Bulletin of the *Transilvania* University of Brasov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 15(64) No. 2 – 2022
https://doi.org/10.31926/but.fwiafe.2022.15.64.2.13

GREEN METHODS FOR EXTRACTION OF INULIN AND ANTIOXIDANTS FROM *CARLINA ACANTHIFOLIA* L. ROOTS – A COMPARATIVE STUDY

Nadezhda PETKOVA[1] Emine SARALIEVA[1] Ivan IVANOV[1]
Dasha MIHAYLOVA[2] Anna LANTE[3] Panteley DENEV[1]

Abstract: *Green extraction methods present perspective approach for isolation of bioacticve molecules. Together with an ecofriendly approach for extraction there are many undervalued plants - a rich of source of prebiotics and phenolic compounds. Carlina acanthifolia L. roots possessed antimicrobial, anti-inflammatory, anti-ulcer properties due to the diverse bioactive compounds in them. The detailed information about inulin-type fructan in its roots is still absent. The object of this work was to define inulin, sugars and phenolic content, the antioxidant activity in water and 70 % ethanol extracts resulted from ultrasound-assisted extraction (UAE) and pressure-liquid extraction (PLE). The results showed that the total fructans reached 12.6±0.2 g/100 g dw. Inulin was found in water extracts (6.82 g/100 g dw) in the prevalence of the ultrasound-assisted extracts. Total phenolic content did to not exceed 15.25 mg GAE/g dw. Additionally, the antioxidant activities was in the range from 5.99 to 205.83 µM TE/g dw. In conclusion, water extracts from UAE demonstrated higher levels of bioactive compounds with antioxidantive properties, probably due to cavitation process. However, this study is the first detailed investigation for fructans and polyphenols content, as well as antioxidant activity of Carlina acanthifolia L. roots in extracts obtained by green methods. Therefore, this research enriched the information of bioactive compounds in Carlina acanthifolia L. roots.*

Key words: *antioxidant activity, Carlina acanthifolia, inulin, pressure liquid extraction, ultrasound-assisted extraction.*

[1] Department of Organic Chemistry and Inorganic Chemistry, Technological Faculty, University of Food Technologies, 4002 Plovdiv, Bulgaria;
[2] Department of Biotechnology, Technological Faculty, University of Food Technologies, 4002 Plovdiv, Bulgaria;
[3] Department of Agronomy, Food, Natural Resources, Animals, and Environment-DAFNAE, Agripolis, University of Padova, 35020 Legnaro, Italy;
Correspondence: Nadezhda Petkova; email: petkovanadejda@abv.bg.

1. Introduction

Carline thistles comprise approximately thirty plant species that belong to the genus Carlina, Asteraceae family. They are widely spread in Europe and Asia as they were commonly used as medicinal plants for healing and in nutrition from ancient times [4, 5, 8, 33]. Among its representatives three species are with a significant importance, because of its phytochemical composition and biological activity: *Carlina vulgaris* L., *Carlina acaulis* L. and *Carlina acanthifolia* All. [4, 30, 31].

C. acanthifolia is biennial, rarely a perennial plant without stem, with leaf rosette and a thick, fleshy, cylindrical, vertical, up to 1 meter long, yellowish brown root and with singular capitula, that reaches to 10 cm in diameter. The leaves are 10-30 cm long, 6-15 cm wide, deeply crested, prickly serrated, arranged in a ground rosette around the flower basket [4]. In Bulgaria it is widely distributed in dry grassy places, stony meadows and slopes, mountain pastures, the mountains (between 800 to 2000 m altitude), and also around the Black Sea coast [2, 10, 33, 34, 36].

The root of *C. acanthifolia* is used as an adulterant for the root of *C. acaulis*. C. *acanthifolia* roots contained 1.0% yellow-coloured essential oil intense with a narcotic odor consisting of nine components, as the principal component (91.5%) in it is carlina oxide (benzyl 2-furylacetylene), that possessed antimicrobial properties [4, 10, 12]. Radix *Carlinae* contains also tannins, inulin, flavonoids, resins and enzymes [5, 10, 28, 32]. Carlina plants are a rich source of chlorogenic acid, as its content in *C. vulgaris* was higher in comparison to its values in *C. acalifolia* and *C. acaulis* [29, 30].

