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Original Research Article

Polyphenolic Compounds, Triterpenes, Carlina Oxide, Antioxidant Activity and Carbohydrate Profile of Different Vegetal Parts of *Carlina vulgaris* L., *Carlina acanthifolia* All. and *Carlina corymbosa* L.

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ABSTRACT

It is known that plants from the Carlina genus possessed many biologic activity due to the bioactive compounds. The current study investigates the phytochemical constituents and antioxidant potential of the different vegetal parts of *Carlina vulgaris* L., *Carlina acanthifolia* All. and *Carlina corymbosa* L. The samples (roots and aerial part) were collected from Bulgaria (Golo Bardo and Vlahina mountains). Total phenols, flavonoids, individual phenolic compounds, triterpenes, phytosterols, carlina oxide, fructans, and individual sugars were determined. Antioxidant potential was evaluated using four methods. The highest total phenolic content was found in ethanol extract from *C. acantifololia* All. roots. Three phenolic acids (chlorogenic acid, ferulic acid, and salicylic acid), three flavonoids (rutin, hesperidin, and quercetin), and triterpenes (lupeol and betulin) were detected in all samples (mainly in roots). However, *p*-Coumaric acid and ursolic acid were detected only in *C. vulgaris*, while carlina oxide was found only in *C. acantifololia* All. roots. The result showed that the roots of *C. acanthifolia* All. were characterized by appreciable amounts of total fructans (20 g/100 g dry weight), while inulin represented 18-12 g/100 g of dry weight. Sugars were found in all plant materials. The current study provides data about the chemical composition of extracts obtained from three members of the Carlina genus and their use as a source of antioxidants, phenolic compounds, carlina oxide, and inulin-type prebiotics.

Keywords: *Carlina* genus, phenolic compounds, antioxidant activity, fructan, inulin, sugars

Introduction

Carlina L. genus belongs to the Compositae family, to the tribe Cardueae, subtribe Carlininae. The plants of the Carlina genus are also known as carline thistles. These species are widely spread across the Canary Islands and the Mediterranean throughout central Siberia and northwestern China.[1-3] The genus *Carlina* L. comprises nearly 50 plant species from Europe and West Africa, but scientists reported observing the highest diversity in the Mediterranean region[4]. In Bulgaria, *Carlina vulgaris* L., *Carlina acanthifolia* All., *Carlina corymbos*a L. and *Carlina lanata* L. have a widespread distribution. [5-7] Common Carline thistle (*Carlina vulgaris* L.) is a biennual thistle and grows well on limestone or calcareous sand. It is natively distributed in Western, Central and Eastern Europe, and has been introduced to North America and New Zealand.[8] In Bulgaria, it is distributed in all floristic regions; from 0 to 1500 meters above sea level.[5,6] The flowering period is mainly during the second part of the year between late June and early August,[5-8] whereas seeds germinate from April to June.[8]

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Carlina acanthifolia All. is an annual/biennial herbaceous plant that grows to a height of 10-50 cm. It is a geographically widespread species across Bulgaria. It can be found from 0 to 1500 meters above sea level in dry, sandy, and rocky places, slopes, grassy places, and mountain pastures. The root is fleshy, with a pleasant smell and it usually reaches a length of 50 cm and 1 m. The plant does not have an aboveground stem. The leaves and bracts of the basket are spiny. The flower basket is very large (up to 12 cm in diameter). The flowers are regular, bisexual, with tubular corollas. It is used as a medicinal and tanning plant.[5,6,9]

Clustered carline thistle *(Carlina corymbosa)* is found in dry and poor habitats of the Mediterranean region and it is present in Albania, Bulgaria, Serbia, Turkey, and Balearic Islands.[10] The plant can reach a height of 10–70 cm. In Bulgaria, it can be found in stony and grassy places such as the Black Sea coast, Strum Valley, Strandzha mountain, Rhodopes mountains (Eastern Rhodopes), Tundzha hilly plain, and Strandzha (0 to 1000 altitude).[5,6] The flowering period is between late June and September. The outer bract of the flower heads is brown-yellow on the adaxial surface. The leaves are deeply pinnately divided, lobed at the top with strong spines.[10]

Wooly carline thistle (*Carlina lanata* L). is 9–30 cm in height and is distributed in Bulgaria, especially on the Black Sea coast, Strandzha, and Rhodopes mountain from 0- 300 –m.[5,7]

