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Description of the Guelder Rose Fruit in Terms of Chemical Composition, Antioxidant Capacity and Phenolic Compounds

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Abstract: The *Viburnum opulus* (guelder rose) fruit is a Polish native fruit that grows across almost the entire territory of the country, except for the Tatra Mountains. The fruits are rich in nutrients and biologically active compounds, however, they are rarely used for food production or as a dietary ingredient. This study assessed this fruit for dry matter (17.3% ± 0.7), extract (13% ± 0.53), titratable acidity (1.78 g/100 g ± 0.48), pectin (1.10% ± 0.97), vitamin C (47.79 mg/100 g ± 1.02), the amount of sugar identified as fructose (6.85 g/100 g ± 0.67), antioxidant activity, ABTS (14.64 ± 0.61 µM Trolox/100 g), FRAP (211.14 ± 1.04 µM Trolox/100 g) and total polyphenolic content TPC (1695.88 ± 0.86 mg GAE/100 g). The analysis of phenolic identification was performed using the ACQUITY Ultra Performance LC (UPLC) system with the binary solvent manager and PDA detector.

Keywords: plants' secondary metabolites; *Viburnum opulus*; organic food system; antioxidants; wild edible fruit

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1. Introduction

Today there is a trend of returning to the forgotten wild edible fruits that were once successfully used in folk medicine or cuisine. In this study, attention was paid to the fruits of the guelder rose due to its health benefits and nutritious qualities, as well as its desirable and unusual color [1].

In Poland, the *Viburnum opulus* (VO) plant, i.e., guelder rose, is one of two species (along with *Viburnum lantana*) naturally occurring in Poland. Genus Viburnum, which belongs to the family Caprifoliaceae, consists of approximately 150 species of shrubs and small trees. The guelder rose is a shrub typical of North America, Western and Central Europe, Asia, Caucasus and Asia Minor. It grows up to 3 to 5 m. Its fruits are red, vitreous and round-shaped. In the beginning, the fruits are green and as they ripen they turn red at the end of September, and when fully mature they are red and shiny [2,3].

The fruits of *V. opulus* are characterized by an unpleasant bitter taste since they contain saponin glycosides and slightly toxic viburnine. The fruits need to be frozen before they can be eaten since freezing makes the fruits' bitter taste less intense [4,5].

Edible fruits are used as ingredients for preparing jams, marmalades, juices, drinks and sauces. In Canada, they are used as substitutes for cranberries and in Russia and Ukraine, the fruits are used in 'kalinnikov' pies. In Turkey, there is the commercially available gilaburu juice—a traditional non-alcoholic beverage, which needs to undergo the fermentation process. During this process, fruits are placed in plastic drums with tap water, in a dark place at room temperature for four months to eliminate the sour flavor.

The fruits can be used after this time. Their juice is commonly used for medicinal purposes (e.g., to treat colds, liver diseases, diabetes or hypertension) [3].

Numerous clinical trials demonstrated an inverse relationship between the fruits' consumption and diseases. There is much evidence to show that a sufficient quantity of these fruits in the diet reduces diseases such as cancer or cardiovascular and neurodegenerative disease [6–8]. The fruits are a wealthy source of bioactive substances which may act as antioxidants. The color of the fruits indicates the presence of chemoprotective substances, which have biological activity in the body [7]. In the research, a lot of attention is paid to the antioxidant content of the fruits but their effectiveness in the fight against free radicals is still unknown. The important medicinal effect of antioxidants is the protection against diseases induced by reactive oxygen species. They are capable of donating electrons to oxidants making them harmless to the cell. The natural bioactive compounds (plants' secondary metabolites) proved to have pharmacological activity in terms of lipids regulations, insulin sensitivity and antioxidant activity [9]. VO fruits are characterized by high amounts of polyphenolics, phenolic acids, flavonoids, procyanidins, catechins, anthocyanins as well as organic acids (e.g., ascorbic acids and L- malic acids) [3,9,10].

Due to these compounds, numerous in vivo studies demonstrated that different morphological parts of VO show anti-microbial, anti-diabetic, anti-obesity, anti-inflammatory and anti-cancer properties. Moreover, a positive effect on the urinary system was observed in animal studies [3].

