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The quantitative content determination of main groups of biologically active substances in batches of *Viburnum opulus* fruits

Abstract

Aim. To determine the quantitative content of the main groups of biologically active substances (BAS) in batches of *Viburnum opulus* fruits using pharmacopoeial methods for their subsequent use in the standardization of the plant raw material.

Materials and methods. For the study, 6 batches of air-dried *Viburnum opulus* fruits crushed to a particle size of 1–2 mm and harvested in different regions of Ukraine in the mass fruiting phase in October–November 2020 were used. The quantitative determination of polyphenols and tannins was performed using the spectrophotometric method at a wavelength of 760 nm calculated with reference to pyrogallol and dried substance. The total amount of hydroxycinnamic acids was determined according to the method of the SPhU (State Pharmacopoeia of Ukraine) 2.0 described in the monograph “Kidney Tea[™]” calculated with reference to rosemary acid. The total amount of organic acids was determined by the titrimetric method according to the method of the monograph of the SPhU 2.1 “Rose hips[™]” calculated with reference to malic acid.

Results and discussion. The quantitative content of polyphenols (calculated with reference to pyrogallol and dried substance) in batches of *Viburnum opulus* fruits varied by about 1.4 times from 1.74 ± 0.01 % to 2.36 ± 0.01 %. According to the results obtained, the quantitative content of tannins (calculated with reference to pyrogallol and dried substance) in batches of *Viburnum opulus* fruits varied by 1.7 times from 0.73 ± 0.01 % to 1.23 ± 0.01 %. The quantitative content of the total amount of hydroxycinnamic acids calculated with reference to rosemary acid in batches of *Viburnum opulus* fruits ranged by 1.2 times from 3.96 ± 0.01 % to 4.73 ± 0.01 %. The quantitative content of the total amount of organic acids calculated with reference to malic acid in batches of *Viburnum opulus* fruits fluctuated more than 1.3 times from 6.80 ± 0.01 % to 9.08 ± 0.01 %.

Conclusions. The quantitative content of the main groups of biologically active substances has been determined in 6 batches of *Viburnum opulus* fruits harvested in different regions of Ukraine using pharmacopoeial methods: polyphenols (varied by 1.4 times), tannins (varied by 1.7 times), the total amount of hydroxycinnamic (varied by 1.2 times), and the total amount of organic acids (varied by 1.3 times) calculated with reference to dried substance. The content of the groups of biologically active substances in batches of *Viburnum opulus* fruits slightly correlates with the place of the raw material harvesting. Thus, the selected methods of the SPhU 2.0 are quite suitable for determining the quantitative content of polyphenols, tannins and of the total amount of hydroxycinnamic and organic acids in batches of *Viburnum opulus* fruits; they can be used in further studies to standardize the raw material.

Keywords: *Viburnum opulus*; fruits; polyphenols; tannins; hydroxycinnamic acids; organic acids; quantitative content

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Визначення кількісного вмісту основних груп біологічно активних речовин у серіях плодів калини звичайної

Анотація

Мета. Визначити кількісний вміст основних груп біологічно активних речовин (БАР) у серіях плодів калини звичайної з використанням фармакопейних методик для подальшого їх застосування у стандартизації сировини.

Матеріали та методи. Для дослідження використовували 6 серій повітряно сухих подрібнених до розміру частинок 1–2 мм плодів калини звичайної, заготовлених у різних регіонах України у фазі масового плодоношення в жовтні-листопаді

2020 року. Кількісне визначення поліфенолів і танінів проводили за допомогою спектрофотометричного методу за довжини хвилі 760 нм у перерахунку на пірогалол і суху речовину, суми гідроксикоричних кислот – за методикою монографії Державної фармакопеї України 2.0 «Нирковий чай^N» у перерахунку на розмаринову кислоту, суми органічних кислот проводили титриметричним методом за методикою монографії «Шипшини плоди^N» Державної фармакопеї України 2.1 у перерахунку на яблучну кислоту.