Nowadays, various species of Carlina genus (*C. acaulis*, *C. acanthifolia*, *C. utzka* (*C. acanthifolia* subsp. *utzka*) and *C. corymbosa*) are mainly used in traditional medicine of the Balkan countries, Hungary, Spain, Italy, Poland and Lithuania, largely because of their cholagogic, diuretic, antibiotic, and cleansing effects.[11-14] It is considered that *C. acanthifolia* exerts an anti-inflammatory effect on the digestive system due to the tannin content. The leaves and stems of *Carlina curetum* are also used for lowering blood glucose levels and

loosing weight. Carlina root decoction is also used in the treatment of rashes, toothache, skin lesions, and catarrh. [15]

Carlina acaulis and *Carlina acantifolia* are used not only as medical but also as food plants. In Alpine regions, it is cooked and consumed as artichoke and its heads are used to prepare liqueurs and snacks.[14] In Italy, thistle rennet or aqueous extracts of *Carlina acanthifolia* All. were used for cheese-making. [16-17] A large quantity of the leaves and petals of Carline thistle (*Carlina acaulis*) was consumed in Slovakia. [18]. In Bulgaria, *Carlina acanthifolia* All. flour was used for preparing dark chocolate bonbons.[19]

Carlina acaulis is one of the most investigated species, but the fact remains that its chemical composition has not yet been investigated in detail. According to earlier studies, inulin (12-20 %),[20,21] essential oil (1-2 %),[20,22] sugars, tannins and resinous substances, dyes[21] and trace amounts of lupeol were found in the root of *C. acaulis*.[23] Another study by Petkova *et al*.,[9] reported that inulin contributed to a large part (nearly 55%) of the total fructan content (12.6 g/100 g dw) of *Carlina acanthifolia*. Different flavonoids,[20] phenolic acids and pentacyclic triterpenes (lupeol, lupeol acetate, *α*-amyrin, *β*-amyrin, *β*-amyrin acetate, betulinic, oleanolic and ursolic acids)[23] were found in *Carlina vulgaris*. Lupeol, *β*-amyrin, and *α*-amyrin were found only in *C. corymbosa* var. *globosa* and *C. oligocephala*.[23] Another study by Strzemski *et al*.[24] estimated the chlorogenic acid, mineral, total phenolic, and total flavonoid content of three Polish populations of *Carlina vulgaris* L. In the roots of *Carlina gummifera* L., it was also detected amino acids, inulin, sugars, latex, essential oil, flavonoid heterosides, and a triglucosyl derivative of luteolin.[25] Even so, scientists have paid insufficient attention to investigating the phytochemical composition of many members of Carlina genus, and a useful bit of information about phytochemicals is still missing. Hence, the current study aimed at investigating the polyphenolic compounds, triterpenes, carlina oxide, carbohydrate profile, and the antioxidant activity of different parts of *Carlina vulgaris* L., *Carlina acanthifolia* All. and *Carlina corymbosa* L.

Material and Methods

Plant material

The plant material from *Carlina acanthifolia*, *Carlina vulgaris*, and *Carlina corymbosa* was collected in October 2020 and it was identified by Assoc. Prof. Ina Aneva from the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences. *Carlina acanthifolia* All. roots and aerial parts were collected from Golo Bardo mountain. *Carlina vulgaris* L. was collected from the „Komatinski rocks"– Vlahina mountain and *Carlina corymbosa* L. was collected from the South Struma Valley. The plants were identified by the references of the Herbarium of the Institute of Biodiversity and Ecosystem Research - BAS, where a voucher specimen for *Carlina acanthifolia* All. (SOM 287349), *Carlina vulgaris* L. (SOM 287350) and *Carlina corymbosa* L. (SOM 287351) were deposited. The samples were air-dried at room temperature and then finely ground in a laboratory homogenizer. The dried Carlina radix was purchased from Dicrassin Ltd. online herbal shop (www.dicrassin-online.com).

Chemicals and reagents

All chemical reagents were of analytical grade. Solvents were purchased from Merck (Germany) and used as they were received. Folin-Ciocalteu reagent, Trolox (6- hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), $Al(NO_3)_3$, DPPH (1,1-diphenyl-2-picrylhydrazyl radical), ABTS, gallic acid, quercetin, TPTZ (2,4,6-tri-(2-pyridyl)-s-triazine), neocuproine, $CuCl_3$, ammonium acetate, glucose, fructose, sucrose, nystose and 1-kestose were purchased from Sigma-Aldrich (Steinheim, Germany).

Determination of moisture content

The moisture content was determined by a moisture analyzer KERN DAB 100-3 (Kern, Germany).