Despite its sour and bitter taste, the VO fruit, after appropriate freezing, shows great potential for use in juice technology, as a food additive, in cosmetics or pharmaceuticals. On the Polish market, only its bark is available for making infusions while fruits are used at home for making jams and liqueurs [8,11].

Some are trying to use the fruit as a food additive, e.g., for pear juice with the addition of the VO juice or cakes enriched with a VO fruit pomace. Some studies indicate the possibility of adding VO fruits to meat, e.g., cooked minced turkey [12] or homogenized meat products [13]. The addition of these fruits also plays a role in delaying oxidative changes.

The aim of this study was to analyze the basic chemical composition (dry matter, sugars, pectins, acidity, vitamin C, ABTS and polyphenols as well as the phenolic compound of fruits from *Viburnum opulus* (native species), cultivated in Poland (Liszna near Sanok). The novelty of this paper lies in the fact that VO fruits were harvested from an ecological place, far away from industrial sites. It is also worth comparing the obtained results with that of other authors who did not know the place where the fruit was harvested, as it was purchased in a shop. Our results can update the current knowledge of the guelder rose fruit.

2. Materials and Methods

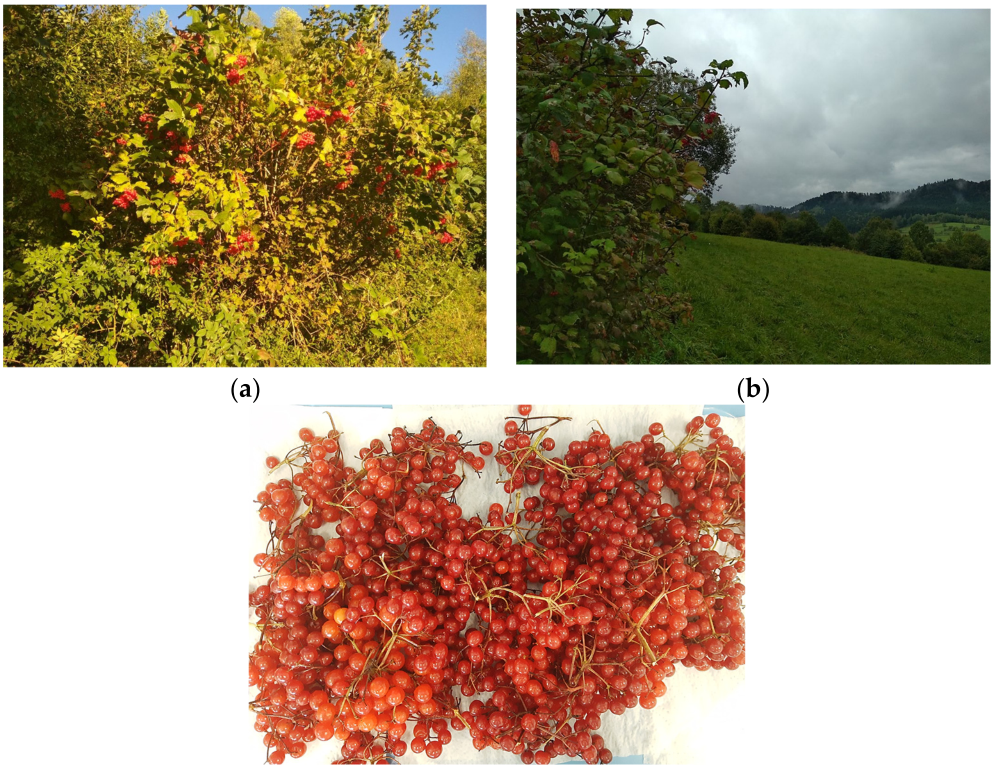
2.1. Plant Material

Matured *Viburnum opulus* fruits (Figure 1a,b) were harvested at the end of September 2020 in the southeastern part of Poland in Subcarpathian Voivodeship (Liszna near Sanok). The collection site was pollution-free, located far from industrial places. The collected fruits (Figure 1c) were stored for one week at a temperature of −18 °C and next they were washed and cleaned, and their seeds were removed. The fruits were ground in a mortar. The pulp was poured onto plates and freeze-dried for two days (LABCONCO Corporation, Kansas City, MI, USA). The lyophilized fruits were vacuum sealed and stored at −18 °C until use. The entire analysis described was based on lyophilized fruits.

