UDC 615.451.13:582.912.4:547.56
DOI: 10.15587/2519-4852.2020.222511

PHYTOCHEMICAL PROFILE AND PHARMACOLOGICAL ACTIVITY OF THE DRY EXTRACT FROM ARCTOSTAPHYLOS UVA-URSI LEAVES MODIFIED WITH PHENYLALANINE

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Diseases of the urinary tract and kidneys occupy a leading place in the structure of diseases all over the world. Arctostaphylos uva-ursi leaves are one of the most widely used types of medicinal plant materials (MPM) with diuretic and uroantiseptic action. However, a decoction from the leaves of Arctostaphylos uva-ursi has certain disadvantages: the duration of the manufacturing process of the dosage form, the lack of standardization before use, the inaccuracy of dosage and the short storage time of the dosage form, which leads to a low complementarity of this remedy, therefore, the development of standardized extracts from this raw material is relevant.

The aim. *The aim of the research was a phytochemical study of a phenylalanine-modified dry extract from the leaves of Arctostaphylos uva-ursi to establish the possibility of creating a new diuretic drug.*

Materials and methods. *The object of the study was dry extract from the leaves of Arctostaphylos uva-ursi, modified with phenylalanine. Phytochemical studies of phenolic compounds and saponins were carried out by HPLC and spectrophotometry. Determination of diuretic activity was carried out according to the method of E.B. Berkhina on outbred rats. Determination of the anti-inflammatory activity of the obtained extracts was carried out by the method of carrageenan edema in white rats. The study of the antibacterial activity of the extracts was carried out by the method of diffusion into agar.*

Results. *In the extracts obtained by HPLC, phenol glycoside (arbutin), 2 phenol carboxylic acids (gallic and ellagic), 6 flavonoids, 8 saponins were identified and their quantitative content was determined. Among flavonoids, hyperoside and catechin were dominant; among saponins, ursolic acid, uvaol, and lupeol were dominant. In the obtained extracts, the content of the main groups of phenolic compounds was determined by spectrophotometry.*

Dry extract of Arctostaphylos uva-ursi, modified with phenylalanine, showed pronounced diuretic, anti-inflammatory and antimicrobial (in relation to St. aureus, E. coli, P. vulgaris, P. aeruginosa, B. subtilis and C. albicans) activity.

Conclusions. *The chemical composition, diuretic, antimicrobial and anti-inflammatory activity of dry extract from Arctostaphylos uva-ursi leaves modified with phenylalanine were determined. The obtained dry extract is noted for better solubility, bioavailability and pharmacodynamics, therefore it is a promising substance for creating new drugs in various dosage forms*

Keywords: *Arctostaphylos uva-ursi, leaves, extract, modification, phenolic compounds, saponins, pharmacological activity*

1. Introduction

Diseases of the urinary tract and kidneys occupy a leading place in the structure of diseases worldwide. In Ukraine, every fourth person is prone to diseases of the urinary system. Signs of chronic kidney and bladder diseases are characteristic for 10 % of the population of Ukraine. Decoction of *Arctostaphylos uva-ursi* leaves is traditionally used to treat these diseases [1–3].

Arctostaphylos uva-ursi leaves are one of the most widely used types of medicinal plant raw materials (MPRM) with diuretic and uroantiseptic action. The method of obtaining a decoction of *Arctostaphylos uva-ursi* leaves is well known [4, 5]. However, it should be noted that the decoction of the leaves of *Arctostaphylos uva-ursi* has certain disadvantages: the duration of the manufacturing process of the dosage form, the lack of standardization before use, inaccurate dosing and short shelf life of the dosage form. All this leads to low complementarity of this medicine and as a consequence of the refusal of most patients to use such drugs [7, 8].

Galenic remedies and dry raw materials of *Arctostaphylos uva-ursi* are part of many drugs and functional supplements. The following drugs are presented on the pharmaceutical market of Ukraine on the basis of BAS of *Arctostaphylos uva-ursi*: Nephrophyt, Detoxiphyt, Phytonephrol, Diuretic tea №1, Prostaplex, Pankova tincture and Cysto Fink [4, 10], but they are all complex. At the same time, there is no standardized domestic galenic or novogalenic drug based on this raw material on the Ukrainian market, so the creation of new modified extracts of *Arctostaphylos uva-ursi*, which would be effective and better pharmacological characteristics, is an urgent task of modern pharmaceutical science.

The main active substances of *Arctostaphylos uva-ursi* leaves and its preparations are simple phenols, hydroxycinnamic acids, flavonoids and tannins. Hydroquinone derivatives are represented by arbutin, methylarbutin and pyroside [6]. The leaves of *Arctostaphylos uva-ursi* contain phenolic acids (gallic and ellagic – up to 6 %), flavonoids (myricetin, hyperoside and quercetin), iridoid glucosides (asperuloside, monotropein and unedoside) [7–9].

