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SHORT COMMUNICATION

Flower heads of *Onopordum tauricum* Willd. and *Carduus acanthoides* L – source of prebiotics and antioxidants

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ABSTRACT

The carbohydrate composition (inulin and sugars) and antioxidant potential of 95% ethanol and subsequent water extracts from flower heads of *Carduus acanthoides* L. and *Onopordum tauricum* Willd. grown in Bulgaria were investigated. The total fructans content in both thistle species was analyzed by ketose-specific assay, while individual inulin and sugars content was defined by HPLC-RID method. The total phenolic content and the antioxidant capacity of the studied extracts were also examined as the antioxidant potential was determined by four *in vitro* assays (DPPH, ABTS, FRAP and CUPRAC). *O. tauricum* Willd. flower heads were evaluated for the first time as a rich source of prebiotics and total phenols. Higher fructans content was found in the water extracts, while the ethanol extracts possessed better antioxidant activity.

Keywords: Antioxidants; Extracts; Inulin; Fructooligosacharides; Thistles

INTRODUCTION

Onopordum tauricum Willd. and *Carduus acanthoides* L. are thistles that belong to the Asteraceae family. *Carduus acanthoides* L. is a plant from *Carduus* genus. In the Bulgarian flora the genus *Carduus* is presented by 14 species, 5 of which are endemic (Stojanov et al., 1967; Tutin et al., 1976; Delipavlov & Cheshmedzhiev, 2003). Phytochemical studies on several Carduus species revealed the presence of various classes of phytochemical constituents like lignans, flavonoids, coumarins, alkaloids, sterols, triterpens (Al-Shammari et al., 2015), inulin-type fructans and polyphenols, as well (Mihaylova et al., 2013; Petkova et al., 2015a). Previous investigations on some Bulgarian *Carduus* and *Onopordum* species showed their antioxidant potential (Zheleva-Dimitrova et al., 2011; Angelov et al., 2012; Petkova et al., 2015a). For the species *Carduus acanthoides* L. (plumeless thistle) phytochemical compounds like flavonoids, quinic acids, phenolic acid glycosides, phenylethanoid glycosides, phenolic acids, coumarins and anthocyanins were established (Zhelev et al., 2013; Li et al., 2014). In addition, Zheleva-Dimitrova et al. (2011) reported the antioxidant activity of ethanol extracts.

The representatives of genus *Onopordum* are native to Europe (Kleonikos, 2006). In Bulgaria this species are commonly spread at dry stony ruderal places and the isolated metabolites from the species of the genus include saponins, alkaloids, sesquiterpen lactones, flavonoids, steroids, triterpenes, lipids and nitrogen containing compounds. The presence of inulin in the roots and receptacles was reported (Petkov, 1982; Van Loo et al., 1995; Petit, 2012) but without mentioning any details. According to the Bulgarian folk medicine these species have refreshing and invigorating effect on the body (Petkov, 1982).

Prebiotics occur naturally in fruits and vegetables, e.g. asparagus, onion, cereals, garlic, *Jerusalem artichoke*, chicory, banana (Bucke & Rastall, 1990). Van Loo et al. (1995) reported that many roots and tubers, and even receptacles of thistles are eaten by indigenous populations, contain inulin or fructooligosacharides (FOSs). Among

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the edible thistles used in human nutrition as a source of inulin consumed mainly in Mediterranean are *O. acanthium* (receptacle, root), *S. marianum* (receptacle, shoots, leaves) and also *C. eriophorum* (receptacle). Previous investigation established *C. thoermeri* flower heads as a potential source of prebiotics (kestose, nystose and inulin) and antioxidants (Petkova et al., 2015a). To the best of our knowledge no detailed information concerning inulin, FOSs and sugars content in the vegetal part of *O. tauricum* and *C. acanthoides*, especially flower heads were available.

Therefore, the aim of the current study was to establish inulin and polyphenols content in extracts obtained from flower heads of *Onopordum tauricum* Willd. and *Carduus acanthoides* L. growing in Bulgaria and to evaluate their *in vitro* antioxidant activity regarding the possible application in the food industry as prebiotics and antioxidants. Moreover, the present study could be considered as the first report for the studied thistles representatives in terms of content of prebiotics.