2.2. Dry Matter, Titratable Acidity, Pectin

Dry matter (DM) was determined according to the gravimetric method based on the Polish Standard PN-90/A-75101/03 [14]. Under thermal drying at 105 °C, a decrease in mass upon removal of water from the product was determined and solid mass was obtained. DM is expressed as a percentage [%] [14], titratable acidity was determined according to the Polish Standard PN-90/A-75101/04 and expressed as g of citric acid per

100 g of sample. [15]. Pectin was determined by Morris's method [16]. All analyses were performed in triplicate and expressed as dry matter.



(a) (b) (c)

Figure 1. Guelder rose shrub. Liszna near Sanok. Author D. Sokół (**a**,**b**). Guelder rose fruit before freezing. Author M. Mazur (**c**).

2.3. Vitamin C as L-Ascorbic Acid

Vitamin C was determined according to Polish Norms PN-90/A-75101/11 [17].

2.4. Analysis of Sugars with HPLC-ELSD Method

The analysis for sugar was according to Kolniak-Ostek [18]. The conditions of the mass spectrometer were as follows: temperature of a source block, 130 °C; temperature of desolvation, 350 °C; voltage capillary, 2.5 kV; voltage cone, 30 V; flow rate of desolvation gas (nitrogen), 300 L/h. The results, provided in triplicate, were expressed as mg/100 g DM.

2.5. Analysis of Antioxidant Activity (ABTS, FRAP, TPC)

Approximately 2.5 g of grounded lyophilized VO fruits were extracted with 30 mL of 80% methanol. The solutions were sonicated at the same temperature of 20 °C (37 Hz, Elma S60, Elmasonic), left at 4 °C for 24 h, and after this time the extract was centrifuged, and the supernatants were used for analysis. ABTS+, radical scavenging activity of the sample was carried out according to the method proposed by Re et al. [19] and Nawirska-Olszańska et al. [20]. The results, provided in triplicate, were expressed as µM Trolox/100 g DM.

Ferric reducing antioxidant power (FRAP) was carried out. The reducing potential of the sample was examined with the use of the method proposed by Benzie and Strain [21]. The results, provided in triplicate, were expressed as µmol Trolox/100 g DM.

Total phenolic content (TPC) determined by the Folin–Ciocalteu spectrophotometric method was described by Olsson et al. [22]. The results were expressed as mg of gallic acid, GAE equivalents per 100 g of dry matter (mg GAE/100 g DM).

2.6. UPLC–MS Method for Identification of Polyphenols

The extraction of polyphenols was determined using the method described by Püssa et al. [23] and previously described by [13] with our own modifications. The samples of lyophilized VO fruits (0.5 g ± 0.01 g) were extracted with a solution, having a pH of 2.58, with 5 mL of methanol (80%) and HCl (0.1%). Next, the samples were shaken for 30 min at room temperature and centrifuged (5000 rpm, 10 min.). After the addition of hexane (10 mL) to the supernatant, the hydrophilic layer was obtained and kept in Eppendorf vials at 18 °C until analysis. Extractions were carried out in duplicate. Before the identification, the sample was diluted with 2% formic acid (1:1 v/v, Sigma-Aldrich, Steinheim, Germany). For the identification of the polyphenols, the protocol proposed by Kolniak-Ostek [18] was used. The identification of the polyphenols in VO extracts was carried out using an ACQUITY Ultra Performance LC system equipped with a photodiode array detector with a binary solvent manager (Waters Corporation, Milford, MA, USA) with a mass detector G2 Q-Tof micromass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative mode.

The single components of the polyphenols were characterized based on the retention time and the accurate molecular masses. The data obtained from UPLC–MS were analyzed in the MassLynx 4.0 ChromaLynx (Application Manager software, Waters). Phenolic acids were monitored at 320 nm.

2.7. Statistical Analysis

For the statistical analysis, Statistica software ver. 8.0. (StatSoft Inc., Kraków, Poland) was used. The results performed in triplicate, (mean ± standard error) were examined using Duncan's multiple range test ($p \leq 0.05$) to identify the statistically significant effects.