Earlier [11] the technology of obtaining dry tincture was substantiated, the essence of the technology, which consists in replacing the solvent with a dry excipient (sucrose, mannitol, sorbitol, etc.). The obtained drug has favorable technological parameters: satisfactory bulk properties, lower hygroscopicity and storage stability, so these achievements were used in the development of the scheme for obtaining a dry modified extract of *Arctostaphylos uva-ursi*. Amino acids in extracts are able to form conjugates, salts and complexes with other groups of BAS, which changes their physicochemical properties, affects bioavailability, solubility and total pharmacological effect. Modification of galenic drugs with different amino acids leads to improved pharmacodynamics and the emergence of new pharmacological effects [12].

In this regard, the creation of modified extracts of *Arctostaphylos uva-ursi* leaves, the study of their chemical composition and pharmacological activity for the development of new standardized substances that would have good pharmacotechnological characteristics, pharmacodynamics and the possibility of use in the manufacture of various dosage forms, is an urgent task of modern pharmacy.

The aim of the study was the phytochemical study of phenylalanine-modified dry extract of *Arctostaphylos uva-ursi* leaves to establish the possibility of creating a new diuretic and anti-inflammatory drug.

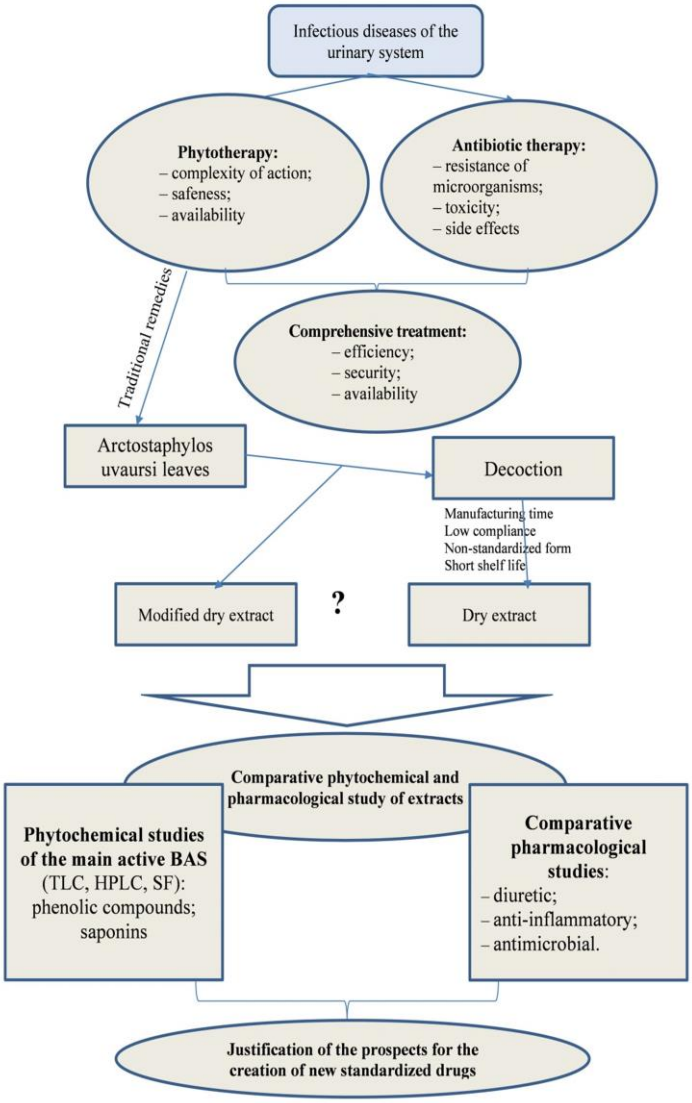

Fig. 1. Scheme of substantiation of relevance and planning of the experiment in the study of extracts of *Arctostaphylos uva-ursi*

2. Planning (methodology) of the research

From the leaves of *Arctostaphylos uva-ursi* pharmaceutical industry get a dry extract modified with phenylalanine, and a dry extract from a decoction of this raw material.

We carried out comparative phytochemical (phenolic compounds and saponins) and pharmacological (antimicrobial, anti-inflammatory and diuretic action) studies of the obtained substances, showed the prospects of using the obtained substance to create a new drug (Fig. 1).

3. Materials and methods

The object of the study was a dry extract of *Arctostaphylos uva-ursi* leaves, modified with phenylalanine. Leaves of *Arctostaphylos uva-ursi L.* (Spreng) was harvested in the botanical garden of Lviv Medical University named after Danylo Halytsky. Voucher specimens no 2015-2019 were deposited at the Department of Pharmacognosy (National University of Pharmacy, Kharkiv, Ukraine). The identity of the plant was established with the consulting assistance of T. Gontova, D.Sc. [13].

Sample preparation, HPLC-DAD analysis and quantification. 50.0 mg (accurately weighed quantity) of the *Arctostaphylos uva-ursi* extracts were weighed in a 5.0 ml measuring tube and brought to the mark with 90 % aqueous methanol. After 30 minutes in an ultrasonic bath, the sample was insisted at room temperature for 3–4 h. Then the test tube was again placed on an ultrasound bath for 15 minutes, then the solution was filtered through a teflon filter with a pore size of 0.45 μm in vial for analysis [14, 15]. Standard substances manufactured by Sigma-Aldrich USA (B9757, O5504, U6753, U6628, L5632 A4256, G7384, E2250, R5143, 83388, Y0001931, Q4951, 17793, C1251, PHL83773), were used for analysis.