Результати та їх обговорення. Кількісний вміст поліфенолів (у перерахунку на пірогалол та суху речовину) у серіях плодів калини звичайної варіював майже в 1,4 раза – від $1,74 \pm 0,01\%$ до $2,36 \pm 0,01\%$. Кількісний вміст танінів (у перерахунку на пірогалол та суху речовину) у серіях плодів калини звичайної варіював в 1,7 раза – від $0,73 \pm 0,01\%$ до $1,23 \pm 0,01\%$. Кількісний вміст суми гідроксикоричних кислот у перерахунку на розмаринову кислоту в серіях плодів калини звичайної коливався в 1,2 раза – від $3,96 \pm 0,01\%$ до $4,73 \pm 0,01\%$. Кількісний вміст суми органічних кислот у перерахунку на яблучну кислоту в серіях плодів калини звичайної коливався в більше ніж 1,3 раза – від $6,80 \pm 0,01\%$ до $9,08 \pm 0,01\%$.

Висновки. У 6 серіях плодів калини звичайної, заготовлених у різних регіонах України, з використанням фармакопейних методик визначено кількісний вміст основних груп БАР: поліфенолів (коливався в 1,4 раза), танінів (коливався в 1,7 раза), суми гідроксикоричних кислот (коливався в 1,2 раза) та суми органічних кислот (коливався в 1,3 раза) у перерахунку на суху сировину. Кількісний вміст груп БАР у серіях плодів калини звичайної незначно корелює з місцем заготівлі сировини. Отже, обрані методики ДФУ 2.0 цілком придатні для визначення кількісного вмісту поліфенолів, танінів та суми гідроксикоричних і органічних кислот у серіях плодів калини звичайної, що може бути використано в подальших роботах зі стандартизації сировини.

Ключові слова: калина звичайна; плоди; поліфеноли; таніни; гідроксикоричні кислоти; органічні кислоти; кількісний вміст

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■ Introduction

So far, we have not found information about the drugs obtained from the fruits of *Viburnum opulus* and available at the pharmaceutical market of Ukraine.

As literature data evidence, *Viburnum* fruits are diversely used in ornamental horticulture, food industry and folk medicine. As of October 21, 2021, the State Register of Plant Varieties Suitable for Distribution in Ukraine includes 9 varieties of *Viburnum* (Rubinova, Ukrainochka, Berehynia, Nasoloda, Bagryan, Ulyana, Anya, Korolova, Velykoplidna) [1]. Today, the food industry produces syrups from this type of the raw material [2], there is information about the freezing of semi-finished products from *Viburnum* fruits [3], and the use of *Viburnum* fruit pomace after juices and wines producing in the bakery industry [4]. Additionally, in folk medicine, *Viburnum* juice has been applied to treatment of tumors, ulcer and used for cosmetic purposes; decoction of the seeds has been shown to possess astringent properties and used to cure dyspepsia [5].

Recent studies have revealed bactericidal properties of *Viburnum* fruits, as well as their pronounced inhibitory activity against *Trichomonas* and *Giardia*. It is also known that the extract from this type of the raw material has a cardiotonic effect similar to digitalis, and there is evidence of the prospects of this raw material in prevention and treatment of obesity-related disorders [6].

The high potential of the *Viburnum* raw material is proven by the geography of its studies, which is represented by Europe, Asia, United States and Turkey [7–9]. According to them, the fruits of *Viburnum* can be used in medicine with several purposes. Thus, fruit extracts have shown positive results *in vivo* studies for treating diseases of the endocrine, cardiovascular and genitourinary systems. In particular, such effects as normalization of glucose and fatty acid absorption [7], the vasorelaxant activity [10], reduction of the endometriosis activity [11] and reduction of the reproductive organ damage by chemicals [12] have been identified. *Viburnum* fruit extracts have also shown a significant antioxidant activity [13],

which is important for maintaining normal functioning of the human body.

