

Phenolics composition and antioxidant capacity of guelder rose fruit, flower and bark extracts

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Received: 4 April 2019 / Available on-line: 4 April 2019

Abstract: *Different parts of plants, including fruits, leaves, roots, bark, stem and flower, are a promising source of bioactive compounds, therefore they are intensively studied for biological activity and the possibility of use in functional foods and dietary supplements. The present research compares the phenolic profile and antioxidant capacity of aqueous extracts obtained from bark, flowers and fruits of guelder rose. Antioxidant activity was evaluated against ABTS, hydroxyl and peroxy free radicals, and as a reducing power by using in vitro test. The total phenolics, flavanols and proanthocyanidins were assessed by spectrophotometric methods, and individual phenolic compounds were also determined using UPLC analysis. Bark water extract proved to be richest in natural antioxidants because it showed the highest antioxidant potential, regardless of the method used. Its very high antioxidant capacity was connected with high phenolic compounds content, especially flavanols and proanthocyanidins. Water extracts of guelder rose fruits and flowers were characterized by the high level of hydroxycinnamic acids, especially chlorogenic acid. It seems that guelder rose bark may be selected as potential source of phytochemicals with high antioxidant potential.*

Keywords: *Guelder rose, fruits, flowers, bark, phenolic compounds, antioxidant capacity.*

Introduction

There is a growing search for new sources of natural compounds with health beneficial effects. Plant derived bioactive ingredients include, among others, vitamins, fiber, essential oils and phenolics. Phenolic compounds are considered to be responsible for multidirectional pro-health activity, such as antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antimicrobial and cardioprotective effects [1]. Biological activities of phenolic compounds depend on their chemical structures, especially degree of hydroxylation, other substitutions and conjugations, and degree of polymerization. People consume phenolics with beverages, fruits, and, to a lesser extent, vegetables and legumes seeds [2]. It is worth noting that others anatomical parts of plant, such as leaves, branches, bark, flowers and stem

have been recognized as valuable sources of these phytochemicals [3-7]. Therefore, other parts of plant than fruits may be used as valuable ingredients in functional foods, herbal teas, dietary supplements as well as natural antioxidants in cosmetics.

Few published data suggest that the guelder rose can be used in this context. First, *in vitro* studies have demonstrated the antioxidant properties of guelder rose fruits, branches, leaves and bark components [8-14]. Secondly, the correlation studies have shown a link between antioxidant activity of guelder rose fruits and their phenolic compounds content [10, 14]. Moreover, ethanolic extract of bark exhibited anti-inflammatory activity [15] when water extract of leaves showed hepatoprotective and hypoglycemic [16] effects. Guelder rose (*Viburnum opulus* L.) also known as European cranberrybush, crampbark tree and snowball bush is a valuable decorative, medicinal and food plant [17]. Its fruits are used in traditional cuisine as a component of e.g. jams, liqueurs and herbal teas [13]. Despite the well documented guelder rose fruits [10, 13, 14, 17, 18], only one research describes the phenolic compounds composition and antioxidant potential of guelder rose bark [9]. To the best of our knowledge, there is no such information about flowers. Therefore, to increase the use of guelder rose in pharmaceuticals and functional foods, a better understanding of its chemical characteristic and pro-health activities is needed.

The objectives of the present study have been: (1) to assess antioxidant capacity of water extracts of commercially available fruits, bark and flowers of guelder rose via 2,2'-azinobis(3-ethyl-benzthiazoline-6-sulphonic acid) cation radical (ABTS), peroxy radical (ORAC) and hydroxyl radical scavenging and ferric reducing antioxidant power (FRAP) methods, (2) to investigate the phenolic compounds composition of the extracts by Ultra-Performance Liquid Chromatography (UPLC) and using spectrophotometric methods for total phenolic, flavanol and proanthocyanidin contents.

Experimental

Materials

Plant materials

The flowers, fruits and bark of guelder rose in a dried form were purchased from “Nanga Przemysław Figura” herbal wholesaler (Złotów, Poland), “Natura Wita Ltd” (Kopernia, Poland) and “FLOS Herbs Packaging Plant” (Mokrsko, Poland), respectively. Droughts (Figure 1) were grind in a coffee grinder and powders were kept in tightly closed containers in a laboratory cabinet without light.