Due to the bioactive compounds in their roots, Carlina plants are used in traditional medicine of many countries because of different activity as cholagogic, diaphoretic, diuretic, stomachic, and cleansing activities [4, 5, 16, 30-32]. In Bulgaria, Carlina roots are mainly applied to cure nervous diseases, kidney disorders, and skin inflammation [11]. The tincture of *Carlina acanthifolia* roots was used for urinary tract disorders , because of its diuretic and anti-inflammatory activity, while for gastrointestinal disorders and as antihemorrhoid agent it is used the water and water-ethanolic root extracts [10]. Therefore, the extracts from carlina thistle present interest due to the pharmacological properties. The selection of proper extraction procedure remains challenges. The novel green extraction technologies are more effective in comparison to conventional ones, because of its energy efficiency, low temperature, speed, and high purity and yield. In recent years the application of green extraction techniques such as pressurized liquid extraction (PLE) or ultrasound-assisted extraction (UAE) to obtain extracts from the medicinal plant material have gained great attention. These two techniques accelerate extraction process, reduce time, costs, solvents in favour of good yield [1]. The PLE of polyphenols extraction found enormous application, while carbohydrates are still slightly investigated [1, 24]. Zhu et al. [37] demonstrated the green techniques for extraction of inulin from plant materials.

However, these extraction methods have not been used in the extraction of biologically active substances from Carline

thistle roots. Moreover, the studies about *Carlina acanthifolia* L. roots remain limited, with no studies focusing on its primary metabolites, such as carbohydrate and secondary metabolites are available. The object of this investigation was to determine the content of bioactive compounds and antioxidant potential in *Carlina acanthifolia* L. root extracts obtained by ultrasound-assisted extraction and pressurized-liquid extraction.

2. Materials and Methods

2.1. Reagents, Plant Material and Extraction Process

All reagents were of an analytical grade obtained from Merck (Darmstadt, Germany). They were used directly as they were purchased. The dry *Carlina acanthifolia* L. roots were bought from the herbal store Decrassin Ltd. (Varna, Bulgaria). The roots were finely ground in a laboratory homogenizer (Bosch, Germany) and sieved though a 0.5 mm sieve. The moisture in the roots was 10 %. They were put in dry plastic tubes.

2.2. Green Extraction Procedures

The different extraction procedures were conducted as follows:

2.2.1 Pressure Liquid Extraction (PLE)

Dried and ground carline thistle sample (98.99 g) were selected from 200 g roots. The PLE was conducted in an automatic equipment (NM LAB/M Deputex 88, Limena, Padova, Italy) for 1.45 h and extracted with 560 ml 70% ethanol, as described by Rossetto et al. [23]. The obtained PLE carline thistle extracts (**Sample A**) were collected and store in dark glass bottles at –20°C before further the analyses. The resulting root residues were extracted with distilled water by ultrasonic power at frequency 35 kHz (**Sample B**) described below.

2.2.2. Ultrasound-Assisted Extraction (UAE)

Five grams dry *Carlina acanthifolia* roots (**Sample C**) and the same amount of its residues after PLE (**Sample B**) were extracted with 50 ml distilled H_2O. The extraction process was done in an ultrasonic bath (SIEL, Gabrovo, Bulgaria) at 35 kHz and power 300 W for 20 min at 75°C. The extraction was repeated twice. The root extracts were filtered, combined and used for other analysis.

2.3. Determination of Total Polyhenolic Content (TPC)

Total phenolic content was analyzed by a five time diluted reagent of Folin-Ciocalteu [17]. Folin-Ciocalteu reagent (1 ml) was added to 0.2 ml extract and then 0.8 ml 7.5% Na_2CO_3 was put in the sample. After 20 min the absorbance was measured at 765 nm against a blank. The results were shown as mg gallic acid equivalent (GAE) per g dry plant material weight (dw).

2.4. Total Flavonoid Content

The total flavonoid content was determined according to Kivrac et al. [13]. *Carlina acanthifolia* roots extracts (0.5 ml) was mixed with 0.1 ml of 1M CH_3COOK, 0.1 ml of 10% $Al(NO_3)_3$, and 3.8 ml of 95% (v/v) ethanol. After 40 min, the absorbance was read at 415 nm. The

results were shown as mg quercetin equivalent (QE)/g dw.

2.5. Antioxidant Activity

2.5.1. DPPH$^{\bullet}$ Radical Scavenging Method

The ability of *Carlina acanthifolia* extracts to donate an electron and scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined [20, 26]. After 30 min the absorbance was measured at 517 nm against methanol. The percent inhibition was calculated. Trolox equivalent antioxidant capacity (TEAC) was calculated and antioxidant activity was presented as the µM per g dw (µM TE/g dw).