Preparation of extracts

Two grams of aerial parts or roots from three Carlina species were extracted with 95 % ethanol in a centrifuge plastic tube (50 ml) employing a solid-to-liquid ratio of 1:15 (v/v) in an ultrasonic bath SIEL UST 5.7-150 bath (Gabrovo, Bulgaria) with the following parameters: 36 kHz frequency and 240 W ultrasonic power. The extraction was done in duplicate. The extracts were filtered and combined for further analysis. The same extraction procedure was repeated as 95 % ethanol was replaced with distilled water.

Total phenolic content

The total phenolic content in the obtained water and 95% ethanol extracts was estimated by the method of Folin–Ciocalteu.[26] The absorbance was measured at 765 nm against a blank sample.[27] The results are presented as milligram equivalents of gallic acid per gram (mg GAE/g dry weight).

Total flavonoids content

The quantity of total flavonoids in carline thistle extracts was evaluated using $Al(NO_3)_3$ reagent.[28] The results were presented as milligram equivalents of mg quercetin (mg QE)/g dw.

Determination of antioxidant activity

DPPH method. Carline thistle extracts (0.15 mL) were mixed with 2.85 mL of freshly prepared DPPH (2,2-diphenyl-1-picrylhydrazyl) (0.1 mM in methanol). After incubation for 15 minutes at 37°C, the reduction in the absorbance was measured at 517 nm.[29]

ABTS method. Carlina extracts (0.15 mL) were mixed with 2.85 mL of freshly prepared ABTS radical solution. After 15 min at 37ºC, the adsorption reduction was recorded at 734 nm. [30]

FRAP method. The carlina thistle extracts (0.1 mL) were mixed with 3 mL of freshly prepared FRAP reagent. After 5 min the absorption was recorded at 593 nm.[27]

CUPRAC method. Carlina thistle extracts (0.1 mL) were added to the plastic centrifuge tube and mixed with reagents in the following order: 1 mL $CuCl_2 \times 2H_2O$, 1 mL ethanol solution of Neocuproine, 1 mL 0.1M ammonium acetate buffer and 1 mL distilled H_2O. The absorbance was recorded at 450 nm after 20 min at 50°C.[31]

All results from antioxidant activity were presented as mM Trolox equivalents per g dry weight (mM TE/g dw).

HPLC analysis of phenolic acids and flavonoids.

Individual phenolic acids and flavonoids were analyzed on a HPLC system equipped with Waters 1525 Binary Pump (Waters, Milford, MA, USA), Waters 2484 Dual Absorbance Detector (Waters, Milford, MA, USA), and a C18 column (Supelco Discovery HS, 5 µm, 25cm × 4.6mm), and Breeze 3.30 software.[31] For flavonoids, separation gradient mode was used with a mobile phase composed of 2.0% (v/v) acetic acid (solvent A) and methanol (solvent B). The injected volume was 20 µL.[32] The results were calculated according to calibration curves.

HPLC-DAD analysis of terpenes, phytosterols, and carlina oxide

The determination of triterpenes, phytosterols, and carlina oxide content was performed 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 betulin, betulinic acid, oleanolic and ursolic acid, *β*-sitosterol and carlina oxide was performed on a reverse-phase column Supelco, Discovery® HS C18 (5 µm, 25 cm × 4.6 mm) operating at 26 °C. The mobile phase was composed of methanol and 0.1% HCOOH in a ratio of 92:8 (v/v), (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) and the separation was conducted in an isocratic mode with a flow rate of 0.4 mL/min. The separation of lupeol and *α*-amyrin (Extrasynthese, Lyon, France) was done on a reverse-phase column Waters Spherisorb C8 (5 µm, 15 cm × 4.6 mm) at 26 °C with a mobile phase acetonitrile:0.1% HCOOH = 92:8 (v/v), (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany) in a isocratic mode with a flow rate of 0.4 mL/min.[32]

Analysis of total fructans

The fructan content was determined spectrophotometrically by the resorcinol-thiourea reagent. The absorbance was measured at 480 nm against a blank sample prepared with distilled water.[9]

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HPLC-RID analysis of inulin and sugars
Analysis of inulin and sugars was performed on a HPLC instrument Elite Chrome Hitachi (Japan), with refractive index detector (RID) Chromaster 5450 at 35°C, as previously described.[33]

Statistical analysis.
Statistical analysis was performed using MS Excel 2010. The data were presented as mean values ± standard deviation (SD) from three replications. Statistical analysis was done using ANOVA, with Tukey's range statistically significant at $p < 0.05$. Different letters within each column show significant differences according to Tukey's test at $p < 0.05$