3. Results and Discussion

Wild edible fruits show rich biodiversity. They are harvested without commercial cultivation, making them a valuable source of bioactive compounds, yet the compound content may differ in various fruits. The chemical properties of the fruits are an important characteristic that determines how a given variety will be used [24].

This study demonstrated that the dry matter of VO fruits amounts to 17.3% ± 0.7 (Table 1). A similar amount of 16.9% was observed in the study by Česioniene et al. [4]. The harvest period has an influence on the dry matter content and in the winter the amounts are higher [25]. The extracted content is one of the basic parameters that assess the suitability of the raw material for food processing. VO fruit extract determined, with the use of the refractometric method, was 13% ± 0.53. VO fruits have a lower extract content as compared to other fruits, e.g., apples (conventional cultivation), 11.7–16.1% [26]; cornelian cherry, 13.8–19.85% [27]; blueberry, 15.0–15.3% [28].

Table 1. The values of dry matter, extract, titratable acidity, pectin, vitamin C and sugar in guelder rose fruit.

Parameter	Unit	Guelder Rose Fruit
Dry matter	%	17.3 ± 0.7
* Extract		13 ± 0.53
* Titratable acidity		1.78 ± 0.48
* Pectin		1.10 ± 0.97
* Vitamin C	mg/100 g	47.79 ± 1.02
* Sugar (fructose)	g/100 g	6.85 ± 0.67

Values expressed as mean ± standard deviation, (n = 3), * Results are expressed as g/100 g of dry mass (DM).

One of the important quality characteristics of these fruits is their acidity and the sugar–acid ratio. Acidity depends on the degree of ripeness, climatic conditions, and varietal characteristics [27]. Viburnum opulus acidity content amounts to 1.78 g/100 g. In the study conducted by Česioniene et al. [4], this content was higher—3.2 g/100 g. The taste of the fruits largely depends on the extract–acidity ratio [27]. The higher the ratio, the greater the acceptance by consumers. According to the obtained results, the Viburnum opulus' ratio is 7.3. In other fruits this ratio is higher, e.g., in apples, from 20.64 to 21.6 [26]; cornelian cherry fruits, from 3.0 to 9.2 [27]; plums, 9.27 [28]; strawberries, from 9.875 to 11.9 [29]. However, in blackcurrants, the ratio is 4.9 [30], which is lower than in VO fruits.

The presence of pectin in fruits is important, and depending on the direction of development, may be desirable or undesirable. For example, in the production of juices and beverages, these compounds make the process less efficient and decrease the clarity of the products, in the case of cloudy products, they stabilize their turbidity, while in the case of gelled products, such as purées or jams, they give them the right texture. The raw materials rich in pectin compounds include currants, blackberries, raspberries, and apples [27]. The studied raw material was characterized by a high pectin content of 1.10% ± 0.97. Guelder rose is suitable for preparing jam and marmalade. In early autumn, the fruit reaches consumable maturity and is rich in pectin [25]. Guelder rose fruits can be classified as valuable sources of vitamin C. Our study revealed that the amount of ascorbic acid (vitamin C) in dried fruits was 47.79 ± 1.02 mg/100 g DM. These values were higher in comparison to other results in fresh fruits: 12.4–41.4 mg/100 g [4] or 27–39 mg/100 g [5]. The sugar content indicates the taste and determines the suitability of the raw material for processing. In the fruits, this content (only fructose was identified) was 6.85 g/100 g ± 0.67. This amount was lower than the one demonstrated in the study conducted by Česonienė et al. [4]. They revealed an amount of 7.6 g/100 g of reducing sugar in fresh fruits. This effect could be due to the period of storage at a low temperature during freezing.

Under this study, VO fruits were also tested for ABTS, FRAP and TPC (Table 2). Phenolic compounds were also identified (Table 3, Figure 2). The antioxidant activity described as ABTS was lower than the one found by other authors and was 14.64 µM Trolox/100 g. Guelder rose fruit is characterized by high antioxidant activity and the results obtained by us are different. Polka et al. [2] determined the ABTS value at 26.57 ± 1.71 µM Trolox/100 g (DM). In the study by Kraujalyte et al. [31], the ABTS value of six VO genotypes ranged from 31.95 to 109.81 µM Trolox/1 g.