MATERIALS AND METHODS

Plant material

The flower heads of *Onopordum tauricum* Willd. and *Carduus acanthoides* L. were obtained from a local herb store in Plovdiv (Bulgaria). The plant material were air-dried in darkness at room temperature and then ground in laboratory homogenizer.

Extract preparation

The flower heads of *O. tauricum* and *C. acanthoides* were extracted as previously described by Petkova et al. (2015a). The obtained 95% ethanol and subsequent water extracts from flower heads of *O. tauricum* and *C. acanthoides* were further analyzed.

Spectrophotometric analysis of total fructans

The total fructans content in the ethanol and water extracts from both thistles was analysed spectrophotometrically at 480 nm by ketose-specific resorcinol-thiourea reagent and the results were expressed as fructose equivalent per dry weight (Petkova & Denev, 2013).

HPLC-RID analysis of carbohydrate content

Chromatographic separations of presented carbohydrates (sugars and inulin) in the thistles extracts were carried out on HPLC Shimadzu, coupled with LC-20AD pump, refractive index detector Shimadzu RID-10A and the software LC solution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan). The analysis was performed on a Shodex® Sugar SP0810 with Pb^{2+} a guard column (50×9.2 mm i.d.), an analytical column (300mm×8.0 mm i.d.) at 85°C, mobile phase d. H_2O with flow rate 1.0 ml/min and the injection volume 20 µl (Petkova et al., 2014).

Determination of total phenolics (TPC)

The TPC was analyzed using the method of Kujala et al. (2000)with some modifications. Each extract was mixed with Folin-Ciocalteu reagent and 7.5% Na_2CO_3. The mixture was vortexed and left for 5 min at 50 ºC. After incubation, the absorbance was measured at 765 nm by room temperature. The TPC was expressed as mg gallic acid equivalents (GAE) per g dry weight (dw).

Determination of antioxidant activity

DPPH• radical scavenging assay

The ability of the extracts to donate an electron and scavenge 2,2-diphenil-1-picrylhydrazyl (DPPH) radical was determined by the slightly modified method of Brand-Williams et al. (1995) as described by Mihaylova et al. (2015). The unit of Trolox equivalent antioxidant capacity (TEAC) was defined the concentration of Trolox having equivalent antioxidant activity expressed as the µM per g DW (µM TE/g dw).

$ABTS^{\bullet+}$ radical scavenging assay

The radical scavenging activity of the extracts against 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) was estimated according to Re et al. (1999). The results were expressed as TEAC value (µM TE/g dw).

Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie & Strain (1999) with slight modification. The FRAP reagent was prepared fresh daily and was warmed to 37 °C prior to use. The absorbance was recorded at 593 nm and the results were expressed as µM TE/g dw.

Cupric ion reducing antioxidant capacity (CUPRAC) assay

The CUPRAC assay was carried out according to the procedure of Ak & Gülcin (2008). Absorbance against a reagent blank was measured at 450 nm after 30 min. The results were expressed as µM TE/g dw.

RESULTS AND DISCUSSION

The chemical composition of the plant extracts and the resulting biological properties of the plant extracts are of particular importance for the discovery of new useful materials for the food industry. The present research paper could be designated as the first detailed comparative carbohydrate composition survey for both examined plant species.

Carbohydrate composition

The results regarding the carbohydrate composition in the flower heads extracts of O. tauricum and C. acanthoides were summarized in Table 1.

