

*Content of Phenolic Compounds in the Genus *Carduus* L. from Bulgaria*

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Abstract. Phytochemical screening of the content of total polyphenols, flavonoids, phenolic acids and anthocyanins in Bulgarian *Carduus* L. species was carried out. The plant materials (inflorescences) from all of the 14 species found in Bulgaria has been collected from natural habitats from different floristic regions, during the period 2011-2013. Chemical analysis of the specimens was carried out in accordance with 11 Russian and 7 European Pharmacopoeia. For some of the plant species the obtained results are the first published data about content of phenolic compounds. The content of flavonoids (1,8-3,2%) and total phenols(1,7-2,3%) was higher in comparison with this of phenolic acids (0,6-2,4%) and anthocyanins (0,5-1,5%). The highest content of total phenols and antocyanins was determined in the *Carduus thracicus*. The three species *Carduus thoermeri*, *Carduus nutans* and *Carduus candicans* ssp. *globifer* were characterized with the highest content of flavonoids. The highest content of phenolic acids was determined in the *Carduus armatus*.

Key words: *Carduus*, phenolic compounds, phytochemical screening

Introduction

Medicinal plants constitute one of the main sources of new pharmaceuticals and healthcare products (DEMIRAY *et al.*, 2009). The role of medicinal plants in disease prevention or treatment been attributed to properties of their constituents, usually associated with a wide range of amphipathic molecules, broadly termed polyphenolic compounds (IVANOVA *et al.*, 2005).

Phenolics are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known (DAI & MUMPER, 2010). Plant phenolics include the groups of phenolic

acids, flavonoids, antocyanins, highly polymerized substances such as tannins and some other compounds. These compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects (BALASUNDRAM & SAMMAR, 2006).

Flavonoids are the most abundant polyphenols in plants, sub-divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins (D'ARCHIVIO *et al.*, 2007). Flavonoids possess a wide spectrum of biological activities in cardio vascular system and they are potential blood circulation enhancers in the

brain (SHOHAIB *et al.*, 2011). Antocyanins are water-soluble pigments, being the most reduced flavonoids. Anthocyanins occur in all tissues of plants, including leaves, stems, roots, flowers and fruits (ELOMAA *et al.*, 2003). One of the most prominent property of the antocyanins is their excellent radical scavenging ability, intimately connected with their oxidation/reduction potential (YOU DIM *et al.*, 2000). The role of anthocyanins in cardiovascular disease protection is strongly linked to oxidative stress protection too (KONG *et al.*, 2003).

Phenolic acids, an other part of phenolic compounds, have attracted considerable interest in the past few years due to their strong antioxidant activities against free radicals (ACIKARA *et al.*, 2013). Free radicals are the cause of many chronic diseases such as cancer, cardiovascular diseases, inflammation, brain dysfunction (GRUZ *et al.*, 2011; KAMATOU *et al.*, 2010). Phenolic acids also exhibit antibacterial, antipyretic, antirheumatic activities (MATTILA & HELLSTRÖM, 2007; MARKOWSKI *et al.*, 1998). Ferulic and sinapic acids, like phenolic acids, are antioxidants (IBTISSEM *et al.*, 2012) They exhibit direct antitumor activity against breast (GELINAS & MCKINNON, 2006) and liver cancer (BEEJMOHUN & FLINIAUX, 2007). Ferulic acid aslo may be effective in preventing cancer induced by exposure to carcinogenic compounds (QUINDE-AXTELL & BAIK, 2006).

The genus *Carduus* is presented in the Bulgarian flora by 14 species, 5 of which are endemic (STOJANOV *et al.*, 1967; DELIPAVLOV & CHESHMEDZHIEV, 2003; TUTIN *et al.*, 1976). The species *Carduus acanthoides* (Plumeless thistle) is used in traditional medicine as a diuretic, cardiotonic, sedative and anti-hemorrhoidal remedy (PETKOV, 1982).

