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THE STUDY OF FATTY AND ORGANIC ACIDS OF *VACCINIUM ULIGINOSUM* LEAVES

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Key words: Ericaceae; bog blueberry; Vaccinium uliginosum leaves; organic acids; fatty acids

Using chromato-mass-spectrometry the analysis of the qualitative composition and the quantitative content of organic and fatty acids in Vaccinium uliginosum leaves has been conducted. The analysis of methyl esters of fatty and organic acids has been performed using a gas chromatograph/mass spectrometer (5973N/6890N MSD/DS Agilent Technologies, USA). Identification of methyl esters of acids has been performed on the basis of the calculation of the equivalent length of the aliphatic chain using the data from the NIST 05 and Willey 2007 mass spectra library with the total number of spectra more than 470000 in combination with AMDIS and NIST programmes for identification; the retention time has been also compared with the retention time of standard compounds (Sigma). In Vaccinium uliginosum leaves 36 organic and fatty acids have been identified, their total content is 5867.84 mg/kg. In leaves 18 fatty acids and 18 organic acids have been found. Dominant components of Vaccinium uliginosum leaves among fatty acids are palmitic acid (1566.74 mg/kg), myristic acid (273.46 mg/kg), tetracosanoic acid (219.73 mg/kg) and arachic acid (214.82 mg/kg), and among organic acids – levulinic acid (600.32 mg/kg) and malonic acid (493.17 mg/kg). Based on the research conducted it has been proven that Vaccinium uliginosum leaves are the promising raw material for further pharmacognostic study.

The family of *Ericaceae* has a special place among small fruits, one of its main representative is *Vaccinium uliginosum* (bog blueberry) [3], which is interesting not only for its biology, ecology, geography and history, but also for its practical value in pharmacy.

The leaves of *Vaccinium uliginosum* increase the activity of the stomach, intestines and heart [1]. In folk and scientific medicine bog blueberry is popular as an antiscorbutic and antidysentric agent [1, 2]. A decoction of branches with leaves in the ratio of 1:10 is used in folk medicine in heart diseases and colitis. Infusion and decoction of leaves are recommended in diabetes, anemia, and to improve metabolism [1]. Moreover, *Vaccinium uliginosum* leaves are also used in various herbal teas and decoctions for the treatment of heart diseases, hypertension, diabetes mellitus, constipations [1, 2].

Earlier the qualitative composition and the quantitative content of some classes of BAS were studied in *Vaccinium uliginosum* leaves, namely simple phenols, derivatives of hydroxycinnamic acids, flavonoids and tannins [4]. Continuing the study of BAS from *Vaccinium uliginosum* leaves and products of their processing our attention was drawn to the fact that the composition of organic acids and fatty acids has been almost unstudied.

Therefore, the aim of our work was to study the qualitative composition and the quantitative content of organic and fatty acids of *Vaccinium uliginosum*.

Materials and Methods

The study of *Vaccinium uliginosum* leaves was conducted in the following way: to 0.50 mg of the dried powdered raw material in a 2 ml vial the internal standard (50 μg of tridecane in hexane) and 1.0 ml of methylating agent – 14% methylene chloride in methanol, Supelco No.3-3033 were added. The mixture was kept in a sealed vial for 8 hours at 65°C. During this time fatty oil was completely extracted from *Vaccinium uliginosum* leaves, and there was re-esterification of fatty and organic acids. The reaction mixture was decanted from the precipitate and diluted with 1 ml of distilled water. To obtain methyl esters of fatty and organic acids 0.2 ml of methylene chloride was added, shaken for 1 hour and subjected to chromatography. The sample injection of 2 μl was carried out to the chromatographic column in a splitless mode, it allowed to introduce the sample without loss in splitting, as well as to increase the sensitivity of the chromatographic method significantly up to 20 times. The sample injection rate was 1 ml/min, the time – 0.2 min. The analysis of methyl esters of fatty and organic acids was performed using a gas chromatograph/mass spectrometer (5973N/6890N MSD/DS Agilent Technologies, USA).