Viburnum opulus is widespread in Ukraine [14]. With this, only in the Ivano-Frankivsk region up to 7 tons of fruits are harvested annually for the production of *Viburnum* fruit powder and tea, and with increasing areas occupied by *Viburnum*, annual harvest of up to 100 tons of fruits is possible [15].

The State Pharmacopoeia of Ukraine (SPhU) 2.0 includes the monograph “*Viburnum* bark” [16]. The pharmacopoeial article “*Viburnum* fruits” was published in the State Pharmacopoeia of the USSR (11th edition), in which the parameters of standardization were the loss on drying (not more than 15%), total ash (not more than 10%); impurities: crude fruits (not more than 4%), fruits blackened, burnt, affected by pests (not more than 1.5%), other parts of *Viburnum* (peduncles, including those separated during the analysis, twigs, seeds, leaves) (not more than 2.5%), organic impurities (not more than 1.0%), mineral impurities (not more than 0.5%). However, the article did not describe a method for determining the quantitative content of biologically active substances in fruits [17]. Recently, the monograph “*Viburnum* fruits” has been included in the SPhU 2.4 [18]. The article controls the following parameters: the loss on drying – not more than 15.0%, impurities (crude fruits – not more than 4.0%, colored fruits – not more than 1.5%, other *Viburnum* particles (peduncles, in particular separated during the analysis, twigs, seeds, leaves – not more than 2.5%), organic particles – not more than 1.0%, mineral particles – not more than 0.5%, the quantitative content of procyanidins – calculated with reference to cyanidin hydrochloride – not less than 0.2%, the quantitative content of organic acids calculated with reference to citric acid – not less than 7.0%.

The growing popularity of *Viburnum* fruits has been confirmed by a large number of scientific papers, such as reviews [5, 19], coverage of morphological diversity [20, 21], chemical composition [22, 23], and other aspects of the raw material application [24]. Thus, *Viburnum* fruits are a promising type of the plant raw material; therefore, it makes sense to study them in depth to determine the quantitative content of the main groups of biologically active substances. The aim of the work was to determine the content of the main groups of biologically active substances (BAS) in batches of *Viburnum opulus* fruits using pharmacopoeial methods to select the optimal ones

with their subsequent use in the standardization of the plant raw material.

■ Materials and methods

For the study, 6 batches of *Viburnum opulus* fruits harvested from single specimens of wild plants, were used. Places and terms of harvesting are given in Table 1.

Air-dried *Viburnum opulus* fruits crushed to a particle size of 1–2 mm were used for the study (bringing to a loose state was carried out by drying in a Gorenje FDK24DW fruit and vegetable dryer at a temperature of 40–50°C for 120 min).

The quantitative determination of polyphenols and tannins was performed using the spectrophotometric method at a wavelength of 760 nm in accordance with the requirements of the Supplement of the SPhU 1.2 (2.8.14) [25]. The quantitative content of each group of compounds was calculated with reference to pyrogallol and dried substance.

The quantitative determination of the total amount of hydroxycinnamic acids was performed according to the spectrophotometric method given in the monograph of the SPhU 2.2 “*Orthosiphon* stamen (kidney tea) leaves^N” [26] at a wavelength of 505 nm and calculated with reference to rosemary acid.

The quantitative determination of the total amount of organic acids was performed by the titrimetric method according to the monograph “*Rose hips*^N” provided by the SPhU 2.1 [16] and calculated with reference to malic acid using 0.1 M solution of sodium hydroxide as a titrant and phenolphthalein as an indicator.