Results and discussion

Total phenolic and flavonoids content
The content of total phenolics and total flavonoids of the 95% ethanol and water extracts from different vegetal parts of Carlina species are shown in Figure 1.
The highest total phenolic content was found in ethanol extract from *Carlina acantifololia* All. roots (6.50±0.77 mg GAE/g dw). In general, ethanol extracts obtained from different vegetal parts were characterized by the highest values of phenolic and flavonoid content. *Carlina corymposa* L. and *Carlina vulgaris* L. ethanol and water extracts demonstrated close results for total phenolic content (between 3.79 and 2.50 mg/dw). The lowest values of total phenolic compounds were detected in water extracts from the aerial part of *Carlina vulgaris* L. Strzemski *et al.* successfully obtained methanolic extracts by ultrasonic irradiation from leaves, flowers, and root of *Carlina vulgaris* L. growing in Poland.[24,33] These scientists found 5.8 mg GAE/g total phenolic content in *Carlina vulgaris* L. root which is two times higher than the results reported in the current research. Kaçar reported that the aerial part of *Carlyna corymbose* contained a higher amount of total phenolics 27.3 mg GAE/g dw,[34] while, in the current study, we found considerably lower values in the root of this plant.
The previous research findings showed that water and 70 % ethanolic extracts had more than three times higher results for the total phenolic and flavonoids content of *Carlina acantifolia* roots[9] in comparison to the current results. This item is the first detailed report on the total phenolic and flavonoids content in three species of Carlina genus.

Antioxidant activity
The antioxidant activity of the obtained extracts from vegetal parts of *Carlina* genus was evaluated by four methods, based on different mechanisms (Table 1). In general, water extracts demonstrated higher antioxidant potential, especially by the CUPRAC method based on electron transfer, followed by the ABTS method based on a mixed mechanism. The obtained data were compared with our previous observation for *C. acannthifolia* ethanol and water root extracts[9]. There are some studies about the antioxidant potential of *Carlina vulgaris* extract,[24,36] as ethyl acetate fractions demonstrated the highest activity by the FRAP method, and the lowest values were found for water extract[36]. According to Stremski *et al.*[24] flower head extracts showed the highest ability to scavenge free radicals, and it was more than 2-fold higher compared to that for the leaf extract. By contrast, root extracts exhibited the lowest activity, and it may be explained by the lower production of antioxidants in the underground part of the plant. In the current research, there is a tendency for aerial part water extract to have a higher antioxidant potential than root extract. The ethanol extract showed higher antioxidant potential by DPPH and FRAP methods compared to water extracts.

Phenolic compounds in carlina thistle extracts
The polyphenolic and flavonoid composition in ethanolic extracts of different parts of the three Carlina species was investigated (Table 2). Chlorogenic acid, ferulic acid, and salicylic acid were identified as major phenolic acid constituents, but only in the roots of *C. vulgaris* *p*-coumaric acid was found in low concentration (Table 2). The amount of chlorogenic acid was two times higher in the aerial parts of *C. acathifolia* compared to the roots. Similar amounts of chlorogenic acid were also found in the aerial and root parts of Serbian and Polish populations. [11,20] Strzemski *et al.* [24] prove the presence of chlorogenic acid in the aerial parts (leaves and flowers) and roots of *C. vulgaris*. The Bulgarian population of *C. vulgaris* showed a certain amount of chlorogenic acid in the aerial parts of this species (0.26±0.02).