Of the secondary metabolites contained in *Carduus* species, the flavonoids are the group, which has been most studied, regarding mostly their qualitative composition (HARBORNE, 1977; AMER *et al.*, 1985; HEDIN & WAAGE, 1986; BAIN & DESROCHERS, 1988; FERNANDEZ *et al.*, 1991; EL-LAKANY *et al.*, 1997; SIMMONDS & GRAYER, 1999; WILLIAMSON *et al.*, 1999; JORDON-THADEN & LOUDA, 2003;

TERENTJEVA & KRASNOV, 2003; ZHANG *et al.*, 2001; XIE *et al.*, 2005; JEONG *et al.*, 2008). Phytochemical studies on *Carduus getulus* revealed the presence of steroids and triterpenoid constituents as well (ABDEL-SALAM *et al.*, 1982). Other authors reported alkaloids and phenolic acids, lignans and coumarins for the species *Carduus acanthoides*, *Carduus crispus* and *Carduus nutans*. (FRYDMAN & DEULOFEU, 1962; ZHANG *et al.*, 2001, 2002; TERENTJEVA & KRASNOV, 2003; XIE & JIA, 2004). Our previous studies of six different *Carduus* species showed significant levels of total phenols and flavonoids (ZHELEVA-DIMITROVA *et al.*, 2011).

Considering the fact that the phenolic compounds contribute most commonly to the biological activity of various medicinal plants, the objective of the present work was phytochemical screening for the main phenolic compounds content, in particular total phenols, flavonoids, phenolic acids and anthocyanins in the Bulgarian *Carduus* species.

Material and Methods

Plant material. The plant material (inflorescences) was collected from natural habitats, during the period 2011-2013, from all fourteen species of the genus *Carduus*, found in Bulgaria (Table 1). Voucher specimens of the studied species were deposited in a herbarium at the Agriculture University of Plovdiv, Bulgaria (Herbarium SOA).

Quantification of total phenols. The determination of total phenols in the plant drugs was performed according to the EUROPEAN PHARMACOPOEIA 7 (2011a) involving Folin-Chiocalteu reagent and pyrogallol as standard. The analyses were carried out at 760 nm. The measurements were carried out using an Ultraspec 3300 pro UV/VIS spectrophotometer (USA). All determinations were performed in triplicate (n=3).

Quantification of flavonoids. The content of the flavonoids in the plant material was spectrophotometrically determined at 430 nm by creating a complex with AlCl₃

according to the RUSSIAN PHARMACOPOEIA 11 (1990). The content of flavonoids was calculated as quercetin. The measurements were carried out using a Ultraspec 3300 pro UV/VIS spectrophotometer (USA). All determinations were performed in triplicate (n=3).

Quantification of phenolic acids. Determination of the total phenolic acids in the plant material was performed according to the EUROPEAN PHARMACOPOEIA 7 (2011b), using rosmarinic acid as a reference. The analyses were carried out at 505 nm. The measurements were carried out using

an Ultraspec 3300 pro UV/VIS spectrophotometer (USA). All determinations were performed in triplicate (n=3).

Quantification of anthocyanins. The determination of anthocyanins in the plant material was performed according to the EUROPEAN PHARMACOPOEIA 7 (2011c). The method comprises sequential extraction with ethanol and butanol. Cyanidin chloride was used as a standard. The analyses were carried out at 505 nm using an Ultraspec 3300 pro UV/VIS spectrophotometer (USA). All determinations were performed in triplicate (n=3).

Table 1. Collection locality, altitude and voucher specimen of the studied *Carduus* species

Species	Collection locality, Floristic region in Bulgaria	Altitude (m)	Voucher specimen in Herbarium SOA-Plovdiv
<i>C. acanthoides</i> L.	Trigrad, Rhodopes Mountains (middle)	1100 m	059719
<i>C. acicularis</i> Betrol. (<i>C. argentatus</i>)	Tzarevo, Black Sea Coast (southern)	30 m	059650
<i>C. armatus</i> Boiss. & Heldr. (<i>C. tmoleus</i> ssp. <i>armatus</i>)	"Balgarka" Nature park, Balkan Range (middle)	1415 m	059781
<i>C. candicans</i> Waldst. & Kit. ssp. <i>globifer</i> (Velen.) Kazmi	Starosel, Thracian valley	349 m	059723
<i>C. carduelis</i> (L.) Gren. (<i>C. alpestris</i>)	Yundola, Rila Mountains	1510 m	059779
<i>C. crispus</i> L.	Ivanski, Northeast Bulgaria	92 m	059725
<i>C. hamulosus</i> Ehrh.	Narechen, Rhodopes Mountains (middle)	588 m	059644
<i>C. kernerii</i> Simonkai ssp. <i>austro-orientalis</i> Franco (<i>C. scardicus</i>)	Beglika, Rhodopes Mountains (western)	1550 m	059651
<i>C. nutans</i> L.	Arkutino, Black sea Coast (southern)	30 m	059660
<i>C. personata</i> (L.) Jacq.	"Balgarka" Nature park, Balkan Range (middle)	815 m	059780
<i>C. pycnocephalus</i> L.	Carevo, Black Sea Coast (southern)	30 m	059649
<i>C. rhodopaeus</i> Velen. (<i>C. adpressus</i> ssp. r.)	Rozhen, Rhodopes Mountains (middle)	1431 m	059776
<i>C. thoermeri</i> Wienm. (<i>C. leiophyllus</i>)	Rozhen, Rhodopes Mountains (middle)	1465 m	059777
<i>C. thracicus</i> Hayek.	Tzatzarovci, Rhodopes Mountains (middle)	956 m	059773