Figure 1. Parts of guelder rose investigated in this study

Preparation of water extract

To 10 g of grounded bark, flowers and fruits was added 200 mL of boiling water followed by heating on the low heat for 5 min. Next the mixture was left at room temperature for 15 min and centrifuged at 5000 rpm for 10 min. The supernatant was evaporated at 40 °C under reduced pressure and lyophilized. The fruits, flower and bark extracts yields were 46.5%, 20.1% and 15.5%, respectively. Stock solution of each extract was prepared at concentration 25 mg/mL in water before analysis.

Methods

Quantification of phenolic compounds by spectrophotometric methods

Total phenolics content was determined using Folin-Ciocalteu reagent as we described in our previous work and was expressed as mg of gallic acid equivalents (GAE) per g of lyophilized extract [19]. Total flavanols content was estimated by vanillin procedure and expressed as mg of CE per g of lyophilized extract and total proanthocyanidins was determined after their acid depolymerization to the corresponding anthocyanidins as described in our previous study [20] and expressed as gram of cyanidin equivalents (CYE)/g of lyophilized extract.

Phenolic profile determination

Phenolic profiles were determined using an ACQUITY Ultra Performance LC system (UPLC) equipped with a photodiode array detector with a binary solvent manager (Waters, Milford, MA). The data were collected by Mass-Lynx™ V 4.1 software. Separation was achieved on a Acquity UPLC HSS T3 column (150 × 2.1 mm, 1.8 μm; Waters). The mobile phase was a binary gradient with A, water/formic acid (95.5:4.5, v/v), and B, acetonitrile, with a flow rate of 0.45 mL/min [21]. The binary gradient was as follows: initial conditions – 99% A (0 min), 12 min – 75% A, 12.5 min – 100% B, 13.5 min – 99% A (12.5-13.5 min). The runs were monitored at the following wavelengths: flavanols at 280 nm, hydroxycinnamic acids at 320 nm, flavonols at 360 nm and anthocyanins at 520 nm. The retention times and spectra were compared to those of the authentic standards. Calibration curves at concentrations ranging from 0.06 to 2 mg/mL ($r^2 \geq 0.96$) were made from chlorogenic acid, cryptochlorogenic acid, neochlorogenic acid, 3-coumaric acid, (+)catechin, (-)epicatechin, procyanidins B1 and B2, quercetin 3-glucoside, rutin, isorhamnetin, isorhamnetin 3-glucoside and isorhamnetin 3-rutinoside. The results were expressed as mg/g of lyophilized extract.

In vitro antioxidant activity assays

Antioxidant potential of guelder rose water extracts was determined in vitro by four methods hydroxyl radical, peroxy radical scavenging capacity by ORAC assay (Oxygen Radical Scavenging Capacity), and by FRAP method. Antioxidant capacity was expressed as μM Trolox equivalents (TE) per g of lyophilized extract.

ABTS^{•+} radical cation scavenging activity (ABTS) and ferric reducing power (FRAP) assays procedures have been detailed in our previous work [19].

Hydroxyl radical scavenging (HRS) activity was evaluated using the method of Racchi et al. [22]. Peroxyl radical scavenging capacity was carried out by the ORAC assay, according to Kevers et al. [23].

Statistical analysis

Data from the present study was presented as the mean \pm standard deviations of three replicates for each sample. Differences between groups were tested by one-way analysis of variance (ANOVA) followed by Duncan's post hoc test. Statistically significant differences were set at $p \leq 0.05$.

Results and Discussion

Phenolic compounds composition

Extraction is a crucial first step for the isolation of phenolics from plant material and its effectiveness is influenced by, among others, phenolic chemical nature and solubility, and presence of interfering substances. So, there is no uniform or completely satisfactory procedure that is suitable for extraction of all phenolic groups in plant materials [24]. Phenolic compounds were extracted from guelder rose fruits using different solvents, such as water, acetonitrile, methanol, ethanol, methanol acidified with acetic acid, and ethyl acetate [11, 13, 17, 18]. According to the work of Erdogan-Orhan et al. [11] methanol extracts of guelder rose branches, leaves and fruits demonstrated the highest phenolic content, followed by water and ethyl acetate. In the present study, different parts of guelder rose were extracted using water according to the recipe of the preparation of bark decoction on the product packaging. Phenolic composition of the studied extracts determined by spectrophotometric methods is presented in Table 1.