The investigation of water and ethanol extracts of the thistle representatives revealed the presence of total fructans in both plants – 0.76±0.10 and 7.90±0.34 g/100 g dw, respectively. The prevalence was in the two extracts of *O. tauricum* - 3.70±0.28 and 7.90±0.34 g/100 g dw, resp. (Table 1). The highest inulin content was found in the water extract of *O. tauricum* – 4.50±0.20 g/100 g dw and the lowest in the water extract of *C. acanthoides* – 0.50 g/100 g dw. By comparing the results the diversity of the carbohydrate components was greater for the *O. tauricum* ethanol extract, whereas the content of fructose, 1-kestose and inulin was significantly higher. This revealed the potential of the thistles representatives to be used as a source of prebiotics. In ethanol extract of *C. acanthoides* low-molecular carbohydrate fraction, mainly fructose, glucose and sucrose, were predominant. In addition, low and high molecular carbohydrates were found in *O. tauricum*, while sugars dominated in *C. acanthoides*. However, a high molecular fraction of inulin was established in both plants in prevail of *O. tauricum*. The previously reported results for inulin and FOSs content in flower heads of *C. thoermeri* (Petkova et al., 2015a) were similar to the data established for *C. acanthoides*. The content of nystose with a pronounce prebiotic effect (Van Loo et al., 1995) was 0.4 g/100g dw, and inulin content was 0.5 g/100g dw. The low value of inulin in the investigated species could be explained with a particular hydrolysis of inulin. Furthermore, HPLC-RID method was used for more detailed analysis and quantitative determination of individual carbohydrate composition in thistles flower head extracts. 1-kestose was evaluated as predominant prebiotic saccharides in *O. tauricum*. In *C. acanthoides* ethanol extracts nystose dominated, followed by 1-kestose, and sugars sucrose, glucose and fructose. The FOSs (1-kestose and nystose) and glucose, fructose and sucrose profile of the *O. tauricum* extracts was similar to artichoke bract waste extract reported by Machado et al. (2015) and *C. thoermeri* (Petkova et al., 2015a). The 1-kestose content in *O. tauricum* reached values comparable with other source of prebiotics as dandelion root (Petkova et al., 2015b) and *Jerusalem artichoke* tubers (Petkova et al., 2013). Therefore, the extracts of *O. tauricum* and *C. acanthoides* could be considered as a potential source of prebiotic sugars: 1-kestose and nystose.

Total phenolic content and antioxidant activity

As following procedure and as informative data for the further potential usage, the investigated samples were subjected to testing of their total phenolic content. The established values varied between 0.96±0.01 and 7.64±0.01 mg GAE/g dw, resp. (Table 2.). Both water and ethanol extracts of *O. tauricum* were richer in polyphenolics compared to the *C. acanthoides* extracts. However, the ethanol extracts showed the best results, which was probably due to the extracting capacity of the solvent.

According the recommendation of usage of a set of reliable methods for antioxidant potential evaluation in the present study four assays were conducted. The established antioxidant capacity was in the range of 6.98±0.61 to 174.29±2.00 μM TE/g dw. Both water and ethanol extracts of *O. tauricum* revealed to possess stronger antioxidant activity in prevalence for the ethanol one (Table 2). The same trend was shown in the total phenolic content results.

Among all investigated samples the 95% ethanol extracts of *O. tauricum* demonstrated the highest antioxidant activity. The highest TEAC value was established by the CUPRAC assay - 174.29±2.00 μM TE/g dw, which is probably due to the mechanism of the assay. Petkova et al. (2015a) reported for *C. thoermeri* water and ethanol extracts 45.76±0.67 and 130.67±3.70 μM TE/g dw, resp. according the CUPRAC assay and Zheleva-Dimitrova et al. (2011) established for *C. acanthoides* 103.1±3.2 μM TE/g dw according the FRAP assay. These obtained results correlated well with the higher concentration of polyphenols in ethanol extracts especially for the *O. tauricum*.

Table 1: Carbohydrate composition in extracts of *O. tauricum* and *C. acanthoides* flower heads, g/100 g dw (mean ± SD)

Plants	Extracts	Total fructans	Fructose	Glucose	Sucrose	1-Kestose	Nystose	Inulin
O. tauricum	ethanol	3.70±0.28	0.22±0.01	0.12±0.01	0.61±0.22	1.35±0.02	nf	nf
	water	7.90±0.34	2.06±0.40	nf	0.73±0.02	nf	0.26±0.05	4.50±0.20
C. acanthoides	ethanol	4.74±0.19	0.54±0.23	0.36±0.29	1.62±0.62	0.23±0.04	0.40±0.03	-
	water	0.76±0.10	nf	nf	nf	nf	nf	0.50±0.00

Table 2: Total phenolic content (mg GAE/g dw) and antioxidant activities (μM TE/g dw) in extracts of *O. tauricum* and *C. acanthoides* flower heads (mean ± SD)