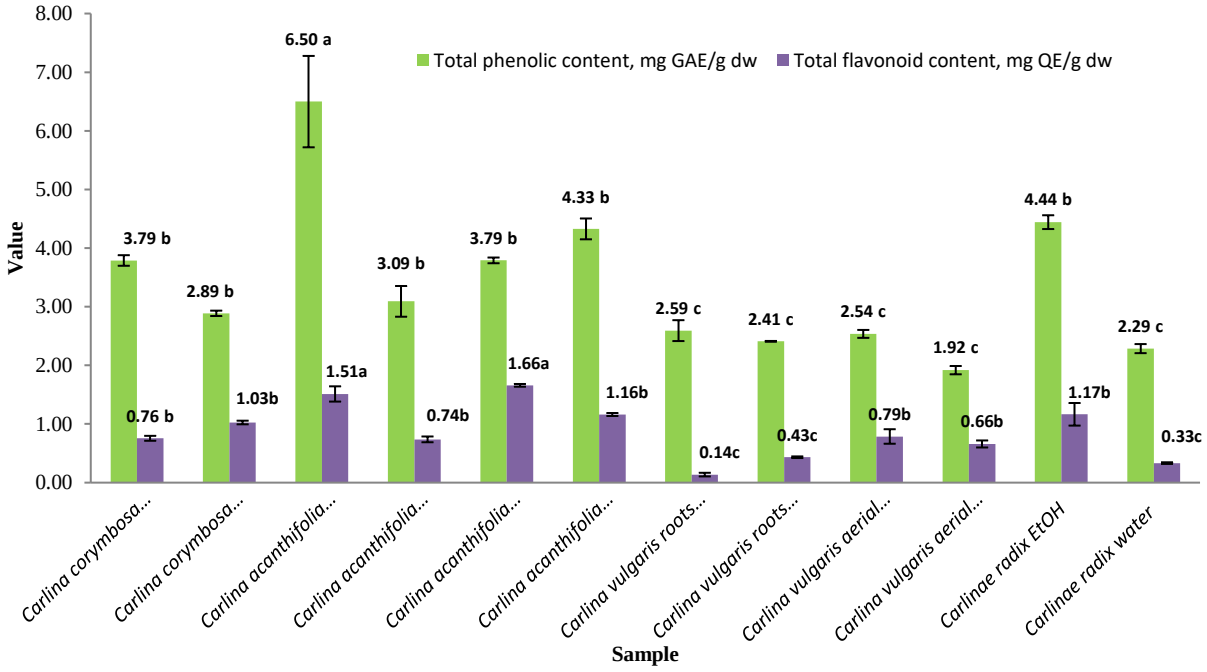

Figure 1: Total phenolic content and total flavonoids in different extracts from vegetal parts of *Carlina* genus representatives
Notes: Values are mean ± standard deviation of three replications. Different letters within each column show significant differences according to Tukey's test at $p < 0.05$

Table 1: Antioxidant activity in different extracts from vegetal parts of *Carlina* genus, mM TE/g dw

Samples	DPPH	ABTS	FRAP	CUPRAC
Carlina corymbosa roots EtOH	14.86 ± 1.95^{a}	19.44 ± 0.19^{d}	16.10 ± 0.08^{c}	43.74 ± 2.14^{c}
Carlina corymbosa roots Water	3.21 ± 0.33^{c}	101.39 ± 2.11^{b}	3.82 ± 0.15^{d}	197.70 ± 2.14^{a}
Carlina acanthifolia roots EtOH	18.09 ± 0.23^{a}	37.60 ± 7.00^{c}	20.10 ± 0.47^{b}	55.28 ± 1.65^{c}
Carlina acanthifolia roots Water	0.59 ± 0.19^{e}	21.96 ± 0.94^{d}	3.38 ± 0.22^{d}	212.66 ± 11.90^{a}
Carlina acanthifolia aerial parts EtOH	18.78 ± 0.05^{a}	25.30 ± 0.02^{d}	20.21 ± 0.95^{b}	47.32 ± 2.42^{c}
Carlina acanthifolia aerial parts Water	7.59 ± 0.60^{b}	33.76 ± 7.44^{c}	6.40 ± 0.17^{d}	204.73 ± 6.36^{a}
Carlina vulgaris roots EtOH	5.02 ± 0.24^{c}	22.12 ± 1.80^{d}	12.32 ± 0.22^{c}	17.37 ± 1.66^{d}
Carlina vulgaris roots Water	2.65 ± 0.10^{d}	19.51 ± 1.23^{d}	3.60 ± 0.20^{d}	153.18 ± 4.71^{b}
Carlina vulgaris aerial EtOH	10.84 ± 0.07^{b}	6.07 ± 4.45^{e}	15.23 ± 0.27^{c}	35.60 ± 3.00^{c}
Carlina vulgaris aerial Water	7.01 ± 0.40^{b}	136.57 ± 4.65^{a}	5.32 ± 0.02^{d}	159.70 ± 7.53^{b}
Carlinae radix EtOH	8.58 ± 0.03^{b}	44.98 ± 0.19^{c}	34.36 ± 1.13^{a}	38.66 ± 1.27^{c}
Carlinae radix water	1.48 ± 1.50^{d}	18.70 ± 0.32^{d}	2.95 ± 0.28^{d}	203.40 ± 10.52^{b}

Notes: Values are mean ± standard deviation of three replications. Different letters within each column show significant differences according to Tukey's test at $p < 0.05$