The detector of the mass spectrometer was quadrupole, the ionization method was electron impact (EI), and the ionization energy was 70 eV. The mode of recording the full ion current was used for analysis. For splitting a HP-INNOWAX capillary column (30 m×250 μm) was used. The stationary phase was INNOWAX. The mobile phase was helium, the flow rate – 1 ml/min. The temperature of the heater of the sample injection was 250°C. The thermostat temperature was programmed from 50 to 250°C. Identification of methyl esters of acids was performed on the basis of the calculation of the equivalent length of the aliphatic chain using the data from the NIST 05 and Willey 2007 mass spectra library with the total number of spectra more than 470000 in combina-

Table

The content of fatty and organic acids in V*accinium uliginosum* leaves

Retention time	Name	The quantitative content, mg/kg	The relative content, %
1	2	3	4
6.194	caproic acid	26.18	0.45
10.723	oxalic acid	80.88	1.38
13.277	malonic acid	493.17	8.40
14.587	fumaric acid	51.51	0.88
14.799	levulinic acid	600.32	10.23
15.223	succinic acid	235.64	4.02
15.792	benzoic acid	153.54	2.62
18.82	phenylacetic acid	14.17	0.24
19.378	salicylic acid	47.37	0.81
20.064	lauric acid	145.10	2.47
22.3	2-hydroxy-3-methylglutaric acid	64.92	1.11
23.728	malic acid	105.42	1.80
24.196	myristic acid	273.46	4.66
26.065	pentadecanoic acid	25.07	0.43
26.349	azelaic acid	94.06	1.60
28.201	palmitic acid	1566.74	26.70
28.892	palmitoleic acid	101.01	1.72
29.835	heptadecanoic acid	66.41	1.13
31.045	citric acid	369.25	6.29
31.642	stearic acid	197.60	3.37
31.92	oleic acid	87.07	1.48
32.021	dodecadicarboxylic acid	48.17	0.82
32.645	linoleic acid	76.02	1.30
33.7	linolenoic acid	53.81	0.92
34.224	vanillic acid	72.58	1.24
34.882	2-oxypalmitic acid	55.97	0.95
34.982	arachic acid	214.82	3.66
36.477	henicosanoic acid	15.24	0.26
38.033	behenic acid	85.64	1.46
39.204	p-hydroxybenzoic acid	51.68	0.88
39.466	tricosanoic acid	26.66	0.45
39.656	syringic acid	20.36	0.35
40.107	gentisic acid	31.27	0.53
40.933	tetracosanoic acid	219.73	3.74
41.887	ferulic acid	97.00	1.65
In total		5867.84	100

Continuation of Table

tion with AMDIS and NIST programmes for identification; the retention time was also compared with the retention time of standard compounds (Sigma).

Results and Discussion

The content of organic acids in *Vaccinium uliginosum* leaves was 5867.84 mg/kg. The relative content of acids was calculated as a percentage of their total content. The retention time of acids is given in Table.

Using chromato-mass-spectrometry the analysis of the qualitative composition and the quantitative content of organic and fatty acids in V*accinium uliginosum* leaves was conducted. In *Vaccinium uliginosum* leaves 36 organic and fatty acids were identified. Palmitic acid dominated among fatty and organic acids; its content was 26.7% of the total amount of acids, and the relative content of fatty acids was 56.76%.

There were 18 fatty acids and 18 organic acids in the leaf. Organic acids of *Vaccinium uliginosum* leaves were presented by levulinic (10.23%), malonic (8.40%) and citric (6.29%) acids. The content of other fatty and organic acids was less than 5%, among them there were such organic acids as succinic acid (235.64 mg/kg), benzoic acid (153.54 mg/kg), malic acid (105.42 mg/kg), ferulic acid (97.00 mg/kg). Myristic acid (273.46 mg/kg), tetracosanoic acid (219.73 mg/kg), arachic acid (214.82 mg/kg), stearic acid (197.60 mg/kg), lauric acid (145.10 mg/kg) and palmitoleic acid (101.01 mg/kg) were present among fatty acids. Such organic acids as phenylacetic acid (14.17 mg/kg) and syringic acid (20.36 mg/kg), and such fatty acids as tricosanoic acid (26.66 mg/kg), caproic acid (26.18 mg/kg), pentadecanoic acid (25.07 mg/kg) and henicosanoic acid (15.24 mg/kg) were in small amounts (<0.5%).