■ Results and discussion

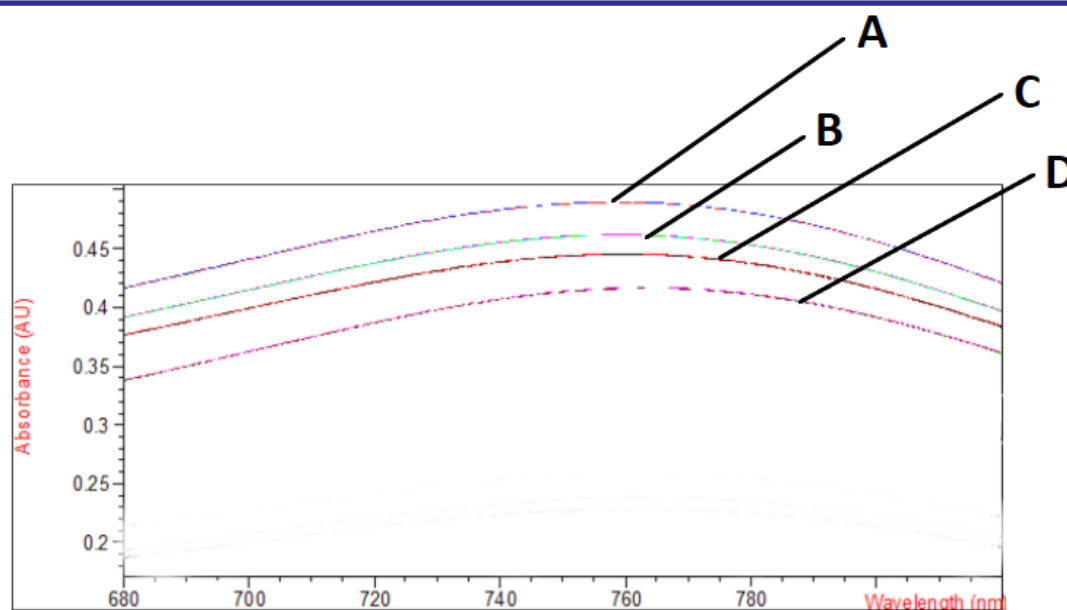
The quantitative content of polyphenols, tannins, the total amount of hydroxycinnamic acids and organic acids in 6 batches of *Viburnum opulus* fruits was determined during the experiment. The results of the quantification are shown in Table 2.

Table 1. Places and terms of harvesting of *Viburnum opulus* fruits

Batch number of the raw material	Harvesting place	Harvesting term
1	Kirovograd region	26.09.2020
2	Lviv region	1.10.2020
3	Luhansk region	26.09.2020
4	Kharkiv region	3.10.2020
5	Ivano-Frankivsk region	27.10.2020
6	Zaporizhia region	20.09.2020

Table 2. The quantitative determination of the main groups of biologically active substances in batches of *Viburnum opulus* fruits calculated with reference to dried substance (n = 5)

Batch number of the raw material	Quantitative content, %			
	polyphenols	tannins	total amount of hydroxycinnamic acids	total amount of organic acids
1	1.88±0.02	0.97±0.01	4.46±0.05	8.42±0.05
2	1.81±0.01	0.92±0.01	4.73±0.04	8.28±0.04
3	1.74±0.02	0.73±0.01	3.96±0.02	6.80±0.03
4	2.24±0.02	1.26±0.02	4.17±0.04	8.88±0.04
5	2.36±0.02	1.23±0.01	4.34±0.03	9.08±0.05
6	1.87±0.01	0.94±0.01	4.18±0.02	7.98±0.04

**Figure 1.** UV spectra of *Viburnum opulus* fruit extracts of batch 1 (A), batch 2 (B), batch 3 (C) and the standard solution of pyrogallol (D) (quantification of polyphenols)

Typical absorption spectra in the UV region of *Viburnum opulus* fruit extracts and the standard solution of pyrogallol with the corresponding reagent are shown in Figure 1. UV absorption spectra of extracts of some *Viburnum opulus* fruit batches treated with the corresponding reagent according to the method of determining the total amount of hydroxycinnamic acids [26] is shown in Figure 2.

From the data of Table 2 it follows that the quantitative content of polyphenols (calculated with reference to pyrogallol and dried substance) in batches of *Viburnum opulus* fruits varied almost by 1.4 times from 1.74±0.01% (batch 3) to 2.36±0.01% (batch 5).