2.5.2. ABTS$^{\bullet+}$ Radical Scavenging Method

The radical scavenging ability of the Carlina root extracts against 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS$^{\bullet+}$) was evaluated [22]. Afterward, the ABTS$^{\bullet+}$ solution was properly diluted, 1.0 ml of diluted ABTS$^{\bullet+}$ reagent was mixed with 100 µL *Carlina acanthifolia* extracts. After 6 min the absorbance was measured and the data were presented as TEAC value (µM TE/g dw).

2.5.3. Ferric-Reducing Antioxidant Power (FRAP) Method

The FRAP method was performed as previously described [3]. One hundred and fifty micro liters of root extracts was added to 2.85 ml freshly prepared FRAP reagent. After 4 min the absorbance was measured at 593 nm against the blank. The results were presented as µM TE/g dw.

2.5.4. Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Method

In a plastic tube 1 ml $CuCl_2$ solution was added follow by addition of 1 ml NH_4Ac, 1 ml neocuproine, 0.1 ml of Carlina root extract, and 1 ml distilled water. The sample was incubated at 50°C for 20 min and then the absorbance was read against a reagent blank at 450 nm. The results were shown as µM TE/g dw [20].

2.6. Analysis of Carbohydrate Content

2.6.1. Total Fructans Assay

The fructans content was analyzed spectrophotometrically using resorcinol [19]. The absorbance was measured at 480 nm against distilled water. The results were presented as fructose equivalent g/100 g dry weight plant material (dw).

2.6.2. Thin Layer Analysis of Inulin and Sugars

Five micro liters of carlina roots extracts were put by a glass capillary tube on silica gel 60 F_{254} plates (Merck, Germany). The mobile phase n-BuOH:iPro:H_2O:CH_3COOH (7:5:4:2) (v/v/v/v) was used. The TLC plates were dipped in diphenylamine-anillineH_3PO_4–acetone [14]. Glucose, fructose, sucrose, inulin and fructooligosacchrides standards were prepared at a concentration of 3 mg/mL.

2.6.3. Inulin and Sugars Analysis by HPLC-RID Method

Inulin and sugars were detected on a HPLC instrument Elite Chrome (Hitachi, Japan),with a column Shodex® Sugar SP0810 (300 mm × 8.0 mm i.d.) with Pb^{2+} and a guard column Shodex SP - G (5 µm ,

6 × 50 mm) at 85°C and with refractive index detector (RID) Chromaster 5450. The mobile phase was distilled water with a flow rate 1.0 ml/min. The injection volume was 20 µl [21].

2.7. Statistical Analysis

The results were shown as an average from two independent experiments performed in triplicates. The data were statistically analyzed using the MS Excel software.

3. Results and Discussion

3.1. Total Polyphenols and Antioxidant Activity

The data for total polyphenols, flavonoids and antioxidant activity were summarized (Table 1). This investigation is the first one about the evaluation of Carlina root extracts resulted from green extraction procedures – pressure liquid extraction and ultrasound-assisted extraction. Moreover, water extracts were not investigated at all.

The total phenols in *Carlina acantifolia* roots extracts ranged between 15.25 and 9.97 mg GAE/g dw, while total flavonoids content was in the range from 5.23 to 2.23 mg QE/g dw. The highest content of total phenolic content, as well as total flavonoids were found in 70 % ethanol extract obtained after PLE - 15.25 mg GAE/g dw (TPC) and 5.23 mg QE/g dw (TFC). These results approve the effectiveness of PLE in the extraction of secondary metabolites, as previously describes [20, 35].

Table 1

Total phenolic content (mg GAE/g dw), total flavonoids (mg QE/g dw) and antioxidant activity (mM TE/g dw) of *C. acannthifolia* extracts

Sample	TPC	TF	DPPH	ABTS	FRAP	CUPRAC
A	15.25±0.05^{a}	5.23±0.04^{a}	166.63±0.96^{a}	134.94±3.61^{a}	129.43±0.88^{a}	205.83±1.05^{a}
B	10.96±0.05^{b}	2.23±0.04^{c}	5.99±0.28^{c}	12.23±0.85^{c}	7.89±0.28^{c}	10.55±0.04^{c}
C	9.97±0.10^{b,c}	3.60 ± 0.75^{b}	16.22±0.03^{b}	34.21±0.85^{b}	16.70±1.01^{b}	12.12±1.59^{b}

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

Strzemski et al. [29] successfully obtained methanol extracts by ultrasonic irradiation from leaves, flowers and root of *Carlina vulgaris* L. growing in Poland. They found the lowest level of phenolic compounds among the investigated vegetal parts. The highest TPC and TFC were detected in *Carlina vulgaris* flowering parts - heads (15.4 mg GAE/g adw and 18.3 RUE mg/g adw), however, for its root, both values were the lowest ones (5.8 mg GAE/g adw and 8.2 RUE mg/g dw, resp.) [29, 30]. Our values for the total phenolic content in *Carlina acantifolia* roots obtained after water ultrasonic extraction was approximately 2 times higher in comparison with *Carlina vulgaris* roots.

