between 12 and 17% in the fresh root (or 50–70% dried weight) or even of 3.6% of fresh material [53]. Ren *et al.* found inulin content  $174.33 \pm 3.68$  mg/g in burdock root water extracts [16]. The variation of inulin and fructooligosaccharide content in burdock roots could be explained by cold climate conditions, geographical origin of burdock, harvest time, and storage conditions [54]. However, the results from this study demonstrated high inulin content 7% that were close to the date of burdock from Korea (9% dw) [55] and other countries [56]. Therefore, the roots of burdock present a good inulin source, and further procedure for the isolation of fructan from inulin-type was performed by microwave-assisted extraction.

### 3.4. Characteristics of isolated fructan.

The isolated fructan was in lower yield 1.2 % in comparison to other scientific studies [7,18,22], where the typical yield was 4-5% (Table 4). This could be explained with applied microwave irradiation that could affect chain length and the physiological characteristics of raw material, climatic conditions, and harvest time. The physicochemical characteristics of isolated inulin were summarized in Table 4. The burdock inulin presented white, brownish tasteless powder. The isolated substances contained 63% inulin calculated as fructose equivalent. Glucose content expressed as reducing groups was 3.5%, lower than detected in dahlia inulin values [40].

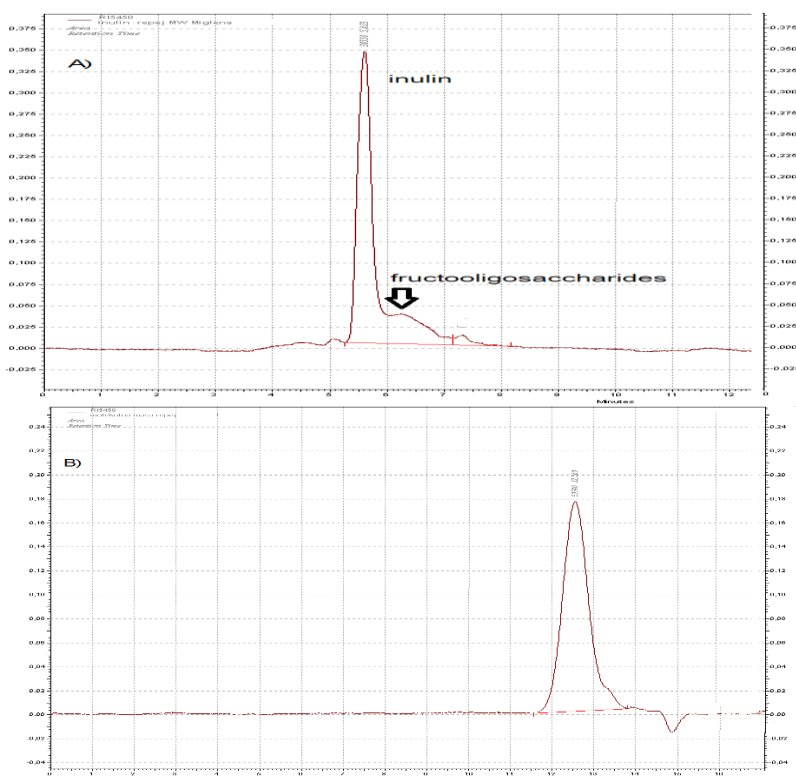
**Table 4.** Characterization of inulin from *Arcticum lappa* L.

Characteristics	Inulin type fructan		
	Current study	linear $\beta$ 1,2-glucofructan [22]	fructan from <i>A. lappa</i> L., var. <i>Herkules</i> [18]
Yield, %	1.2 $\pm$ 0.3	5.2; 14.2	5.2
Purity, %	55.1 $\pm$ 0.5	-	-
Melting points, °C	176-178	-	-
Fructose content, %	63.5 $\pm$ 1.3	-	-
Reducing groups, %	3.5 $\pm$ 0.5	glucose in traces	5.0 $\pm$ 0.2
DPn by spectrophotometric method	20	-	-
Mm, kDa	3.82	2.95 5.0–5.7	2.95 (cold water) 4.85 (hot water)
Mn, kDa	3.70	-	-
DP by HPLC_SEC	24	18-19	14-15
Polydispersity index	1.03	-	-
Appearance	White brownish powder	a white water soluble powder	7.5 $\pm$ 0.2
Swelling capacity, ml/g	8.0 $\pm$ 0.3	-	-
Water-holding capacity, g water/g	4.3 $\pm$ 0.4	-	-
Oil-holding capacity, g oil/g	6.7 $\pm$ 0.3	-	-

- Data not available in literature

### 3.4.1. Purity, homogenisty and molecular weight of burdock inulin.

The purity of burdock inulin was evaluated by HPLC-RID analysis (Table 3). The presented chromatograms demonstrated the presence of a single peak (inulin purity 52%), followed by tailing due to the presence of fructooligosaccharides fraction (Figure 3A). The retention time of the analyte ( $R_t = 5.85$  mins) coincided with the reference -chicory inulin (DP=22).