Studies of phenolic compounds of Arctostaphylos uvaursi extracts were performed by HPLC on a liquid chromatograph Shimadzu LC20 Prominence in a modu-

lar system equipped with a four-channel pump LC20AD, column thermostat STO20A, automatic sampler SIL20A in the following conditions: (2), size 250 mm x 4.6 mm, particle size 5 μm; column temperature - 35 C; detection wavelength - 330 nm (for hydroxycortic acids, glycoside flavonoids), 370 nm (for flavonoid aglycones), 280 nm (for tannins), 340 nm (coumarins); the flow rate of the mobile phase - 1 ml / min; the volume of the injected sample - 5 μl; mobile phase: Eluent A: 0.1% solution of trifluoroacetic acid in water; Eluent B: 0.1% solution of trifluoroacetic acid in acetonitrile (Table 1).

Identification of phenolic compounds was performed by retention time of standards and UV spectral characteristics (Fig. 2) [16, 17].

The qualitative composition and quantitative content of saponins were studied by the method of high-performance liquid chromatography (HPLC) on a Shimadzu LC20 Prominence liquid chromatograph in the modular system equipped with the 4-channel pump LC20AD, the column thermostat CTO20A, the automatic sampler SIL20A, the diode array detector SPDM20A and Chem Station LC20 in the following chromatographic conditions: the X-Bridge C18 chromatographic column with the size of 150 × 4.6 mm and the particle size of 5 μm(Waters company); the column temperature – 30 °C; the detection wavelength – 205 nm; the flow rate of the mobile phase – 1.0 ml/min; the injection volume – 20 μl; the mobile phase – methanol for HPLC : 0.2 % solution of ammonium acetate (pH 6.75) in the ratio of 80:20; the elution mode – isocratic. Identification of the components was carried out by the retention time and compliance of UV spectra with reference substances [14]. Triterpenic saponins was detected at 205 nm based on their absorption maximum at 200–210 nm. The quantitative determination of individual components in ethanol extracts of the raw material was carried out using the external solutions of reference samples (Table 2 and Fig. 3).

Table 1

Conditions of chromatography in the analysis of phenolic compounds

Chromatography time (min.)	Eluent A, %	Eluent B, %
0–5	95	5
5–35	95 → 75	5 → 25
35–40	75	25
40–60	75 → 50	25 → 50
60–65	50 → 20	50 → 80
65–70	20	80
70–85	95	5