In most of the reports, it was demonstrated that the radical scavenging activity of Carlina roots was due to Carlina oxide [6, 16]. CH_2Cl_2 extract of *C. acaulis* was used in the DPPH assay, showing a half maximal effective concentration (EC_{50}) of 122 µg/mL. *C. acaulis* root methanol extract showed EC_{50} of 208 µg/mL, while the essential oil of *C. acanthifolia* demonstrated EC_{50} of 13.6

µg/mL according to DPPH assay. According some authors the anti-inflammatory and gastroprotective properties of this plant could be explained with the antioxidant activity [6, 25, 29]. Additionally, CH_2Cl_2 extract of the *C. acaulis* roots demonstrated *in vitro* antioxidant activity by 2,2-diphenyl-1-picrylhydracyl assay and *in vivo* antioxidant activity was shown for *C. elegans* mode [15].

In our study the highest antioxidant potential was demonstrated by 70% ethanol PLE extract (Table 1), as this sample showed better metal reducing activity by CUPRAC assay, followed by radical scavenging activity of DPPH radical. The water extracts contained 1.5 lower content of polyphenols and flavoinods which cause seriously impact on 100 times decrease in the antioxidant potential of these samples. The lowest values were detected in this water extracts from root wastes after PLE.

The correlation between total antioxidant activity evaluated by DPPH, ABTS, FRAP and CUPRAC methods, total phenolics and total flavonoids in *Carlina acantifolia* roots extracts were summerized (Table 2).

Table 2

Correlation coefficient (r^2) between total phenolic content, total flavonoids and antioxidant activities

	DPPH	ABTS	FRAP	CUPRAC	Total flavonois
Total phenolic content	0.9753	0.9446	0.9735	0.9851	0.8025
Total flavonoids	0.9144	0.9539	0.9177	0.8931	-

The results showed positive linear correlations between total antioxidant activities, total phenolic contents and total flavonoids (coefficient of correlation r^2 >0.90). Total phenols in *Carlina acantifolia* roots extracts provided the high antioxidant activity. The highest correlation was observed between total phenolic content and metal reducing method CUPRAC, (r^2 >0.9851), while total flavonoids demonstrated the highest correlation with ABST assay (r^2 >0.9539). In addition, total flavonoids are weakly correlated with the CUPRAC assay. The antioxidant potential evaluated by the DPPH method and antioxidant assays based on single electron transfer as FRAP and CUPRAC were influenced mainly by the content of total phenols in the *Carlina acantifolia* roots extracts. A similar tendency for a high correlation between antioxidant activity and phenolic content was reported by other researches [18, 19, 22].

3.2. Carbohydrate Composition

This is the first detailed study for individual carbohydrate composition in C. *acanthifolia* root extracts (Table 3).

The TLC chromatogram of extracts from the carlina thistle root showed that carbohydrates (sugars and inulin) were extracted (Figure 1). The presence of glucose (Rf = 0.51), fructose (Rf = 0.50), sucrose (Rf = 0.44), 1-kestose (Rf = 0.37), nystose Rf = (0.32) equivalent with used fructooligosacchride standard Frutafit CLR with degree of polymerization 7-9 (Sensus, The Netherlands), and inulin was found in water extracts form root residue

(Sample B), while in UAE water extract only inulin and sugars were found.

Table 3

Inulin and sugars content in different extracts of *C. acanthifolia* roots, g/100 dw

Sample	Total fructans	Inulin	Nystose	1-Kestose	Sucrose	Glucose	Fructose
A	5.30±0.15^{ns}	n.d	n.d	n.d	0.52±0.05^{b}	1.21±0.15^{b}	3.70±0.30^{b}
B	5.50±0.10^{ns}	5.01±0.11^{b}	tr	0.29±0.05^{a}	0.37±0.05^{b}	0.39±0.10^{c}	0.20±0.02^{c}
C	12.61±0.2^{a}	6.82±0.21^{a}	n.d	n.d	1.11±0.12^{a}	2.51±0.12^{a}	6.59±0.10^{a}