Plant	Extracts	TPC	ABTS	DPPH	FRAP	CUPRAC
O. tauricum	ethanol	7.64±0.01	65.90±0.80	91.21±1.27	92.08±0.61	174.29±2.00
	water	4.64±0.04	49.78±0.10	71.06±0.40	65.34±1.21	88.40±0.97
C. acanthoides	ethanol	1.76±0.01	28.41±0.77	29.47±0.60	33.44±0.33	44.57±0.37
	water	0.96±0.01	13.92±0.64	6.98±0.61	16.08±0.18	18.41±0.18

Table 3: Correlation coefficients (r) for relationships between assays

	TPC	CUPRAC	FRAP	DPPH
ABTS	0.9818	0.9661	0.9982	0.9969
DPPH	0.9728	0.9466	0.9933	
FRAP	0.9915	0.9774		
CUPRAC	0.9907			

Correlations

Correlation analyses between total phenolics content and antioxidant capacity are shown in Table 3. The established correlation coefficients ranged from 0.9661 to 0.9982, which correspond to very good and high correlation. Based on this, our results suggested that the phenolic compounds mostly contributed to the antioxidant capacity of the investigated thistles extracts. Numerous investigations of plant extracts have confirmed a high linear correlation between the values of total phenolics concentration and antioxidant activity (Katalinić et al., 2006; Mihaylova et al., 2013).

CONCLUSIONS

The present paper investigated the carbohydrate composition and the antioxidant capacity of the *Onopordum tauricum* Willd. and *Carduus acanthoides* L. in respect of their potential as a source of prebiotics and antioxidants. The obtained results enriched the information about phytochemical compounds in the flower heads of *O. tauricum* and *C. acanthoides*. The established content of carbohydrates and especially of fructooligosachaides, and inulin revealed the potential of these plants to be applied as a source of prebiotics and soluble dietary fibres for the human nutrition. Due to the presence of inulin and antioxidants the flower head extracts of the two thistles could be used in food and herbal cosmetics, as a natural source of bioactive compounds and phytonutrients.

Authors contributions

Nadezhda Petkova designed the study, did analyses of carbohydrate composition and wrote the article. Dasha Mihaylova was involved in overall planning, supervision and critical revision and did the analyses of total phenolic content and antioxidant activity estimation and the correlation evaluation as well.

REFERENCES

Ak, T. and I. Gülcin. 2008. Antioxidant and radical scavenging properties of curcumin. Chem. Biol. Interact. 174: 27-37.

Al-Shammari, L. A., W. H. B. Hassan and H. M. Al-Youssef. 2015. Phytochemical and biological studies of *Carduus pycnocephalus* L. J. Saudi Chem. Soc. 19: 410-416.

Angelov, G., S. Georgieva and K. Petkova-Parlapanska. 2012. Antioxidant activity of extracts from cotton thistle (*Onopordum acanthium* L.). Sci. Technol. 2(3): 19-23.

Benzie, I. F. and J. J. Strain. 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 299: 15-27.

Brand-Williams, M. E., W. Cuvelier and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. 28: 25-30.

Bucke, C. and Rastall. 1990. Synthesising sugars by enzymes in reverse. Chem. Br. 26: 675-678.

Delipavlov, D. and I. Cheshmedzhiev, (Eds.). 2003. Key to the plants in Bulgaria. Academic Publishing House of the Agricultural University, Plovdiv, (Bulgaria). Pp. 405-407. (In Bulgarian).

Katalinić, V., M. Miloš, T. Kulišić and M. Jukić. 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 94(4): 550-557.

Kleonikos, G. S. 2006. Wild Edible Plants of Crete. Rethymnon Crete, Privately Published, ISBN 960-631-179-1..

Kujala, T. S., J. M. Loponen, K. D. Klika and K. Pihlaja. 2000. Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: Distribution and effect of cold storage on the content of total phenolics and three individual compounds. J. Agric. Food Chem. 48: 5338-5342.

Li, R., L. Sui-ku, W. Song, Y. Wang, Y. Li, X. Qiao, H. Liang and M. Ye. 2014. Chemical analysis of the Tibetan herbal medicine *Carduus acanthoides* by UPLC/DAD/qTOF-MS and simultaneous determination of nine major compounds. Anal. Methods. 6: 7181-7189.