**Figure 3.** HPLC chromatograms of inulin form *Arcticum lappa* L., where **A)** HPLC-RID chromatogram for purity; **B)** HPLC-SEC chromatogram for homogeneity and molecular weight

The dominant polysaccharides component, separated by HPLC-SEC displayed a single and symmetrically sharp peak corresponding to  $M_w=3.82$  kDa for inulin (Figure 3 B). Similar homogeneity for burdock fructan was reported by Liu et al. [7], who obtained fraction with

molecular weight 4600 Da and yield 4.8% using precipitation with 95% ethanol. Water-soluble branched fructan (yield 4.4%) from *Arctium lappa* roots with molecular weight  $5.12 \times 10^3$  Da was also isolated [26]. In our study molecular weights of inulin from burdock were presented in Table 4. Weight molecular weight (Mw) and number molecular weight (Mn) were evaluated using dextran as standards. The weight molecular weight was 3.82 kDa that was near to obtained glucofructan from *Arctium lappa* L. roots 2.9 kDa obtained by some other authors [18,22]. The polydispersity of inulin from *Arctium lappa* L. roots was 1.03, which was near dahlia inulin [40].

### 3.4.2. Functional properties.

Until now, information about the functional properties of inulin from burdock roots is absent. Lou *et al.* [8] evaluated functional properties of burdock root by-product powder resulted after inulin extraction. This is the first detailed study that revealed the functional properties of burdock inulin. The swelling capacity of burdock inulin was 8.0 ml/g sample (Table 4) was higher than the reported data for some inulin from plant sources. In comparison with inulin from chicory, salsify, agave, Jerusalem artichoke burdock inulin showed higher swelling and water holding capacities [57-59]. Pectin from *A.lappa* roots had a WHC of  $6.95 \pm 0.65$  g water [25], which was near to WHC of inulin reported in our study – 4.3 g water/g sample. The oil-holding capacity of burdock inulin was higher than the water holding capacity. The determined oil holding capacity was 6.7 g oil/g sample, which was near to reported values of salsify inulin [57], and twice higher than the oil-holding capacity of chicory inulin (3.5 g oil/g), agave inulin (3.3 oil g/g) [58] and six-time higher than Jerusalem artichoke inulin (1.02 g/g) [59]. These results revealed the possible application of inulin from burdock roots as a stabilizer in oils and/or lipid-soluble components in food technology and pharmacy. Moreover, WHC of dietary fiber could contribute to its anti-constipation effect [25].

### 3.4.3. FTIR spectra of inulin from *Arctium lappa*.

FTIR spectra of inulin isolated from *Arctium lappa* L. by microwave irradiation is shown (Figure 4). The detailed assignment of wavelength was summarized in Table 5. The FTIR spectra contain typical bands for inuline type fructan that coincided with literature reports [7,18,22,57].



**Figure 4.** FTIR spectra of inulin from roots of *Arctium lappa* L. obtained after microwave irradiation.

The broadband at  $3330 \text{ cm}^{-1}$  was assigned with free hydroxyl groups in inulin molecule. The band at  $2933 \text{ cm}^{-1}$  was due to C-H stretching vibrations. The bands between  $1160$  and  $990 \text{ cm}^{-1}$  were due to C-O stretching vibrations. The presence of  $\alpha$ -glucopyranosyl residue

connected to fructofuranosyl units by  $\alpha$ -1 $\rightarrow$ 2 bonds were clearly observed in the fingerprint region with typical bands at 934  $\text{cm}^{-1}$ .

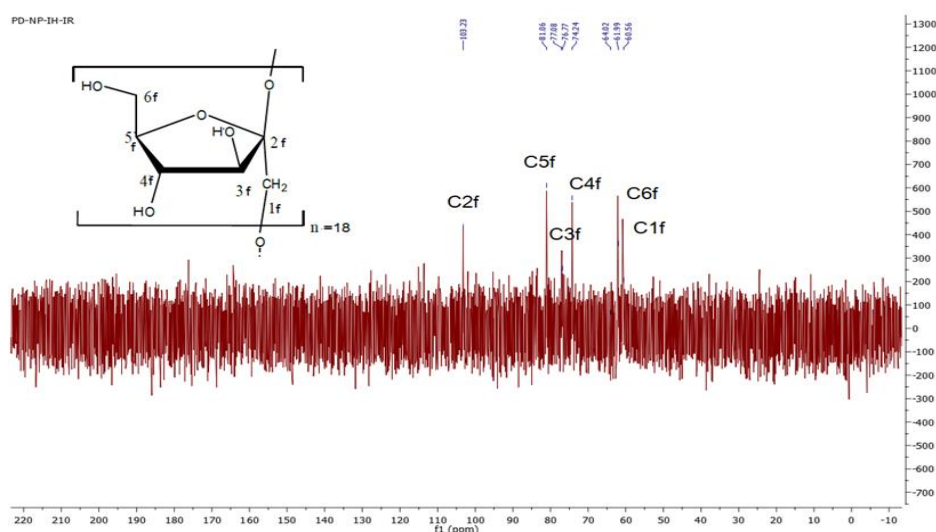
**Table 5.** Assignment of characteristic bands of inulin from *Arcticum lappa* L. roots