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

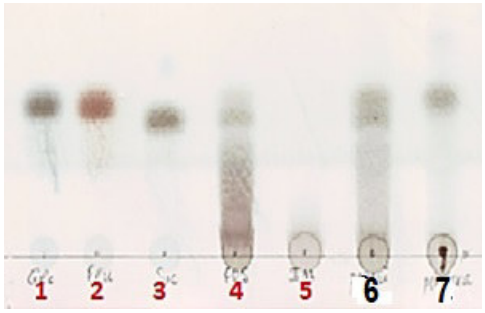

Fig 1. *TLC chromatograms of extracts from C. acanthifolia roots, where: 1 – glucose; 2 – fructose; 3 – sucrose; 4 – FOSs including 1-kestose nystose and 7-8 oligomers; 5 – inulin; 6 – sample B; 7 – sample C*

The individual carbohydrate content in *C. acannthifolia* root was presented in Table 3. The highest content of total fructans (12.61±0.21 g/100 g dw) expressed as fructose equivalent were found in the water extract (Sample 3). In 70 % ethanol extract after PLE (Sample A) and the subsequent water extraction from the root residue (Sample B) (Figure 2) the total fructan was between 5.3 and 5.50 g/100 g, while 90% of it is due to inulin in Sample B. Inulin was detected only in the water extracts of *C. acannthifolia* roots (Sample B and C), due to it water solubility, as its content is 5-6.8 g/100 g dw (Figure 2). Inulin content found in *C. acanthifolia* roots from Bulgaria was 4 times lower than reported values for some representatives of Carlina genus. Some authors mentioned that *C. acaulis* root had about 20 % of inulin [4, 6, 7, 27], while according to Herrmann et al. [9] inulin is the main compounds of Carlina spp. (18-20%). It was considered that the diuretic activity of *C. acanthifolia* roots was due to high content of inulin [10]. The detected in our study total fructans was near to the levels in leuzea (*Rhaponticum carthamoides*) roots (7-14%, with mean level 4.6 g/100 g dw) [19]. However, the detailed data about the presence of inulin and fructooligosacchrides have not been reported yet. HPLC-RID chromatogram of water extracts revealed the detailed profile of the following carbohydrates: inulin, nystose (GF3), 1-kestose (GF2), sucrose (GF), glucose (G) and fructose (F) (Figure 2).

Nystose were detected in trace amounts below 0.01g/100 g dw (Table 3). Disacchride sucrose was found in all extracts, together with monosacchride glucose. In 70% ethanol extract after PLE (Sample A) was detected only sugars:

sucrose, glucose and fructose. This could be explained with the solubility of sugars in water–alcohol mixture. Polysacharide inulin was absent in 70% ethanol because it is insoluble in such solvent (Figure 2a). The highest content of sugars was found in UAE water extracts (Sample C). Fructose was the dominant monosaccharides in water and 70% ethanol extracts.