Table 2: Phenolic compounds in vegetal parts of *Carlina* genus

Compound	Concentration, mg/g dw					
	C. corymbose **root**	***C. acanthifolia*** **root**	**aerial parts**	***C. vulgaris*** **root**	**aerial parts**	**Carlinae radix**
Phenolic acids						
Gallic acid	nf	nf	nf	nf	nf	nf
Protocatehuic acid	nf	nf	nf	nf	nf	nf
Chlorogenic acid	0.50 ± 0.01^{b}	0.46 ± 0.01^{b}	0.91 ± 0.02^{a}	nf	0.26 ± 0.02^{c}	0.59 ± 0.02^{b}
Vanillic acid	nf	nf	nf	nf	nf	nf
Caffeic acid	nf	nf	nf	nf	nf	nf
Syringic acid	nf	nf	nf	nf	nf	nf
p-Coumaric acid	nf	nf	nf	0.02 ± 0.01	nf	nf
Ferulic acid	4.50 ± 0.03^{a}	0.17 ± 0.02^{c}	0.21 ± 0.02^{c}	1.05 ± 0.04^{b}	0.84 ± 0.02^{b}	0.06 ± 0.01^{c}
Salicylic acid	1.38 ± 0.02^{b}	2.03 ± 0.03^{a}	0.48 ± 0.02^{c}	0.86 ± 0.02^{b}	0.59 ± 0.02^{c}	0.43 ± 0.01^{c}
Flavonoids						
(+)-Catechin	nf	nf	nf	nf	nf	nf
(-)-Epicatechin	nf	nf	nf	nf	nf	nf
Rutin	0.02 ± 0.01^{c}	0.14 ± 0.01^{b}	0.20 ± 0.01^{a}	nf	nf	0.06 ± 0.01^{c}
Hesperidin	nf	1.38 ± 0.02^{a}	nf	0.33 ± 0.01^{b}	nf	0.54 ± 0.01^{b}
Quercetin	0.05 ± 0.01^{a}	0.02 ± 0.01^{b}	nf	0.06 ± 0.01^{a}	nf	nf

Notes: nf – not found. Values are mean ± standard deviation of three replications. Different letters within each column show significant differences according to Tukey's test at $p < 0.05$.

Furthermore, the phenolic acid composition was studied in *C. corymbose*. It was evident that three phenolic acids chlorogenic acid (0.50±0.01 mg/g dw), ferulic acid (4.50±0.03mg/g dw), and salicylic acid (1.38±0.02 mg/g dw) were quantified and identified for the first time. In addition, significant concentrations of ferulic acid and salicylic acid were found in various parts of *C. acanthifolia* and *C. vulgaris*. In the roots of *C. acanthifolia*, salicylic acid was found in an amount of 2.03±0.03 mg/g dw, while in *C. vulgaris* ferulic acid reached 1.05±0.04 mg/g dw. Both phenolic acids were observed for the first time in Bulgarian populations. In Polish populations of *C. acantifolia*, on the other hand, only chlorogenic acid and protocatechuic acid[11] were found, while in Serbian only the presence of chlorogenic acid was reported.[20] In three studied Polish populations of *C. vulgaris*, only chlorogenic acid was detected.[24] A relatively high concentration of hesperidin was found in the roots of the Bulgarian populations of *C. acanthifolia* (1.38±0.02 mg/g dw) and *C. vulgaris* (0.33±0.01 mg/g dw).

The quercetin glycoside rutin was found only in *C. acanthifolia* (aerial part and root) and *C. corymbosa* (root). It was found that quercetin was present in trace amounts in the roots of all three investigated carlina species.

Triterpenes, phytosterols, and carlina oxide

The results of the triterpenes and phytosterols chromatographic analysis are summarized in Table 3. It was evident that different constituents such as betulin, betulinic acid, oleanolic acid, ursolic acid, lupeol, *α*-amyrin and *β*-sitosterol were identified and quantified. Interestingly, a great diversity of triterpenes and phytosterols was observed in the aerial parts of *Carlina vulgaris* and *C. acanthifolia* (Table 3). Oleanolic acid,