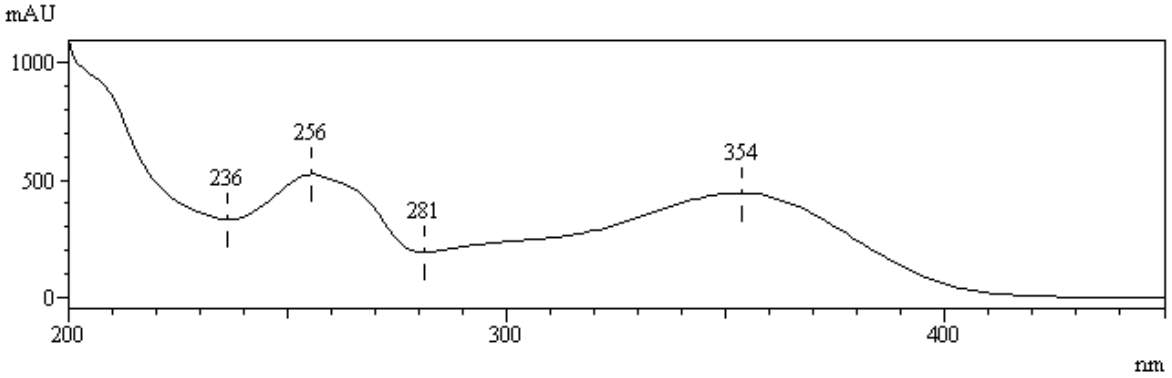

Fig. 2. UV spectrum of hyperoside

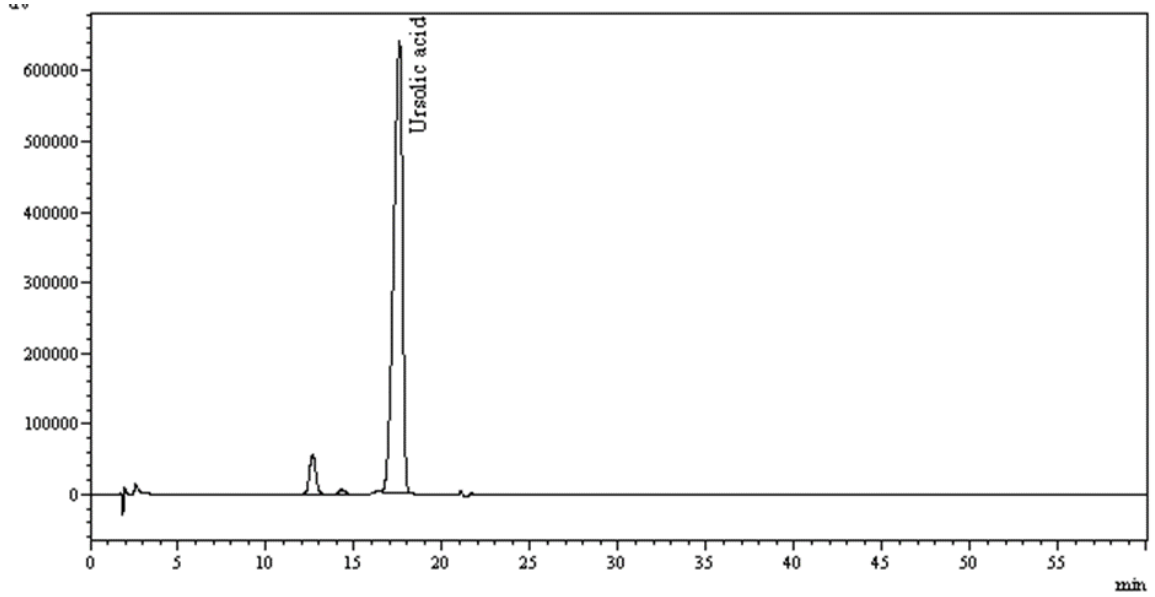

Fig. 3. Chromatogram of ursolic acid standard

Table 2

The chromatographic analysis of solutions of the reference samples (вопрос 2)

Compounds	Retention time, min	λ_{max}, nm
Euscaphic acid	8.53	200
Tormentic acid	12.68	200
Betulin	14.57	200, 234, 322
Oleanolic acid	16.25	200
Ursolic acid	17.29	200
Uvaol	22.80	200
Lupeol	48.13	203, 230

The content of substances in the liquid extracts was calculated by the formula:

$$X = \frac{A_{pr} * m_{st} * P}{A_{sr} * V_{st} * 100},$$

where A_{pr} – is the peak area of the substance on thechromatogram of the test solution; A_{st} – is the peak area of the substance on the chromatogram of the reference solution; m_{st} – is the weight of the standard sample of the substance in the reference sample solution, mg; V_{st} – is dilution of the reference solution, ml; P – is purity of the reference sample, %.

The quantitative content of the basic groups of BAS in the *Arctostaphylos uva-ursi* extracts were determined by the method of absorption spectrophotometry on the spectrophotometer Evolution TM 60S UV-Visible (Thermo Fisher Scientific, USA) [18, 19]. In all the *Arctostaphylos uva-ursi* extracts the extractive substances were determined gravimetrically [5] after complete drying at room temperature taking into account the loss on drying; the sum of the hydroxycinnamic acid derivates was determined by direct spectrophotometry (as chlorogenic acid, λ = 325 nm) [20, 21]; flavonoids were quantified by the method of dierential spectrophotometry with aluminium chloride (as rutin, λ=410 nm) [4, 19]; polyphenols were quantified by direct spectrophotometry (as gallic acid, λ=270 nm) according to Koshovyi et al. [14]. All assays were performed in triplicate.

Diuretic activity. Determination of diuretic activity was performed by the method of E.B. Berkhin [22] on outbred rats weighing 150-220 g, which were kept in standard conditions on a normal diet with free access to water and food. The animals were divided into three groups of 5 rats each. The diuretic effect of the extracts was assessed by the amount of urine excreted 2 and 4 hours after the start of the experiment. Prior to the experiment, the animals were kept for 2 hours without food with free access to water. Test extracts were administered orally as aqueous solutions at doses of 25, 50, 75 and 100 mg/kg 60 minutes before the start of the experiment. The study was performed with a water load of 3 % of the animal's body weight. The control rats received the appropriate volume of saline. Animal care was in line with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Anti-inflammatory activity. Determination of anti-inflammatory activity of the obtained extracts was performed according to the guidelines of the study of drugs edited by Stefanov O.V. [23] on white rats weighing 180–220 g. The test extract was administered orally as an aqueous solution in doses of 25, 50, 75 and 100 mg/kg 60 minutes before the start of the experiment. Animals were injected with an appropriate volume of saline. The reference drug was sodium diclofenac at an effective dose of 8 mg/kg. Acute aseptic inflammation was replicated by administering a 1 % solution of carrageenan administered to rats subplantarly in a volume of 0.1 ml per animal 1 hour after administration of the study drugs. Measurements of paw edema in rats with acute exudative inflammation were performed using a mechanical oncometer according to A.S. Zakharevsky in the dynamics of carrageenan edema - after 1, 2, 3, 4 hours. The anti-exudative activity of the studied extracts was determined by the ability to reduce the development of edema in comparison with the group of control pathology, which was calculated and expressed as a percentage [24]. *Antimicrobial activity*. The study of the antibacterial activity of *Arctostaphylos uva-ursi* complexes with amino acids was performed by the method of diffusion into agar in the laboratory of biochemistry of microorganisms and nutrient media of the Institute of Microbiology and Immunology named after I. I. Mechnikov under the leadership of Ph.D. Osolodchenko T. P. According to the WHO recommendation, reference strains of *Staphylococcus aureus 25923 ATCC*, *Escherichia coli ATCC 25922*, *Proteus vulgaris ATCC 4636*, *Pseudomonas aeruginosa ATCC 27853*, *Basillus subtilis ATCC 6633* and *Candida albicans 653/885 ATCC* were used to assess drug activity. For the study 1 % solutions of extracts were used, the solvent was saline [15, 14, 18].