Results and Discussion

Content of total polyphenols. The amount of total polyphenols (including all water soluble phenolic compounds in plant) was expressed as pyrogallol equivalent in % (Fig. 1). The amounts ranged from 1,72% to 2,25%. Species, characterised by higher contents of these substances were *C. thracicus* (2.25%), followed by *C. armatus* (2,12 %) and *C. nutans* (1,97 %). In most of the investigated species contents of total

polyphenols between 1,7 - 1,8% was established.

The established quantities of total phenols in the studied genus *Carduus* were similar to those reported for the closely related genera *Silybum* (WOJDYŁO *et al.*, 2007) and *Centaurea* (EROL-DAYI *et al.* 2011). Relatively lower values were determined by NAZARUK (2008) - 0,012 - 0,8% and YIN *et al.* (2008) - 0,022 % for species of the genus *Cirsium*. The obtained results revealed that

the Bulgarian *Carduus* species have good content of these valuable biologically active ingredients, confirming our previous data for six *Carduus* species (ZHELEVA-DIMITROVA *et al.*, 2011), as well as the fact that the environmental conditions of growth location do not significantly affect the accumulation of common water-soluble polyphenols in a genus *Carduus* (ZHELEV *et al.*, 2011).

Content of flavonoids. The phytochemical analysis for flavonoid content showed that this group of phenolic compounds was predominant in the studied *Carduus* species - from 1.78% to 3.20% (Fig. 2). *C. thoermeri* showed the highest flavonoid content of 3.20%, followed by *C. nutans* (3.00%) and *C. candicans* ssp. *globifer* (2.95%). Other species, with flavonoid contents more than 2% in descending order were *C. pycnocephalus*, *C. crispus*, *C. acanthoides*, *C. kernerii* ssp. *austro-orientalis* and *C. thracicus*.

Among the investigated phenolic compounds in the studied plant species the presence of flavonoids was established as predominant, although they are part of the total phenolic compounds group. This might be explained by the different extracting solvents - the polyphenols determination was carried out in the water extracts where the most flavonoids are not soluble. For quantification of the flavonoids 90% ethanol was used. More than a half of the studied species demonstrated significant

levels of flavonoids, which confirm our previously reported results, especially for *C. thoermeri*, *C. nutans* and *C. candicans* ssp. *globifer* (ZHELEV *et al.*, 2011; ZHELEVA-DIMITROVA *et al.*, 2011). The quantitative accumulation of total flavonoids is influenced not only by the species differences, but also by the conditions of growth locations (ZHELEV *et al.*, 2011). In comparison, study of NAZARUK & SZOKA (2009) on species from the related genus *Cirsium* show significant lower values in range from 0.016 to 0,07%, compared to 2.31% flavonoids in *Cirsium japonicum* (Yin *et al.*, 2008).

Content of phenolic acids. Research on the quantity of phenolic acids has been carried out for the first time for the genus *Carduus*. The obtained results showed that this group of phenols varies in wide range 0,57% - 2,43% (Fig. 3). Among the investigated plants with higher content (more than 2%) were established to be *C. armatus* (2,43%), *C. personata* (2,15%), *C. hamulosus* (2,03%) and *C. carduelis* (1,96%). In comparison lower values of phenolic acids were reported by NAZARUK & SZOKA (2009) in species of the genus *Cirsium* 0,15% - 1,31%.