Table 1. The content (mg/g) of total phenolics, flavanols and proanthocyanidins in extracts of guelder rose flowers, bark and fruits. Values are expressed as mean \pm SD ($n \geq 3$), mean values within a row with different letters are significantly different at $p \leq 0.05$ ($n \geq 3$)

Factor	Flowers	Bark	Fruits
Total phenolics	78.60 \pm 1.14 ^b	171.02 \pm 3.16 ^c	50.64 \pm 1.53 ^a
Total flavanols	3.57 \pm 0.35 ^a	97.79 \pm 3.71 ^c	11.82 \pm 1.19 ^b
Total proanthocyanidins	3.27 \pm 0.15 ^a	50.29 \pm 1.80 ^c	5.81 \pm 0.31 ^b

The statistically significant differences ($p \leq 0.05$) between fruit, bark and flower extracts in terms of total phenolics, flavanols and proanthocyanidins were observed. The bark extract was characterized by the highest level of total phenolics (171.02 mg GAE/g) as well as other determined groups of polyphenols, such as flavanols (97.79 mg CE/g) and proanthocyanidins (50.29 mg CYE/g). The lowest content of total phenolics (50.64 mg GAE/g) was observed in guelder rose fruit extract while the flower extract contained the least flavanols (3.57 mg CE/g) and proanthocyanidins (3.27 CYE/g). The data obtained by Erdogan-Orhan

et al. [11] for total phenol content in water extracts of *Viburnum opulus* branch (162.67 mg GAE/g) and fruits (49.06 mg GAE/g) correspond to our results. The water extraction of *V. opulus* pomace, carried out by Kraujalis et al. [25], gave the extract with a higher content of polyphenols (174.9 mg GAE/g) compared to the fruit extract. Probably, our extract of fruits contained other water-soluble fruit ingredients, such as sugars, acids and proteins, which in the pomace were found in trace amounts due to their extraction into the juice. According to Perova et al. [13] proanthocyanidins, along with hydroxycinnamic acids, are the main group of polyphenols of the guelder rose fruits. In our study they accounted for 29.4, 11.5 and 4.2% of total phenolic content in bark, fruit and flower extracts.

Data on the composition of individual phenolic compounds are very important because the structure of phenolics significantly affects their properties. In connection with the above, the water extracts of guelder rose fruits, flowers and bark were analysed for the content of individual phenolic compounds using UPLC system (Figure 2). The results of qualitative and quantitative phenolic composition of extracts are summarized in Table 2.

Table 2. Content (mg/g) of individual phenolic compounds of guelder rose extracts determined by UPLC method. Values are expressed as mean \pm SD (n = 3), mean values within a row with different letters are significantly different at $p \leq 0.05$ (n = 3). -, not detected. Peak numbers of compounds are in accordance with those in Figure 2.

Peak no.	Compound	λ_{\max}	Flowers	Bark	Fruits
1	Procyanidin B1	278.7	2.91 \pm 0.00 ^b	25.58 \pm 0.03 ^c	1.15 \pm 0.01 ^a
2	(+)-Catechin	278.7	3.70 \pm 0.01 ^a	55.57 \pm 0.05 ^b	-
3	Procyanidin B2	278.7	-	5.57 \pm 0.03	-
4	(-)-Epicatechin	277.7	-	4.89 \pm 0.06	-
	Flavanols		6.61\pm0.01^b	91.61 \pm 0.06^c	1.15 \pm 0.01^a
5	Neochlorogenic acid	323.7	1.38 \pm 0.00 ^b	2.22 \pm 0.00 ^c	0.42 \pm 0.00 ^a
6	Chlorogenic acid	325.7	117.12 \pm 0.25 ^c	16.78 \pm 0.02 ^a	62.70 \pm 0.04 ^b
7	Cryptochlorogenic acid	325.7	3.36 \pm 0.00 ^c	1.14 \pm 0.00 ^b	0.32 \pm 0.00 ^a
8	3-Coumaric acid	277.7	-	0.52 \pm 0.01	-
	HCA		121.86\pm 0.25^c	20.66 \pm 0.03^a	63.44 \pm 0.04^b
9	Rutin	353.7	0.69 \pm 0.00 ^b	-	0.35 \pm 0.02 ^a
10	Quercetin 3-glucoside	353.7	5.10 \pm 0.06 ^b	-	0.06 \pm 0.00 ^a
11	Isorhamnetin 3-rutinoside	353.7	-	-	0.12 \pm 0.00
12	Isorhamnetin 3-glucoside	352.7	3.34 \pm 0.00	-	-
13	Isorhamnetin	370.7	0.25 \pm 0.00	-	-
	Flavanols		9.38\pm0.06^b	-	0.53\pm 0.02^a
	Phenolics Total		137.85\pm0.32^c	112.27 \pm 0.07^b	65.12 \pm 0.04^a