Statistical analysis. Statistical properties of random variables with n-dimensional normal distribution are given by their correlation matrices, which can be calculated from the original matrices. Pharmacological research material was processed by the method of variational statistics with the calculation of the arithmetic mean and its standard error; the reliability of the compared values was estimated using the Student, Wilcoxon, Mann-Whitney criteria with the probability level of ≤0.05 on a computer using Statistica 6.0 and Word Exel programs [4, 25].

4. Research results

To obtain extracts, 100 g of leaves of *Arctostaphylos uva-ursi*, crushed to a particle size of 2–3 mm, were placed in a flask, filled with 500 ml of 50 % ethyl alcohol solution, extracted overnight at room temperature. The extraction was repeated again with a new portion of the extractant (500.0 ml). The resulting extracts were pooled, decocted overnight, filtered and sterilized. To 400 ml of the filtrate was added 1.485 g of phenylalanine and the solution was decocted overnight. The filtrate was evaporated by rotary evaporation to a dry extract. From the leaves of *Arctostaphylos uva-ursi* by a known classical technology obtained a decoction [4], which was then dried in a vacuum rotary apparatus to dry extract.

The content of the main BAS of phenolic nature and saponins was determined by HPLC in the obtained extracts (Table 3, Fig. 4, 5).

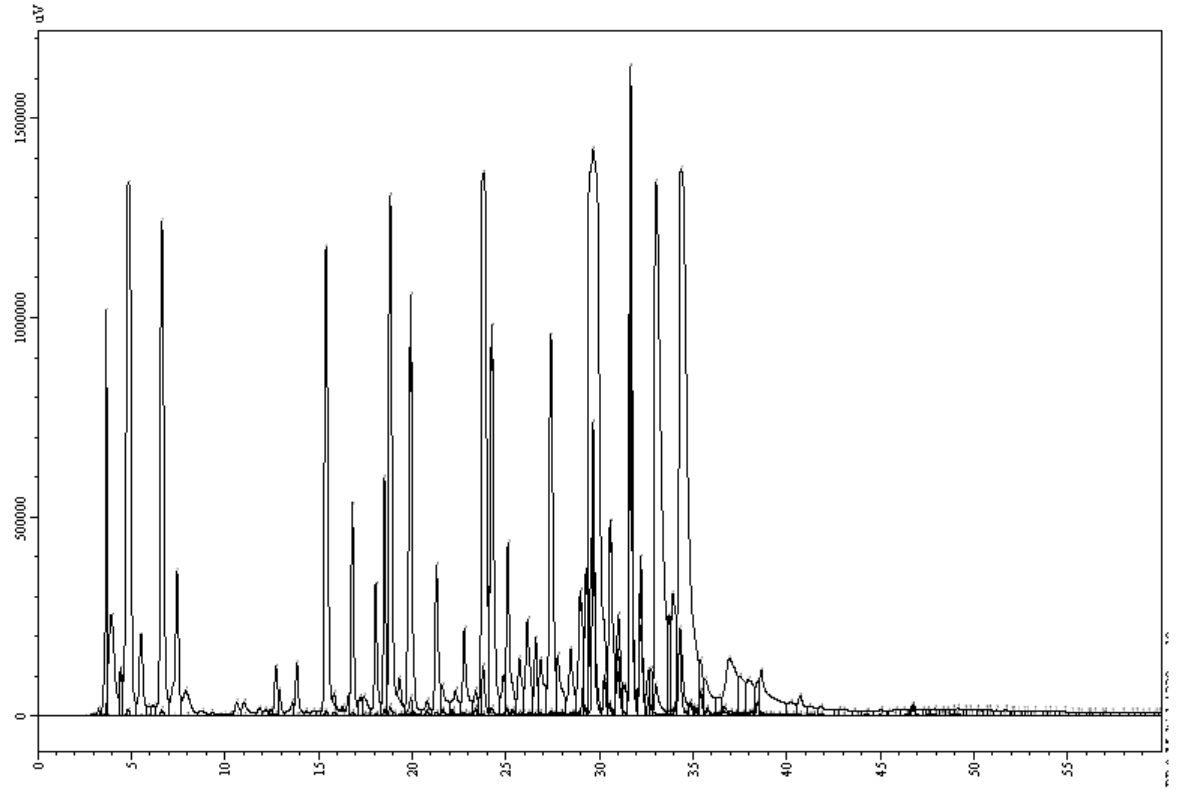

Fig. 4. Chromatogram of phenolic compounds of dry extract of *Arctostaphylos uva-ursi* leaves