Machado, M., K. Gláucia, S. Vieira, F. Menegalli, J. Martínez and M. Hubinger. 2015. Prebiotic oligosaccharides from artichoke industrial waste: Evaluation of different extraction methods. Ind. Crops Prod. 76: 141-148.

Mihaylova, D., L. Georgieva, I. Zhelev and I. Dimitrova-Dyulgerova. 2013. Influence of the extraction conditions over the antioxidant activity of *Carduus thoermeri*. Bull. J. Agric. Sci. 19(2): 61-64.

Mihaylova, D., A. Lante and A. Krastanov. 2015. Total phenolic content, antioxidant and antimicrobial activity of *Haberlea rhodopensis* extracts obtained by pressurized liquid extraction. Acta Aliment. 44(3): 326-332.

Olennikov, D., L. Tanhaeva and A. Rokhin. 2009. Glucofructans from *Taraxacum officinale* roots. Chem. Nat. Compd. 45: 141-144.

Petit, D. 2012. Significance of spines in Cardueae (asteraceae), bioclimatic stages, and mammalian grazing. Rev. agrobiol. 3: 27-32.

Petkov, V. 1982. Modern Phytotherapy. State Publishing House "Medicina i fizkultura", Sofia. p513. (Bg).

Petkova, N. and P. Denev. 2013. Evaluation of fructan content of the taproots of Lactuca serriola L. and Sonchus oleraceus L. Scientific Bulletin, Series F "Biotechnologies", 17: 117-122.

Petkova, N., P. Denev, M. Ivanova, R. Vlaseva and M. Todorova. 2013. Influence of harvest time on fructan content in the tubers of *Helianthus tuberosus* L. Nutrihort Proceeding Papers Ghent. Pp. 284-289.

Petkova, N., R. Vrancheva, P. Denev, I. Ivanov and A. Pavlov. 2014. HPLC-RID method for determination of inulin and fructooligosacharides. Acta Sci. Nat. 1: 99-107.

Petkova, N., D. Mihaylova,·P. Denev and·A. Krastanov. 2015a. Evaluation of biological active substances in flower heads of *Carduus thoermeri* Weinm. Rom. Biotechnol. Lett. 20(4): 10592-10599.

Petkova, N., I. Ivanov, S. Topchieva, P. Denev and A. Pavlov. 2015b. Biologically active substances and *in vitro* antioxidant activity of different extracts from Dandelion (*Taraxacum officinale*) roots. Sci. Bull. Ser. F. Biotechnol. XIX: 190-197.

Re, R., N. Pellegrini, A. P. Pannala, M. Yang, C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26(9/10): 1231-1237.

Stojanov, N., B. Stefanov and B. Kitanov. 1967. Flora Bulgarica, 4th ed., Vol. 2. Leguminosae to Compositae Sofia, Nauka i Izkustvo. (In Bulgarian).

Tutin, T. G., V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters and D. A. Webb, (Eds.). 1976. Flora Europaea, Vol. 4. Plantaginaceae to Compositae (and Rubiaceae), Cambridge University Press, Cambridge, UK.

Van Loo, J., P. Coussement, L. De Leenheer, H. Hoebregs and G. Smits. 1995. On the presence of Inulin and Oligofructose as natural ingredients in the western diet. Critic. Rev. Food Sci. Nutr. 35(6): 525-552.

Zhelev, I., I. Dimitrova-Dyulgerova, D. Belkinova and R. Mladenov. 2013. Content of phenolic compounds in the Genus *Carduus* L. from Bulgaria. Ecol. Balkanica. 5(2): 13-21.

Zhelev, I., P. Merdzhanov, M. Angelova-Romova, M. Zlatanov, G. Antova, I. Dimitrova-Dyulgerova and A. Stoyanova. 2014. Lipid composition of *Carduus thoermeri* Weinm., *Onopordum acanthium* L. and *Silybum marianum* L., growing in Bulgaria. Bulg. J. Agric. Sci. 20: 622-627.

Zheleva-Dimitrova, D., I. Zhelev and I. Dimitrova-Dyulgerova. 2011. Antioxidant activity of some *Carduus* species growing in Bulgaria. Free Radic. Antiox. 1(4): 15-20.