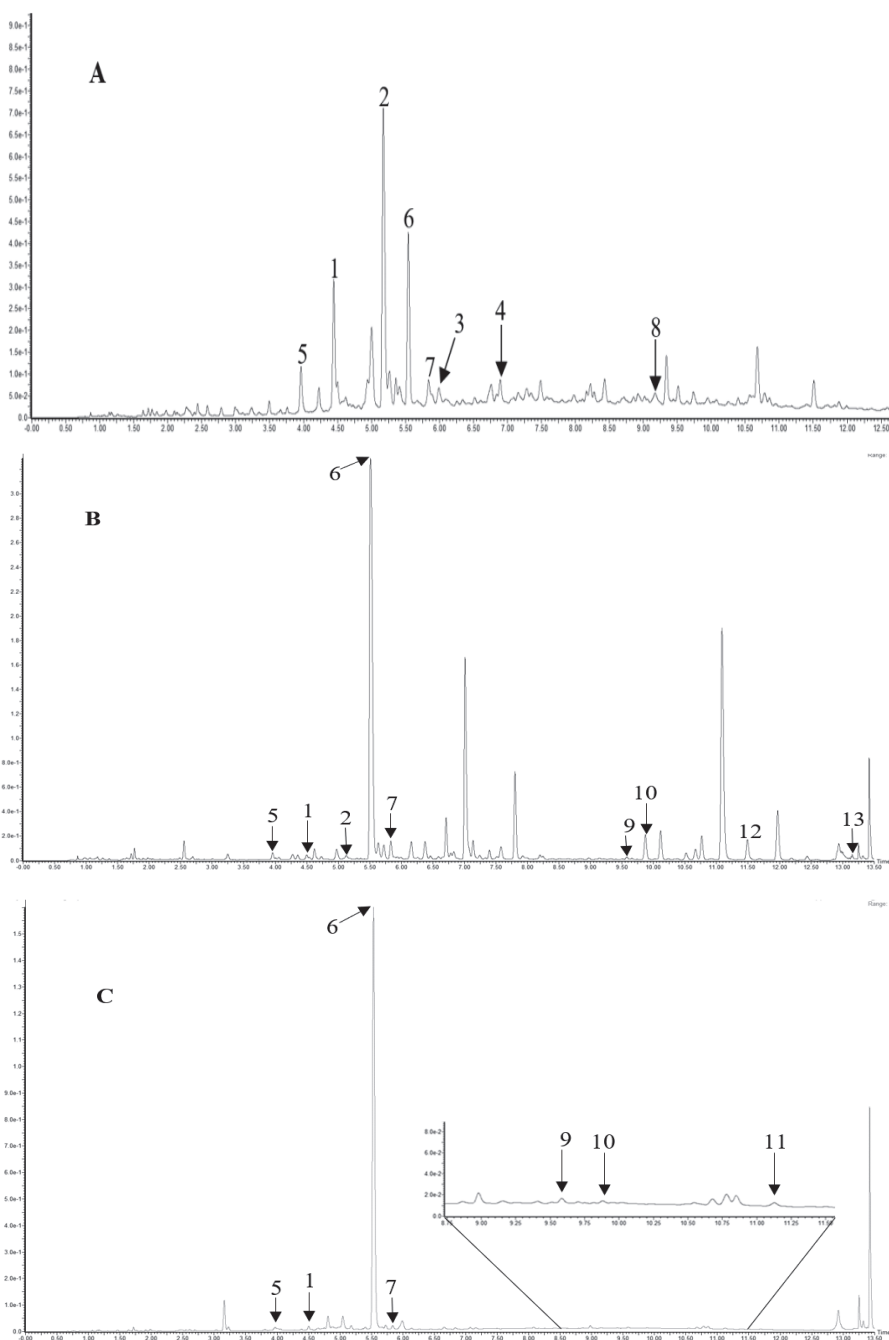


Figure 2. Chromatograms of extracts of guelder rose bark (A), flowers (B) and fruits (C) at 280 nm. Peak numbers of compounds are in accordance with those in Table 2