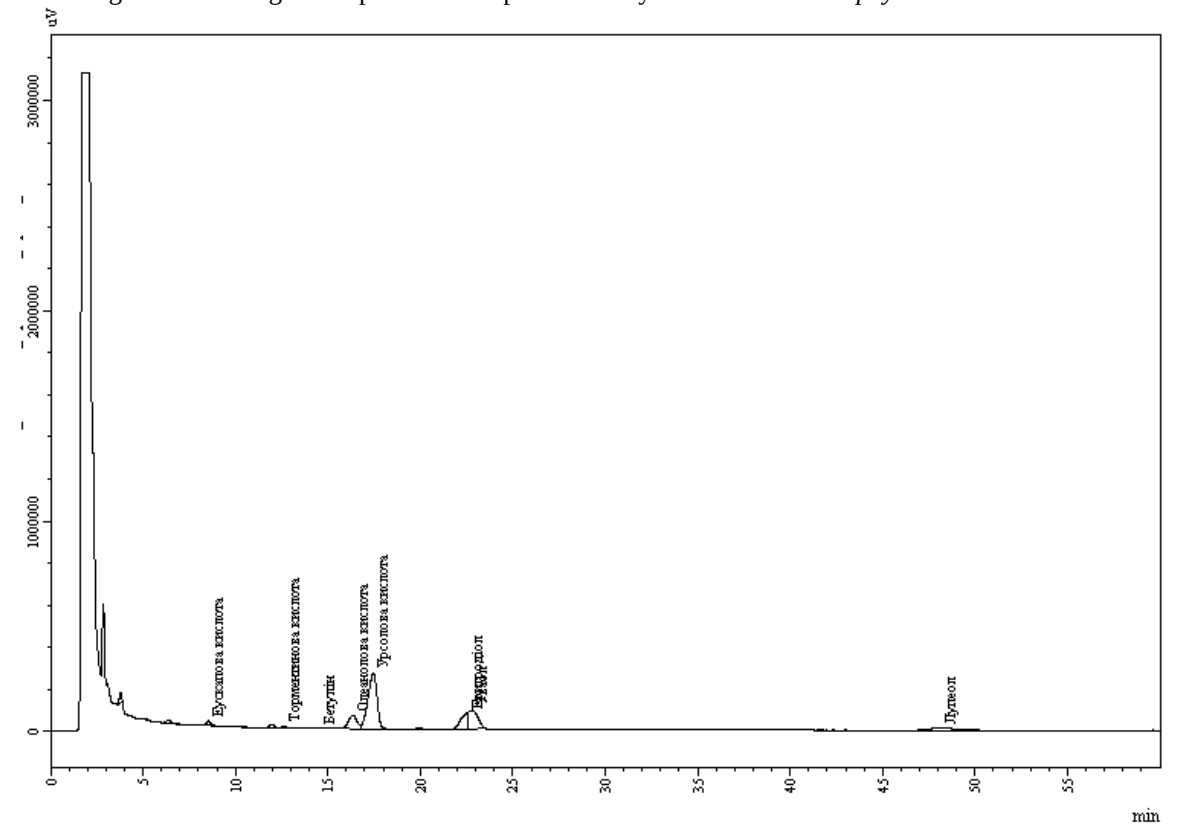

Fig. 5. Chromatogram of saponins of dry extract of *Arctostaphylos uva-ursi* leaves

Table 3

The results of HPLC analysis of dry extracts from the leaves of *Arctostaphylos uva-ursi*

Substance	**Content of the substance,** mg/100 g	
	Dry extract of a decoction of the leaves of *Arctostaphylos uva-ursi*	Dry leaf extract of *Arctostaphylos uva-ursi* with phenylalanine
Arbutin	2956.72±57.9	2819.81±49.8
Phenolic acids		
Gallic acid	147.31±4.2	140.49±3.9
Ellagic acid	64.33±1.2	61.33±1.7
Flavonoids		
Rutin	11.82±0.03	11.23±0.02
Hyperoside	446.23±13.2	425.57±12.7
Quercitrin	14.55±0.5	13.87±0.02
Quercetin	3.58±0.01	3.44±0.02
Isoquercitrin	0.01	0.01
Catechin	277.57±5.5	264.72±6.3
Saponins		
Ursolic acid	1067.47±83.2	1018.06±73.8
Euscapic acid	38.53±0.72	36.747±0.63
Tormentinic acid	17.49±0.05	16.68±0.04
Uvaol	347.63±10.4	331.54±9.6
Oleanolic acid	164.52±5.7	156.90±7.1
Erythrodiol	160.34±8.1	152.91±7.5
Betulin	130.82±5.8	124.76±4.5
Lupeol	369.52±11.5	352.41±12.4

In the obtained extracts, the content of the main groups of BAS was determined by spectrophotometry (Table 4, Fig. 6, 7).

Among herbal diuretics, a special place is occupied by arbutin-containing plants, such as *Arctostaphylos uva-ursi, Vaccinium vitis-idaea, Pyrola elliptica*, etc. In addition to the diuretic effect, arbutin-containing medicinal plant raw materials have anti-inflammatory, antibacterial and antioxidant effects. It is known that extracts from the leaves of *Arctostaphylos uva-ursi* due to the content of hydroquinone derivatives have diuretic activity [26, 27]. But it is advisable to prove experimentally that it is complexes with phenylalanine, potentiate the diuretic effect.