Content of anthocyanins. Anthocyanins, the most reduced flavonoids, were presented in *Carduus* inflorescences in range from 0.47% to 1.45% (Fig. 4) as *Carduus thracicus* was the species with the highest established amount of these purple or blue pigments. Among

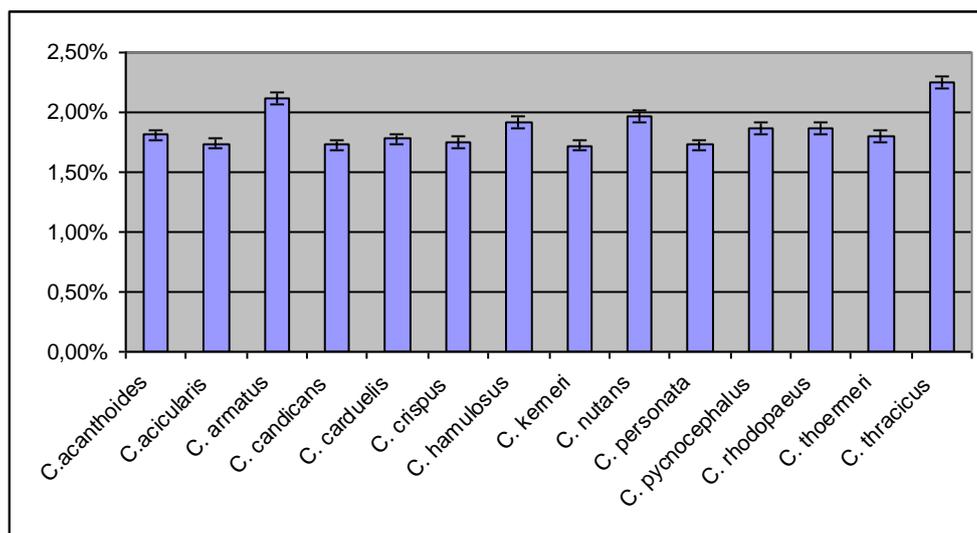


Fig. 1. Content of total water soluble polyphenols in Bulgarian *Carduus* species (%).

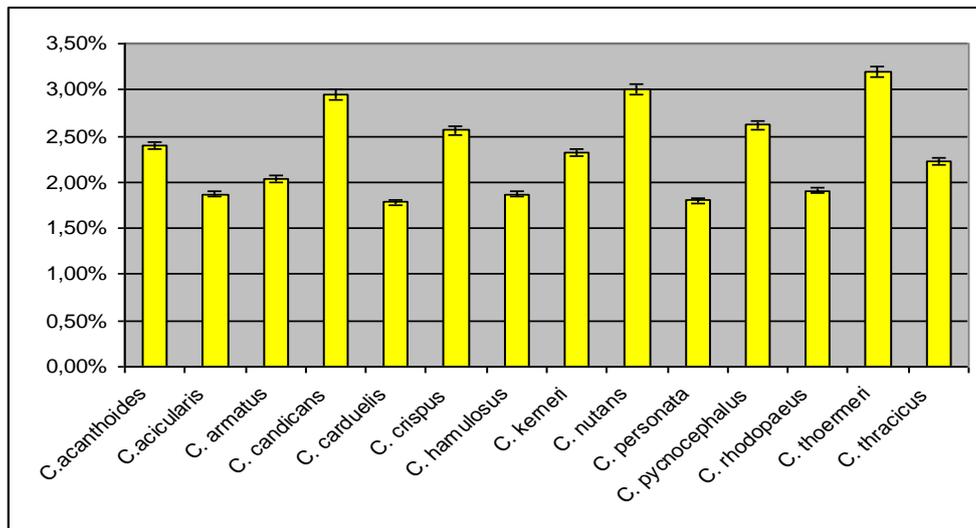


Fig 2. Content of flavonoids in Bulgarian *Carduus* species (%).