Table 2. The values of ABTS, FRAP and TPC in guelder rose fruit.

Parameter	Unit	Guelder Rose Fruit
ABTS	µM Trolox/100 g	14.63 ± 0.61
FRAP	µM Trolox/100 g	211.14 ± 1.04
TPC	mg GAE/100 g	1695.88 ± 0.86

ABTS—Radical scavenging spectrophotometric assay. FRAP—Ferric reducing antioxidant power. TPC—Total antioxidant content. Values expressed as mean ± standard deviation, (n = 3).

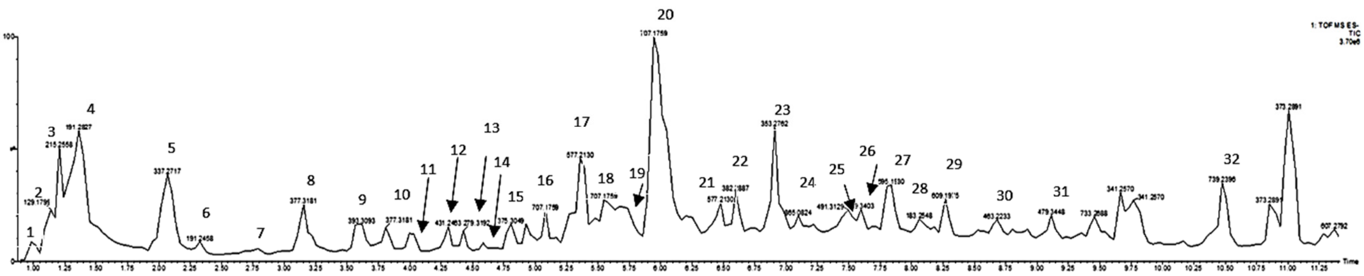


Figure 2. Representative UPLC–MS chromatogram of VO fruit. It complements Table 3.

The obtained result for the FRAP assay was 211.14 µM Trolox/100 g., while another study by Polka et al. [2] observed the value of 19.29 µM Trolox/100 g. Moreover, there are significant differences in the results obtained in another study by Kajszczak et al. [3].

The content of polyphenols in VO fruit was 1695.99 mg GAE/100 g DM. In other studies, a lower amount of TPC in frozen fruits was reported as compared to our study.

These differences may be due to different growing conditions or a different climate. Ersoy et al. [5] reported a polyphenol content of 621 to 987 mg GAE/100 g, Česioniene et al. [4] reported TPC of 1106 mg GAE/100g, Rop et al. [25] reported the content of 829 mg/100 g in V. opulus 'Tajożnyje Rubiny', and Akbulut et al. [32] reported the content of 325 mg/100 g. In comparison to popular fruits in Poland, guelder rose fruit has a higher polyphenol content than e.g., apples 60–210 60 mg/100 g or plums 220–500 mg/100 g [33].

The identification of phenolic compounds is presented in Table 3 and Figure 2. The main phenolic compound identified in the studied fruits was chlorogenic acid. Moreover, as in the study conducted by Karaçelik et al. [10], chlorogenic acid occurs in the form of dimers and one negative molecular ion. Additionally, in this study, like in previous studies, the peak was dominant [1,3,10].

Moreover, we found the following components in VO fruits: quinic acid, apigenin -7-0-glucoside, p-coumaric acid cinnamyl ester, loganic acid, pelargonidin 3-O-robinobioside, procyanidin B1, procyanidin C, and quercetin 3-rutinoside (commonly called rutin).

B- and C-type procyanidin identified under this study was also mentioned in a review study by Kajszczak et al. [3]. Moreover, rutin [1,10] and quinic acid [10] were also identified by other authors.

We also observed small amounts of compounds that are not presented in the table. These were: syringetin-3-O-galactoside, hydrate (RT 2.791 min, *m/z* 525.2838), vitexin-$2''$-O-rhamnoside (RT 3.808, *m/z* 413.2820), quercetin-3-galactoside-$6''$-rhamnoside-$3'''$-rhamnoside (RT 3.808/9.457; *m/z* 771.2408/769.2241), (+)-catechin hydrate (RT 4.307, *m/z* 291.3129), and quercetin-3-O-alpha-L-rhamnopyranosyl(1-2)-beta-D-glucopyranoside-7-O-alpha-L-rhamnopyranoside (RT 5.547, *m/z* 757.1452) [34,35].