CONCLUSIONS

The qualitative composition and the quantitative content of organic and fatty acids in V*accinium uliginosum* leaves have been studied. In all, 36 substances have been identified. Dominant components of *Vaccinium uliginosum* leaves were palmitic acid, levulinic acid and malonic acid. Further pharmacognostic study of Vaccinium uliginosum leaves is promising for creation of new medicines.

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ДОСЛІДЖЕННЯ ЖИРНИХ ТА ОРГАНІЧНИХ КИСЛОТ ЛИСТЯ ЛОХИНИ ЗВИЧАЙНОЇ

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Ключові слова: *Вересові; лохина звичайна; листя лохини; органічні кислоти; жирні кислоти*

За допомогою методу хромато-мас-спектрометрії проведений аналіз якісного складу та кількісного вмісту органічних та жирних кислот у листі лохини. Аналіз метилових естерів жирних та органічних кислот проводили з використанням хромато-мас-спектрометра 5973N/6890N MSD/DS Agilent Technologies (США). Ідентифікацію метилових естерів кислот проводили на основі розрахунку еквівалентної довжини аліфатичного ланцюга (ECL) з використанням даних бібліотеки мас-спектрів NIST 05 і Willey 2007 з загальною кількістю спектрів більше 470000 у поєднанні з програмами для ідентифікації AMDIS і NIST; також порівнювали час утримання з часом утримання стандартних сполук (Sigma). У листі лохини звичайної було ідентифіковано 36 органічних та жирних кислот, загальний вміст яких становив 5867,84 мг/кг. У листі знайдено 18 жирних кислот та 18 органічних кислот. Домінуючим компонентом листя лохини звичайної серед жирних кислот були пальмітинова кислота (1566,74 мг/кг), міристинова кислота (273,46 мг/кг), тетракозанова кислота (219,73 мг/кг) та арахінова кислота (214,82 мг/кг), а з органічних кислот були левулинова кислота (600,32 мг/кг) та малонова кислота (493,17 мг/кг). На підставі проведених досліджень доведено, що листя лохини звичайної є перспективною сировиною для подальшого фармакогностичного вивчення.

ИССЛЕДОВАНИЕ ЖИРНЫХ И ОРГАНИЧЕСКИХ КИСЛОТ ЛИСТЬЕВ ГОЛУБИКИ ОБЫКНОВЕННОЙ

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Ключевые слова: *Вересковые; голубика обыкновенная; листья голубики; органические кислоты; жирные кислоты*

Методом хромато-масс-спектрометрии проведен анализ качественного состава и количественного содержания органических и жирных кислот в листьях голубики. Анализ метиловых эфиров жирных и органических кислот проводили с использованием хромато-масс-спектрометра 5973N/6890N MSD/DS Agilent Technologies (США). Идентификацию метиловых эфиров кислот проводили на основе расчета эквивалентной длины алифатической цепи (ECL) с использованием данных библиотеки масс-спектров NIST 05 и Willey 2007 по общему количеству спектров более 470000 в сочетании с программами для идентификации AMDIS и NIST; также сравнивали время удержания со временем удержания стандартных соединений (Sigma). В листьях голубики обыкновенной было идентифицировано 36 органических и жирных кислот, общее содержание которых составило 5867,84 мг/кг. В листьях найдены 18 жирных кислот и 18 органических кислот. Доминирующим компонентом листьев голубики обыкновенной среди жирных кислот были пальмитиновая кислота (1566,74 мг/кг), миристиновая кислота (273,46 мг/кг), тетракозановая кислота (219,73 мг/кг) и арахиновая кислота (214,82 мг/кг), а из органических кислот были левулиновая кислота (600,32 мг/кг) и малоновая кислота (493,17 мг/кг). На основании проведенных исследований доказано, что листья голубики обыкновенной являются перспективным сырьем для дальнейшего фармакогностического изучения.