Wavelength, $\text{cm}^{-1}$	Experimental bands, $\text{cm}^{-1}$	Assignment [7,22,60,61]
3200 - 3400	3330	O-H stretching vibrations, intra and inter hydrogen bonds
2933 - 2981	2929	asymmetric stretching vibrations of C-H from $\text{CH}_2$
2850 - 2904	2889	symmetric stretching vibrations of C-H ( $\text{CH}_2$ )
1664 - 1634	1656	absorption of water
1455 - 1470	1433	stretching vibration C-Hs ( $\text{CH}_2$ ) in pyranosyl ring
1335 - 1336	1326	$\beta$ -OH (OH)
1125 - 1162	1124	stretching vibration (C-O-C), glycoside linkage
1015 - 1060	1029	Stretching vibration C-O
925 - 930;935	934	$\alpha$ -D-Glcp residue in chain
873	873	$\rho\text{CH}_2$ in ring, $\beta$ -anomer bendings C1-H, ring vibration of (2-ketofuranose)
818	818	2-ketose in pyranosyl of furanosyl ring

Bands at 873 and 817 $\text{cm}^{-1}$  in inulin spectra confirmed  $\text{CH}_2$  ring vibration of  $\beta$ -anomer and the structure of 2-ketofuranose. Similar bands for fructan from *Arcticum lappa* were reported [7].

#### 3.4.4. NMR spectra of burdock inulin.

In  $^{13}\text{C}$  NMR spectra of inulin, chemical shifts typical only for fructose units were observed (Figure 5):  $^{13}\text{C}$  NMR (126 MHz,  $\text{D}_2\text{O}$ )  $\delta$  103.28, 81.06, 76.77, 74.28, 61.99, 60.96 ppm. The spectra contained prominent shifts for C1–C6 carbons (C1 60.96 ppm, C2 103.28 ppm, C3 76.77 ppm, C4 ~74 ppm, C5 ~81 ppm, and C6 ~61.99 ppm) of fructosyl residue due to fructose repeated units. The  $^{13}\text{C}$  shifts from glucose were not observed (Figure 5) due to the low quantity in the sample. Similar shifts in  $^{13}\text{C}$  NMR spectrum were reported for fructans isolated from *Arcticum lappa*, which demonstrated the presence of linkage  $\rightarrow$ 1)-Fruf-(2 $\rightarrow$  and Fruf-(2 $\rightarrow$  [7,18,22, 26].



**Figure 5.**  $^{13}\text{C}$  NMR spectra of inulin from roots of *Arcticum lappa* L. obtained after microwave irradiation.

The superposition of glucose shifts was observed in other studies and was reported for inulin from echinacea, dahlia, and stevia [40, 62, 63]. In the  $^{13}\text{C}$  NMR spectra were observed only one shift at 103.28 ppm corresponding to the fructose C-2 carbon involved in  $\beta$ -(2 $\rightarrow$ 1)-

D-fructofuranosylfructose bonds. Similar shifts at 103.2 ppm were reported [26], but they also found branched fructan with a shift at 103.7 ppm due to a  $\beta$ -(2,6)-linked fructosyl residue. In our case, we found linear fructan with typical  $\beta$ -(2 $\rightarrow$ 1)-bonds. In DEPT 135 spectra (data were not shown) were identified methinic carbon atoms C5f, C3f, C4f as positive shifts. Two methylene carbon C6f and C1f appeared as negative shifts in DEPT 135 spectra, while the absence of anomeric carbons peaks in DEPT 135  $^{13}\text{C}$  NMR spectra of the polysaccharide indicated that quaternary carbon atoms existed in the compound.

#### 4. Conclusions

The present study investigated the bioactive compounds in different fractions obtained from roots of *Arctium lappa* L. The main detected components were oleanolic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, and inulin. The isolated polysaccharide from water fraction by microwave-assisted irradiation was characterized as inulin-type fructan with an average degree of polymerization 20–24, a molecular weight of 3.82 kDa. For the first time, functional properties of burdock inulin were demonstrated, as it demonstrated promising swelling properties and better oil-holding capacity. The results for antioxidant potential, antimicrobial activity, and phytochemicals are fundamental knowledge for applying this plant in medicinal cosmetics and as a functional ingredient in healthy foods enriched with fibers and antioxidants.

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#### Conflicts of Interest

The authors declare no conflict of interest.

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