According to the results obtained, the quantitative content of tannins (calculated with reference to pyrogallol and dried substance) in batches of *Viburnum opulus* fruits varied by 1.7 times (from 0.73±0.01% in the raw material

of batch 3 to 1.23±0.01% in the raw material of batch 5).

The quantitative content of the total amount of hydroxycinnamic acids calculated with reference to rosemary acid in batches of *Viburnum opulus* fruits varied by 1.2 times: from 3.96±0.01% (batch 3) to 4.73±0.01% (batch 2).

The quantitative content of the total amount of organic acids calculated with reference to malic acid in batches of *Viburnum opulus* fruits varied by more than 1.3 times: from 6.80±0.01% (batch 3) to 9.08±0.01% (batch 5).

During the experiment it was found that the difference between the quantitative content of each of the groups of biologically active substances in batches of *Viburnum opulus* fruits is insignificant. The methods of the SPhU 2.0 selected for our research are quite suitable for determining the quantitative content of polyphenols, tannins and the total amount of hydroxycinnamic and

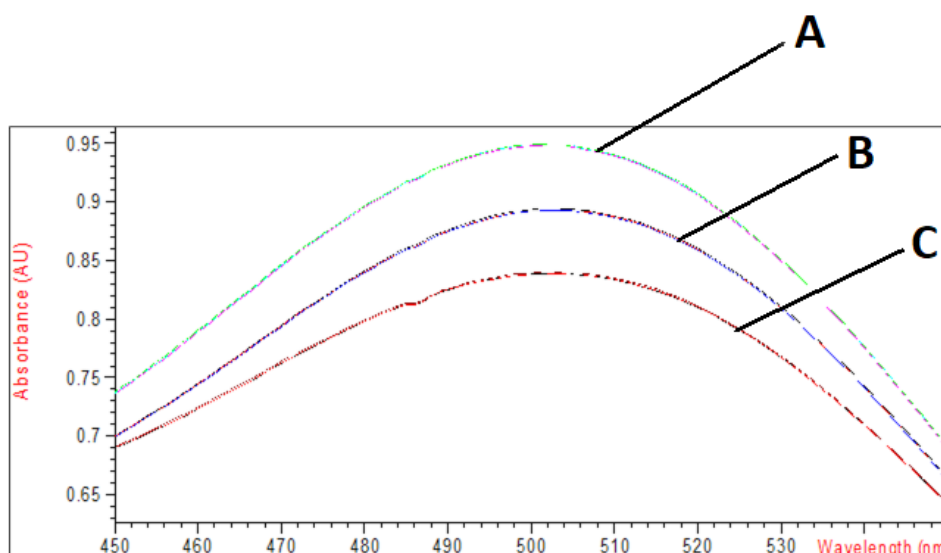


Figure 2. UV spectra of *Viburnum opulus* fruit extracts of batch 1 (A), 2 (B) and 6 (C) (quantification of the total amount of hydroxycinnamic acids)

organic acids in batches of *Viburnum opulus* fruits, They can be used in further studies of the raw material.

■ Conclusions

The quantitative content of the main groups of biologically active substances has been determined in 6 batches of *Viburnum opulus* fruits harvested in different regions of Ukraine using pharmacopoeial methods: polyphenols (varied by 1.4 times), tannins (varied by 1.7 times), the total amount of hydroxycinnamic (varied by

1.2 times), and the total amount of organic acids (varied by 1.3 times) calculated with reference to dried substance.

The content of the groups of biologically active substances in batches of *Viburnum opulus* fruits slightly correlates with the place of the raw material harvesting. Thus, the selected methods of the SPH 2.0 are suitable for determining the quantitative content of polyphenols, tannins and of the total amount of hydroxycinnamic and organic acids in batches of *Viburnum opulus* fruits; they can be used in further studies to standardize the raw material.

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