In our study, the different biological parts of guelder rose were characterised by a large variation in the amount of individual phenolic compounds tested. The results showed that hydroxycinnamic acids dominated in the extracts of guelder rose fruits and flowers (97.4 and 95.6% of sum of phenolic compounds) while flavanols in bark extract (81.6% of sum of phenolics). Additionally, flavonols were not found in bark extracts. The authors of the only report on the polyphenol composition of guelder rose bark have reported the presence of caffeic, chlorogenic, *p*-hydroxybenzoic and gallic acids [9]. In the present study, a comparison of the retention time and UV-Vis absorption spectra to those of the reference compounds allowed us to determine four flavanols and four hydroxycinnamic acids in bark extract with (+)-catechin and procyanidin B1 as dominant phenolic compounds followed by chlorogenic acid. Chlorogenic acid was the main phenolic compound in the fruit and flower extracts. The studies on the phenolic composition of guelder rose fruits have shown presence of hydroxybenzoic and hydroxycinnamic acids, catechins and procyanidins as well as flavonols and anthocyanins [13, 17, 26]. According to Özrenk et al. [26] the most abundant components in *V. opulus* fruits were (+)-catechin and gallic acid while Velioglu et al. [17] indicated chlorogenic acid as the main component followed by (+)-catechin. The fruit and flower extracts contained flavanols, which were marked in fruits by others authors [13, 17]. Anthocyanins were not found in fruit extract studied although they were identified in fresh fruits [10, 13, 27]. Probably, they were destroyed during the drying of fruit.

Antioxidant capacity

The antioxidant activity of phenolics is due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers and as metal chelators. Antioxidant properties of guelder rose extracts of flowers, bark and fruits were estimated by four different methods, as scavenging potential toward stable, synthetic ABTS^{•+} radical cation (ABTS) and toward reactive oxygen species such as •OH radical (HRS), and peroxy radical (ORAC), and as the potential to reduce ferric to ferrous ion (FRAP). The results for antioxidant capacity of guelder rose extracts, expressed as Trolox Equivalent (TE), are presented in Table 3. Significant differences ($p < 0.05$) were found among the analyzed extracts in the antioxidant capacity determined by ABTS and FRAP methods. The scavenging activity toward hydroxyl radical was similar for all extracts tested. The difference between extreme TE values in the ABTS method was 4.7-fold, in FRAP method 3.7-fold, and 5.8-fold in ORAC method. The antioxidant capacity of extracts of guelder rose different parts investigated was in the following order: bark > flowers > fruits.

Table 3. Antioxidant potential ($\mu\text{M TE/g}$) of extracts of guelder rose flowers, bark and fruits

Method	Flowers	Bark	Fruits
ABTS	475.95 \pm 10.80 ^b	1792.16 \pm 40.10 ^c	380.36 \pm 23.35 ^a
FRAP	463.91 \pm 9.42 ^b	1160.30 \pm 51.60 ^c	311.34 \pm 14.98 ^a
ORAC	4283.41 \pm 147.40 ^b	4386.19 \pm 72.41 ^b	754.46 \pm 29.11 ^a
HRS	188.12 \pm 14.62 ^a	191.42 \pm 14.52 ^a	184.00 \pm 20.30 ^a

Correlations between antioxidant activity assays with phenolic compounds tested by spectrophotometric assays (Table 1) are presented in Table 4. High correlations were found between total phenolics, flavanols and proanthocyanidins with ABTS, HRS and FRAP assays ($r \geq 0.786$). Correlation analysis also evidenced a positive relationship between the total phenolics with ORAC test ($r = 0.698$). On the other hand, the Pearson's coefficients listed in Table 4 suggest weak correlation between the total content of flavanols and proanthocyanidins and ORAC assay.

Table 4. Pearson's correlation coefficients (r) between groups of phenolic compounds and antioxidant activity assays

	ABTS	FRAP	ORAC	HRS
Total phenolics	0.987	0.999	0.698	0.935
Total flavanols	0.990	0.969	0.452	0.786
Total proanthocyanidins	0.994	0.976	0.480	0.805

Summary

This research has investigated the phenolics composition and antioxidant capacity of extracts from different parts (fruits, flowers and bark) of guelder rose (*Viburnum opulus*) using water as an extracting agent. The results demonstrated the differences in the phenolic compounds composition, and antioxidant properties of the extracts tested. This is the first report on the phenolic compounds and antioxidant properties of guelder rose flower extract. According to our UPLC data, the extract of flowers was more abundant in hydroxycinnamic acids, flavanols, and flavonols compared to fruits. The main phenolic compound in this extract was chlorogenic acid which also dominated in fruit extract. The extract from the guelder rose bark exceeded fruit and flower extracts in terms of total polyphenols, total flavanols and proanthocyanidins, and antioxidant potential. The highest antioxidant potential of bark extract is probably related to the presence of flavanols, especially (+)-catechin and procyanidin B1.

Acknowledgments

The present research was performed under the financial support of the Polish National Science Centre (project nr 2016/23/B/NZ9/03629).

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