betulin, and betulinic acid dominated in *Carlina vulgaris* (5.04±0.06, 4.08±0.08 and 2.92±0.06 mg/g dw, respectively), whereas betulin (4.44±0.03 mg/g dw) was found in *C. acanthifolia*. The *β*-sitosterol content in *Carlina acanthifolia* (35.83±0.05 mg/g dw) was nearly twofold higher than that found in *Carlina vulgaris* (19.05±0.05 mg/g dw). It was found that the concentration of triterpenes in the roots of the investigated species was considerably low. These findings are similar to those reported in previous studies on Polish cultivated plants. [23,24] In the current study, for the first time, we found the triterpenes lupeol (1.39±0.02 mg/g dw) and betulin (0.44±0.01 mg/g dw) in the extracts of *Carlina corymbose* roots. Table 3 contains important data relating to the levels of polyacetylene carlina oxide. It is interesting to note that the quantity of carlina oxide was considerably higher in the roots of *Carlina acanthifolia* (15.06±0.07 mg/g dw, Carlinae radix - 10.62±0.05 mg/g dw) by comparison with the aerial parts of the species (2.04±0.01 mg/g dw). Our results for *C. acanthifolia* roots are the same as the results for *C. acanthifolia*, and *Carlina acaulis* roots (1-2%) of a previous study, where the content of carlina oxide accounted for 98-90% of essential oil.[37] Contrary to the report of Sörensen & Sörensen[38] in the current research carlina oxide was not found in *Carlina vulgaris*. Other scientists [39,40] have also investigated an essential oil of the roots of *C. vulgaris*. Carlina oxide (33.7%) and 13-methoxy carlina oxide (11.5%) represented a high percentage of the oil.

Correlation between total phenolic content, total flavonoids and antioxidant activity

Table 4 shows the correlation (r^2) between antioxidant activity and the total phenolic content and total flavonoids (Table 4).

As can be seen, there was a positive linear correlation between CUPRAC and total phenolic content and total flavonoids (r^2>0.85) suggesting that polyphenols in carlina thistle extracts were responsible for the high antioxidant activity exhibited by ABTS and CUPRAC methods. In addition, the highest correlation was also observed between total phenolic content and metal-reducing method CUPRAC, (r^2 >0.9260). The total flavonoids showed the highest correlation with CUPRAC and DPPH methods (r^2 >0.79).

Table 3: Triterpenes and carlina oxide in different vegetal parts of *Carlina* genus

Compound	**Concentration, mg/g dw**					
	***C. corymbose* root**	***C. acanthifolia* root**	**aerial parts**	***C. vulgaris* root**	**aerial parts**	***Carlinae* radix**
Triterpenes						
Betulin	0.44 ± 0.01^b	0.68 ± 0.03^b	4.44 ± 0.03^a	0.50 ± 0.02^b	4.08 ± 0.08^a	0.08 ± 0.01^c
Betulinic acid	nf	1.05 ± 0.02^c	nf	1.76 ± 0.03^b	2.92 ± 0.06^a	nf
Oleanolic acid	nf	0.20 ± 0.01^b	nf	nf	5.04 ± 0.06^a	nf
Ursolic acid	nf	nf	nf	nf	0.60 ± 0.03	nf
Lupeol	1.39 ± 0.02^a	0.10 ± 0.01^b	0.21 ± 0.01^b	tr	0.35 ± 0.02^b	0.02 ± 0.00^c
α-Amyrin	nf	tr	0.02 ± 0.01^b	0.03 ± 0.01^b	0.06 ± 0.01^a	nf
Phytosterols						
β-Sitosterol	nf	nf	35.83 ± 0.05^a	nf	19.05 ± 0.05^b	nf
Polyacetylenes						
Carlina oxide	nf	15.06 ± 0.07^a	2.04 ± 0.01^c	nf	nf	10.62 ± 0.05^b

Notes: nf – not found, tr-traces. Values are mean ± standard deviation of three replications. Different letters within each column show significant differences according to Tukey's test at $p < 0.05$

Table 4: Correlation coefficient (r^2) between antioxidant activities and total phenolic content, and total flavonoids

	Total phenolic content		**Total flavonoids**	
	Ethanol	**Water**	**Ethanol**	**Water**
DPPH	0.5425	0.2902	0.7917	0.5161
ABTS	0.7320	0.3474	0.4562	0.2929
FRAP	0.4289	0.5218	0.4999	0.6516
CUPRAC	0.7338	0.9260	0.8571	0.6742
Total flavonoids	0.6616	0.7657	-	-