Table 3. The phenolic compounds identified in guelder rose fruit.

Peak Number	RT (min)	[M-H]- (*m/z*)	[2M-H]-(*m/z*)	Compound	Refs.
1	0.984	305.1158		Glutathione (oxidized form)	[34,35]
2	1.123	129.1799		UI organic acid	[35]
3	1.206	215.2558		UI	
4	1.361	191.2826		Quinic acid	[36]
5	2.069	337.2717		5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranosyl 5′-monophosphate	[34,35]
6	2.,325	191.2458		Quinic acid	[36]
7	2.791	321.2785		UI	
8	3.154	377.3181		UI	
9	3.619	393.3092		UI flavonoid	[35]
10	3.808	377.3181		UI	
11	3.997	211.2830		UI	
12	4.307	431.2463		Apigenin-7-O-glucoside	[34,35]
13	4.428	279.3191		p-Coumaric acid cinnamyl ester	[37]
14	4.583	361.3319		UI	
15	4.806	375.3049		Loganic acid	[38]
16	5.082		707.1758	Chlorogenic acid	[39]
17	5.358	577.2130		Pelargonidin 3-O- robinobioside	[34,35]
18	5.547	707.1758		UI	
19		579,2114		UI	
20	5.945		707.1758	Chlorogenic acid	[39]

Table 3. *Cont.*

Peak Number	RT (min)	[M-H]- (*m*/*z*)	[2M-H]-(*m*/*z*)	Compound	Refs.
21	6,477	577.2130		Procyanidin B1	[34,35]
22	6.599	382.2887		1,3-dicoumaroylglycerol	
23	6.909	353.2762		Chlorogenic acid	[39]
24	7.097	865.0824		Procyanidin C	[34,35]
25	7.495	491.3129		Oleanane-type triterpenoids	
26	7.596	479.3403		Paeoniflorin	[39]
27	7.839	595.1930		Quercetin 3-O-glucosyl-xyloside apigenin	[34,35]
28	8.061	183.2548		3,4-Dihydroxymandelic	
29	8.270	609.1974		Quercetin 3-rutinoside	
30	8.682	463.2233		Ellagic acid-hexoside	
31	9.113	479.3448		Myricetin-3-Galactoside	
32	10.474	739.2396		Kaempferol-3-Galactoside-6″-Rhamnoside-3‴-Rhamnoside	[34,35]

UI—unidentified.

4. Conclusions

Guelder rose fruits are characterized by diversity in chemical composition, which is also confirmed by results obtained by other authors. In this study, guelder rose fruits obtained from virgin, "clean" areas far from industrial sites were selected as raw material. It was shown that the dry matter content of these fruits was comparable to the values obtained by other authors. Guelder rose fruits are characterized by a lower extract content as compared to that of popular fruits, i.e., apples, cornelian cherry and blueberry.

The acidity of VO fruits was lower than in another study. Based on the extract–acidity ratio, which indicated the taste of the fruit, VO fruits were characterized by a lower ratio than in apples, plums and strawberries, but higher than in blackcurrants. Under this study, it was demonstrated that guelder rose fruits are a valuable source of pectin and vitamin C.

Moreover, the antioxidant activity described as ABTS was lower than in the studies of other authors, but the obtained result for FRAP was higher than in another study. In general, the obtained results vary significantly. This study demonstrated that VO fruits are characterized by a high amount of TPC, significantly higher than the one shown in other studies. The identification of phenolic compounds demonstrated, just like in previous studies by another author, that the dominant compound is chlorogenic acid.

Since the guelder rose fruit is still not very popular in Poland, it is important to focus on its chemical and health properties and to search for ways of using it in the diet. The presented work aimed to update previous knowledge and point out differences between the obtained results and those of other authors, especially the ones in which self-obtained fruits were used since so far other authors have focused on studies on the composition of the raw material of unknown origin.

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