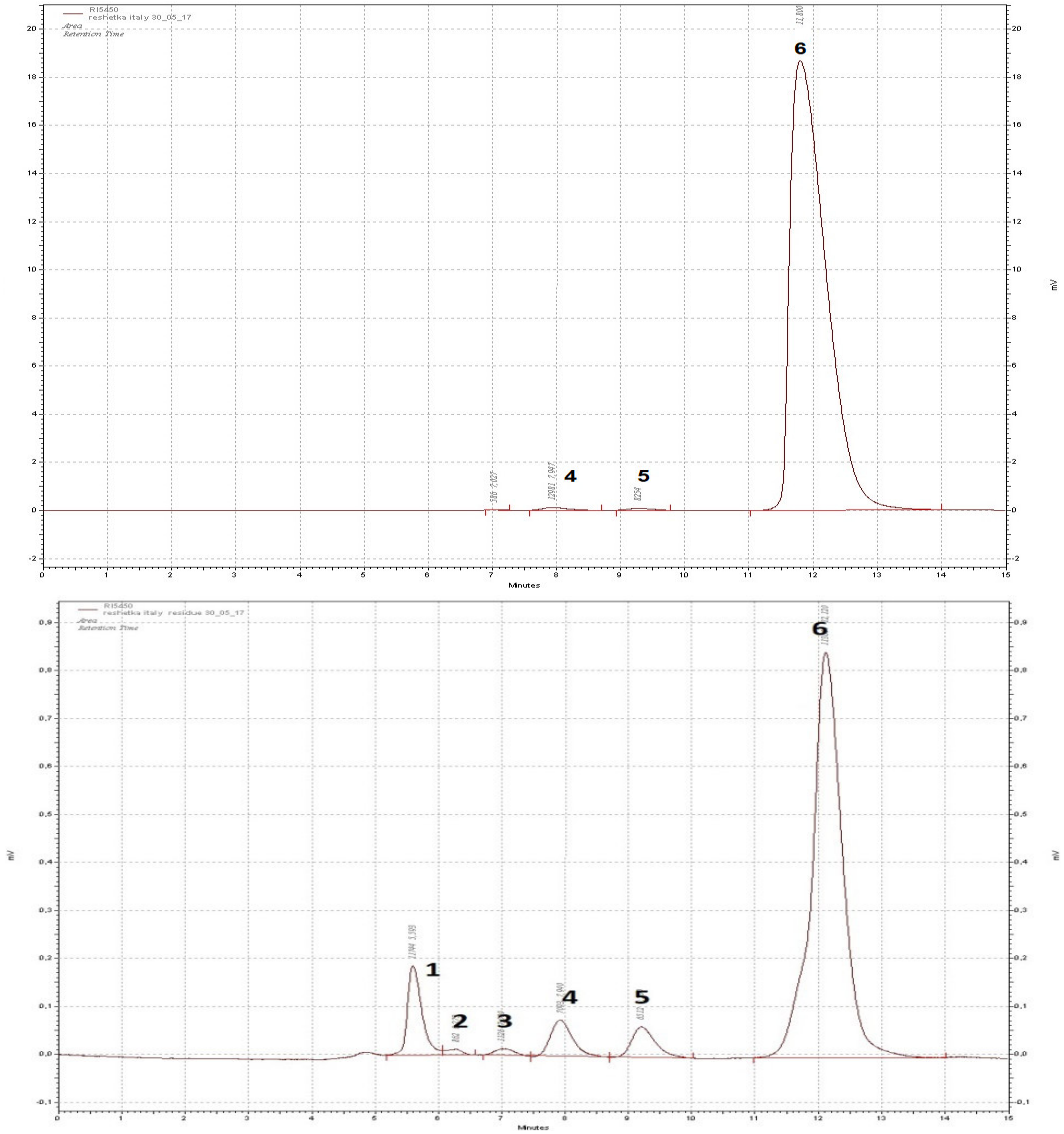

Fig. 2. *HPLC-RID chromatogram of water Carlina acantifolia extract obtained by UAE 35 kHz (sample A and B), where: 1 – inulin; 2 – nystose; 3 – 1-kestose; 4 – sucrose; 5 – glucose; 6 – fructose*

4. Conclusions

This research was the first detailed report for the carbohydrate composition of the carline thistles roots. The current research showed the potential of *Carlina acantifolia* roots to be used as a promising source of inulin and fructans, where their contents were in the range of 5 to 12% dw. Furthermore, the 70% ethanol and water extracts of this plant present a promising source of polyphenols, as antioxidant potential was the highest in 70% PLE ethanol extracts. This study reveals future perspectives for isolation of phenolic component and inulin from *Carlina acantifolia* roots using green extraction approaches.

References

1. Ameer K., Shahbaz H.M., Kwon J.-H., 2017. Green extraction methods for polyphenols from plant matrices and their byproducts: A review. In: Comprehensive Reviews in Food Science and Food Safety, vol. 16(2), pp. 295-315. DOI: 10.1111/1541-4337.12253.
2. Bancheva S., Delcheva M., 2018. Diversity of *Asteraceae* family in the Black Sea coast floristic region of Bulgaria. In: Acta Zoologica Bulgarica, vol. 11, pp. 57-62.
3. Benzie F., Srain J., 1996. Ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power". The FRAP assay. In: Analytical Biochemestry, vol. pp. 70-76.
4. Djordjevic S., Petrovic S., Ristic M. et al., 2005. Composition of *Carlina acanthifolia* root essential oil. In: Chemistry of Natural Compounds, vol. 41(4), pp. 410-412. DOI: 10.1007/s10600-005-0163-2.
5. Đordevic S., Lakusic B., Petrovic S. et al., 2004. Morpho-anatomical characteristics of *Carlina acaulis* subsp. *caulescens* and *C. acanthifolia* subsp. *utzka* (Asteraceae). In: Archive of Pharmacy, vol. 54, pp. 773-783.
6. Dordević S., Petrović S., Dobrić S. et al., 2007. Antimicrobial, anti-inflammatory, anti-ulcer and antioxidant activities of *Carlina acanthifolia* root essential oil. In: Journal of Ethnopharmacology, vol. 109(3), pp. 458-463. DOI: 10.1016/j.jep.2006.08.021.
7. Đordevic S., Tadic V., Petrovic S. et al., 2012. Bioactivity assays on *Carlina acaulis* and *C. acanthifolia* root and herb extracts. In: Digest Journal of Nanomaterials and Biostructures. vol. 7(3), pp. 1213-1222.
8. Guarrera P., Savo V., 2016. Wild food plants used in traditional vegetable mixtures in Italy. In: Journal of Ethnopharmacology, vol. 185, pp. 202-234. DOI: 10.1016/j.jep.2016.02.050.
9. Herrmann F., Hamoud R., Sporer F. et al., 2011. Carlina oxide - A natural polyacetylene from *Carlina acaulis* (Asteraceae) with potent antitrypanosomal and antimicrobial properties. In: Planta Medica, vol. 77(17), pp. 1905-1911. DOI: 10.1055/s-0031-1279984.
10. Ivancheva S., Nikolova M., Tsvetkova R., 2006. Pharmacologicalactivities and biologically active compounds of Bulgarian medicinal plants. In: Phytochemistry: Advance Research, vol. 8, pp. 87-103.