The results obtained during the study of diuretic activity are given in Table 5.

The results of the study of anti-inflammatory activity of the extract from the leaves of *Arctostaphylos uva-ursi* are shown in Fig. 8.

The results of studies of antimicrobial activity of extracts from the leaves of *Arctostaphylos uva-ursi* are given in Table 6.

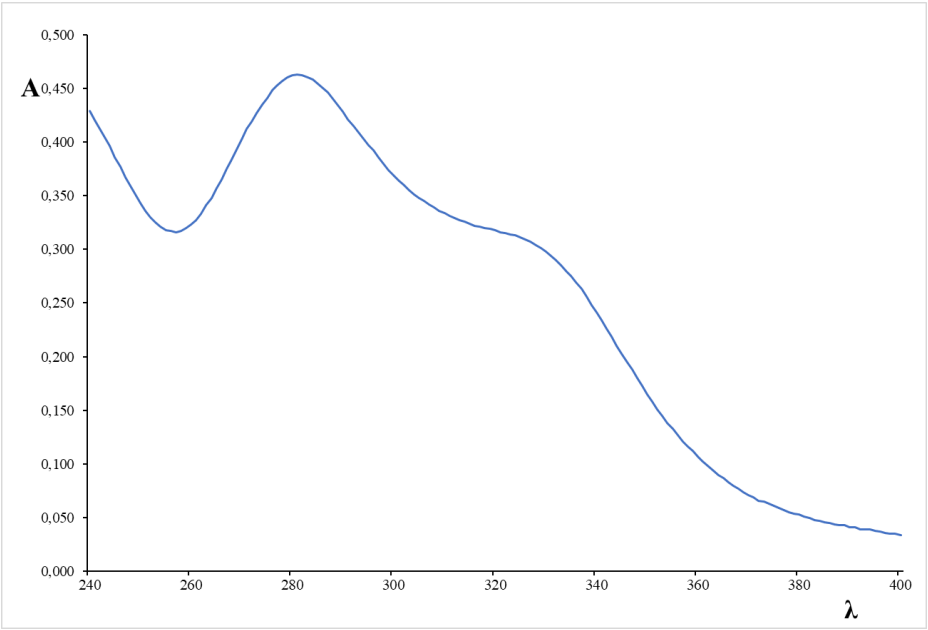

Fig. 6 Absorption spectrum of the original extract of *Arctostaphylos uva-ursi* leaves

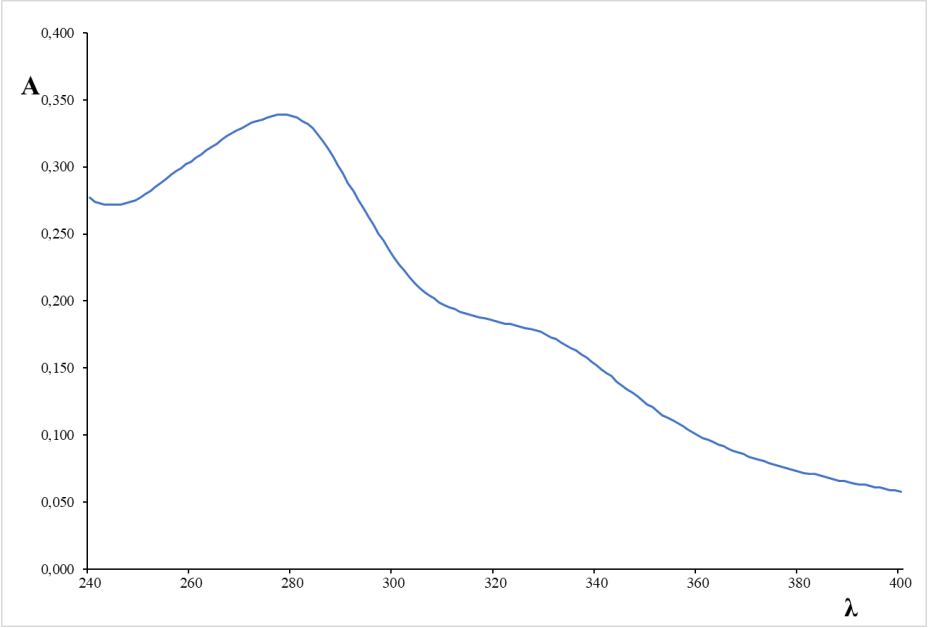

Fig. 7 Absorption spectrum of phenylalanine-modified *Arctostaphylos uva-ursi* leaves extract

Table 4

The content of the main groups of BAS in dry extracts from the leaves of *Arctostaphylos uva-ursi*

Group of BAS	Content of the substance, %	
	Dry extract of a decoction of the leaves of *Arcto-staphylos uva-ursi*	Dry leaf extract of *Arcto-staphylos uva-ursi* with phenylalanine
The sum of hydroquinone-derived compounds (in terms of arbutin)	6.98±0.05	5.06±0.04
Hydroxycinnamic acids (in terms of chlorogenic acid)	2.88±0.02	3.15±0.02
Flavonoids (in terms of rutine)	4.30±0.06	3.05±0.03
The sum of phenolic compounds (in terms of gallic acid)	17.68±0.09	19.80±0.06