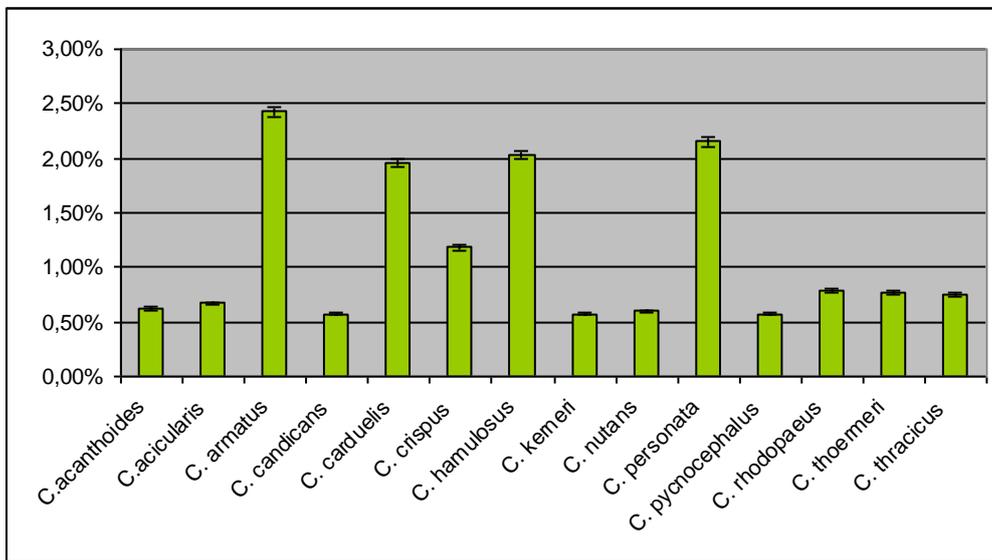


Fig. 3. Content of phenolic acids in Bulgarian *Carduus* species (%)

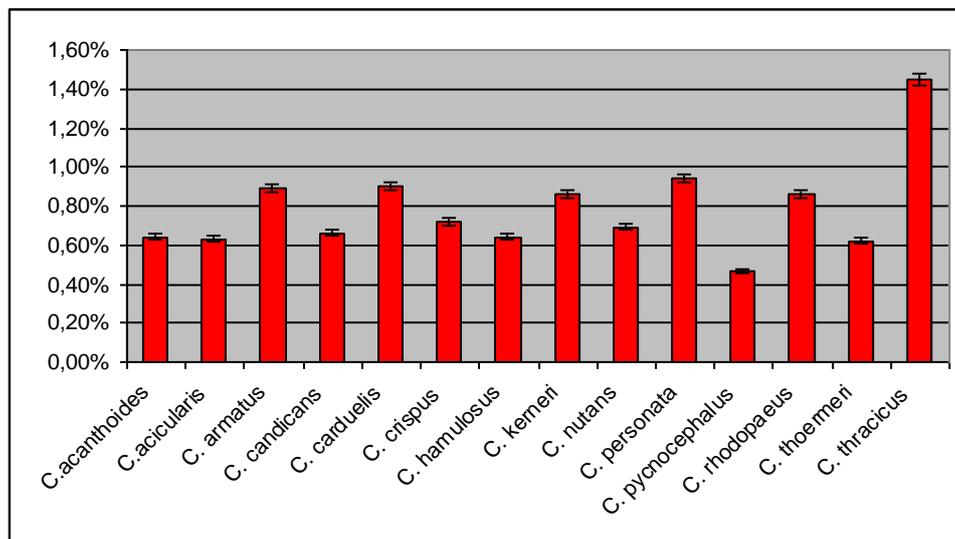


Fig 4. Content of anthocyanins in Bulgarian *Carduus* species (%)

the investigated plants with anthocyanins content in range of 1% were *C. personata* (0.94%), *C. carduelis* (0.90%), *C. armatus* (0.89%), *C. rhodopaeus* and *C. kernerii* (0.86% both).

It is notable that plant species from mountain areas at the above 800m altitude, contained higher quantities of these substances. However, comparison of the results is not possible, because there are no previous data on anthocyanins content in *Carduus* genus and related genera at all.

The investigations of other researchers conducted on phenolic compounds content were mainly related to their good antioxidant properties (DEMIRAY *et al.*, 2009; DAI & MUMPER, 2010; MIHAYLOVA *et al.*, 2013). Our previous study on six *Carduus* species showed that all tested extracts exhibit significant antioxidant activity (ZHELEVA-DIMITROVA *et al.*, 2011). The antioxidant activity values for *C. thoermeri*, *C. candicans* ssp. *globifer* and *C. nutans* were similar compared to the standard compound (L-ascorbic acid). This could be an objective for further investigations on the qualitative composition and biological activity of the investigated groups of phenolic compounds in the species of this genus.

Conclusions

In summary, as a result of the conducted phytochemical screening of genus *Carduus* in Bulgaria in terms of main phenolic compounds content, their presence in the *Carduus* inflorescences was established. Flavonoids and total phenols were revealed as dominant constituents, followed by phenolic acids and anthocyanins. Species with the highest amount of these valuable biologically-active substances were *Carduus thoermeri*, *Carduus nutans*, *Carduus candicans* ssp. *globifer*, *Carduus thracicus* and *Carduus armatus*.

The present study provides for the first time scientific data for the quantity of the phenolic acids and anthocyanins occurring in all Bulgarian *Carduus* species. The total polyphenols and flavonoids content have been also studied for the first time for the

mentioned species - *C. armatus*, *C. carduelis*, *C. hamulosus*, *C. rhodopaeus* and *C. thracicus*, respectively.

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