11. Jordanov D., Nikolov P., Bojcinov A., 1966. Fitoterapija, Medicina i fizkultura, Sofia, Bulgaria.
12. Jović J., Mihajilov-Krstev T., Žabar A. et al., 2012. Influence of SOLVENT on antimicrobial activity of Carlinae radix essential oil and decoct. In: Biologica Nyssana, vol. 3(2), pp. 701-710.
13. KivrakI., Duru M., Öztürk M. et al., 2009. Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. In: Food Chemistry, vol. 116(2), pp. 470-479. DOI: 10.1016/j.foodchem.2009.02.069.
14. Lingyun W., Jianhua W., Xiaodong Zh. et al., 2007. Studies of the extraction technical conditions of inulin from Jerusalem artichoke tubers. In: Journal of Food Engineering, vol. 79(3), pp. 1087-1093. DOI: 10.1016/j.jfoodeng.2006.03.028.
15. Link P., Roth K., Sporer F. et al., 2016. *Carlina acaulis* exhibits antioxidant activity and counteracts Aβ toxicity in *Caenorhabditis elegans*. In: Molecules, vol. 21(7), ID article 871. DOI: 10.3390/molecules21070871.
16. Lunz K., Stappen I., 2021. Back to the roots - An overview of the chemical composition and bioactivity of selected root-essential oils. In: Molecules, vol. 26(11), ID article 3155. DOI: 10.3390/molecules26113155.
17. Padureanu C., Maier A., Padureanu V. et al., 2022. The Total content of polyphenols and the antioxidant properties of several berry vinegars In: Bulletin of the Transilvania University of Brasov. Series II: Forestry, Wood Industry, Agricultural Food Engineering, vol. 15(64), no. 1, pp. 145-156. DOI: 10.31926/but.fwiafe.2022.15.64.1.11.
18. Parzhanova A., Petkova N., Ivanov I. et al., 2018. Evaluation of biologically active substance and antioxidant potential of medicinal plants extracts for food and cosmetic purposes. In: Journal of Pharmaceutical Sciences and Research, vol. 10(7), pp. 1804-1809.
19. Petkova N., Hambarlyiska I., Angelova E. et al., 2021. Fructans, polyphenols and antioxidant activity in edible roots and thistles from seven medicinal plants. In: Chiang Mai University (CMU) – Journal of Natural Sciences, vol. 20(4), ID article e2021082. DOI: 10.12982/CMUJNS.2021.082.
20. Petkova N., Ivanov I., Mihaylova D. et al., 2020. Effect of pressure liquid extraction and ultrasonic irradiation frequency on inulin, phenolic content and antioxidant activity in burdock (*Arctium lappa* L.) roots. In: Acta Scientiarum Polonorum, Hortorum Cultus, vol. 19(3), pp. 125-133. DOI: 10.24326/asphc.2020.3.11.
21. Petkova N., Vrancheva R., Denev P. et al., 2014. HPLC-RID method for determination of inulin and fructooligosacharides. In: Acta Scientifica Naturalis, vol. 1, pp. 99-107.
22. Re R., Pellegrini N., Proteggente A. et al., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. In: Free Radical Biology and Medicine, vol. 26(9-10), pp. 1231-1237. DOI: 10.1016/S0891-5849(98)00315-3.
23. Rossetto M., Lante A., Vanzani P. et al., 2005. Red chicories as potent scavengers of highly reactive

radicals: a study on their phenolic composition and peroxyl radical trapping capacity and efficiency. In: Journal of Agricultural and Food Chemistry, vol. 53(21), pp. 8169-8175. DOI: 10.1021/jf051116n.