Table 5: Fructan content and individual sugars in water extracts, g/100 g dw

Sample	**Total fructans**	**Inulin**	**Nystose**	**1-Kestose**	**Sucrose**	**Glucose**	**Fructose**
Carlina corymbosa roots	3.11 ± 0.18^c	1.02 ± 0.36^c	0.45 ± 0.06^a	nd	0.82 ± 0.20^b	0.29 ± 0.01^c	0.33 ± 0.14^b
Carlina acanthifolia roots	15.47 ± 0.07^b	12.14 ± 0.34^b	nd	nd	3.03 ± 0.73^a	0.65 ± 0.18^b	1.56 ± 0.41^a
Carlina acanthifolia aerial parts	2.06 ± 0.24^a	nd	nd	nd	nd	2.54 ± 0.01^a	0.80 ± 0.01^b
Carlina vulgaris roots	1.13 ± 0.08^c	0.06 ± 0.01^c	0.20 ± 0.01^b	0.03 ± 0.01	0.25 ± 0.02^c	0.25 ± 0.08^c	0.14 ± 0.02^c
Carlina vulgaris aerial parts	3.31 ± 0.94^c	0.28 ± 0.03^c	0.58 ± 0.01^a	nd	0.35 ± 0.01^c	0.29 ± 0.23^c	0.57 ± 0.37^b
Carlinae radix	20.29 ± 0.07^a	18.10 ± 0.05^a	nd	nd	1.85 ± 0.42^b	1.00 ± 0.13^b	1.21 ± 0.46^a

Notes: n.d. – not detected, Values are mean ± standard deviation of three replications. Different letters within each column show significant differences according to Tukey's test at $p < 0.05$

The total flavonoids were weakly correlated with the ABTS and FRAP assay. A similar tendency for phenolic content to correlate highly with antioxidant activity was reported by other researchers.[9] A high correlation existed between TPC/TFC, TPC, and DPPH/ABTS, and TFC and DPPH/ABTS (r > 0 79, r > 0 61, and r > 0 68) was also reported.[23,24]

Total fructans, inulin, and sugar content in Carlina thistles extracts

The results for sugar composition and fructan content in different plant materials of *Carlina corymbosa*, *Carlina acanthifolia*, and *Carlina vulgaris* were summarized in Table 5. This item is the first detailed study that gives information about the sugar and inulin content of the investigated *Carlina* species. As shown (Table 3), the roots contained a higher quantity of inulin polysaccharide, whereas the aerial parts contained very small amounts (0.3 g/100 g dw). Glucose and fructose constituted all vegetal parts of the plants. Fructooligosaccharides (1-kestose and nystose) were detected mainly in the roots. The highest content of inulin was detected in commercial Carlina radix - 18.10 g/100 g dw, followed by *Carlina acanthifolia* roots – 12.14 g/100 g dw. In an earlier study, it was reported, that *Calina acaulis* roots contained 20 % of inulin.[20] Another study[40] it was suggested that inulin is the main compound of *Carlina* spp. (18-20 %), while a previous study by Petkova *et al*. found that inulin content in commercial *Carlina acanthifolia* roots reached 5-6.8 g/100 g dw.[9] Furthermore, Table 5 shows that *Carlina corymbosa* roots contained a considerably lower amount of inulin in comparison to *Carlina acanthifolia* roots. The lowest values of inulin were detected in *Carlina vulgaris* roots and vegetal parts (< 0.3 g/100 g dw). The roots of *Carlina vulgaris* L. and *Carlina corymbosa* L., on the other hand, showed a level of inulin below 1%. The last two species contained 0.5 % nystose, while it was completely missing in *Carlina acanthifolia* All. There was not any amount of 1-kestose in most of the samples. Our results indicated that a majority of sugars were present in the aerial part of the plants than in the roots, especially for *Carlina vulgaris* L.

Our findings led to the conclusion that *Carlina acanthifolia* All. could serve as a better source of fructans and inulin in comparison with chicory and Echinacea plants.

Conclusion

This research is the first detailed study about phytochemical constituents in different plant parts of *Carlina vulgaris* L., *Carlina acanthifolia* All. and *Carlina corymbosa* L. Extracts of Carlina species exhibited antioxidant potential mainly by CUPRAC method. In addition, there was a higher correlation between the total phenolic compounds and the antioxidant activity values by ABTS and CUPRAC methods. The detected phenolic acids, flavonoids, and triterpenes were in the highest concentration in *Carlina vulgaris* L and *Carlina acanthifolia* All., while in *Carlina acanthifolia* All. roots predominated carlina oxide and inulin. Our study reveals for the first time the carbohydrate profile of *Carlina vulgaris* L., *Carlina acanthifolia* All. and *Carlina corymbosa* L. and evaluated *Carlina acanthifolia* as a rich source of inulin-type fructans. Owing to the various phytochemical compounds, the extracts from the investigated carlina thistles can be used in food, pharmaceutical, and cosmetic formulations.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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