24. Ruiz-Aceituno L., García-Sarrió M.J., Alonso-Rodriguez B. et al., 2016. Extraction of bioactive carbohydrates from artichoke (*Cynara scolymus* L.) external bracts using microwave assisted extraction and pressurized liquid extraction. In: Food Chemistry, vol. 196, pp. 1156-1162. DOI: 10.1016/j.foodchem.2015.10.046.
25. Sanpinit S., Chonsut P., Punsawad C. et al., 2021. Gastroprotective and antioxidative effects of the traditional thai polyherbal formula phy-blica-d against ethanol-induced gastric ulcers in rats. In: Nutrients, vol. 14(1), ID article 172. DOI: 10.3390/nu14010172.
26. Selamoglu Z., Sevindik M., Bal C. et al., 2020. Antioxidant, antimicrobial and DNA protection activities of phenolic content of *Tricholoma virgatum* (Fr.) P. Kumm. In: Biointerface Research in Applied Chemistry**, v**ol. 10 (3), pp. 5500-5506. DOI: 10.33263/briac103.500506.
27. Stojanović-Radić Z., Čomić L., Radulović N. et al., 2012. Commercial *Carlinae* radix herbal drug: Botanical identity, chemical composition and antimicrobial properties. In: Pharmaceutical Biology, vol. 50(8), pp. 933-940. DOI: 10.3109/13880209.2011.649214.
28. Strzemski M., Wójciak-Kosior M., Sowa I. et al., 2016. Carlina species as a new source of bioactive pentacyclic triterpenes. In: Industrial Crops and Products, vol. 94, pp. 498-504. DOI: 10.1016/j.indcrop.2016.09.025.
29. Strzemski M., Wójciak-Kosior M., Sowa I. et al., 2017a. Application of Raman spectroscopy for direct analysis of *Carlina acanthifolia* subsp. *utzka* root essential oil. In: Talanta, vol. 174(1), pp. 633-637. DOI: 10.1016/j.talanta.2017.06.070.
30. Strzemski M., Wójciak-Kosior M., Sowa I. et al., 2017b. *Carlina vulgaris* L. as a source of phytochemicals with antioxidant activity. In: Oxidative Medicine and Cellular Longevity, vol. 2017, ID article 1891849. DOI: 10.1155/2017/1891849.
31. Strzemski M., Wójciak-Kosior M., Sowa I. et al., 2019. Historical and traditional medical applications of *Carlina acaulis* L. - A critical ethnopharmacological review. In: Journal of Ethnopharmacology, vol. 239, ID article 111842. DOI: 10.1016/j.jep.2019.111842.
32. Strzemski M., Wojnicki K., Sowa I. et al., 2017. In vitro antiproliferative activity of extracts of *Carlina acaulis* subsp. *caulescens* and *Carlina acanthifolia* subsp. *utzka*. In: Frontiers in Pharmacology, vol. 8, ID article 371. DOI: 10.3389/fphar.2017.00371.
33. Vasheniak I., Dmytrash-Vatseba I., Markivska L. et al., 2020. Phytosociology, ecology and conservation status of *Carlina acanthifolia* subsp. *utzka* on its northern distribution limit (Poland and Ukraine). In: Biologia, vol. 75, pp. 637-651. DOI: 10.2478/s11756-020-00436-8.
34. Vitkova A., Evstatieva L., 2002. Plants rich in inulin from family Asteraceae. In: Godishnik na Sofiskiya Universitet

"Sv. Kliment Okhridski", Botanika, vol. 92, pp. 107-111.
35. Wianowska D., Gil M., 2019. Critical approach to PLE technique application in the analysis of secondary metabolites in plants. In: TrAC Trends in Analytical Chemistry, vol. 114, pp. 314-325. DOI: 10.1016/j.trac.2019.03.018.
36. Yaneva I., Karanesheva T., 2018. Origin of the Bulgarian names of medicinal plants. In: Bulgarian Journal of Public Health, vol. 10(4), pp. 70-78.
37. Zhu Z., He J., Liu G. et al., 2016. Recent insights for the green recovery of inulin from plant food materials using non-conventional extraction technologies: A review. In: Innovative Food Science and Emerging Technologies, vol. 33, pp. 1-9. DOI: 10.1016/j.ifset.2015.12.023.