Table 5

Diuretic activity of extracts from the leaves of *Arctostaphylos uva-ursi*

Complex	Dose, mg/kg	Diuresis through			
		2 hours		4 hours	
		(M ± m), ml	in % to control	(M ± m), ml	in % to control
Control		0.90±0.15	100	1.22±0.26	100
Dry leaf extract of *Arctostaphylos uva-ursi* with phenylala-nine	25	1.12±0.15*	124	1.54±0.12$^{*,\#}$	126
	50	1.24±0.11*	138	1.57±0.12*	129
	75	1.49±0.06$^{*,\#}$	166	1.74±0.13$^{*,\#}$	143
	100	1.43±0.14$^{*,\#}$	159	1.88±0.14$^{*,\#}$	154
Dry extract of a de-coction of the leaves of *Arctostaphylos uva-ursi*	25	1.00±0.16	111	1.30±0.11	107
	50	1.25±0.09*	139	1.51±0.12	124
	75	1.23±0.21*	137	1.55±0.06*	127
	100	1.22±0.14*	136	1.56±0.21	128

*Note: * – p≤0.05 in relation to control animals; # – p≤0.05 in relation to experimental animals that received a dry extract from a decoction of Arctostaphylos uva-ursi leaves*

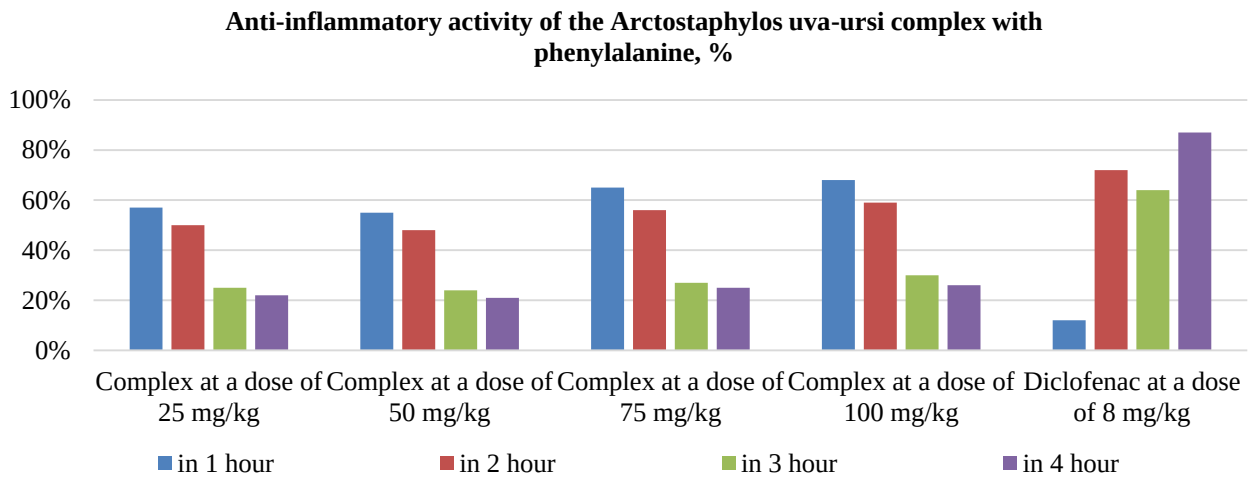

Fig. 8. Anti-inflammatory activity of the complex of *Arctostaphylos uva-ursi* extract with phenylalanine

Table 6

The results of the study of antimicrobial activity of dry extracts from the leaves of *Arctostaphylos uva-ursi*

Microorganism	Growth retardation zone, mm		
	Extract from a decoction of the leaves of Arctostaphylos uva-ursi	Arctostaphylos uva-ursi extract with phenylalanine	Chlorophyllipt
Staphylococcus aureus ATCC 25923	21.0±1.0	18.0±1.0	20±0.2
Escherichia coli ATCC 25922	20.0±1.0	17.7±0.6	14±0.2
Proteus vulgaris ATCC 4636	22.0±1.0	20.0±1.0	Growth
Pseudomonas aeruginosa ATCC 27853	22.0±1.0	23.7±0.6	Growth
Bacillus subtilis ATCC *6633*	20.0±1.0	19.3±1.5	Growth
Candida albicans ATCC 885/653	23.0±1.0	22.3±1.5	Growth

5. Discussion of the results

From the leaves of *Arctostaphylos uva-ursi* was developed a method of obtaining a dry modified extract with the addition of phenylalanine and a dry extract from a decoction of this raw material. Obtained extracts from the leaves of *Arctostaphylos uva-ursi* are ordinary dry loose powders of brown color with a specific odor. The yield of dry modified extract with phenylalanine was 15.9 %, dry extract from the decoction - 16.8 %. The basic scheme of obtaining a dry modified extract was proposed for the first time, in contrast to previous studies [6, 24, 26] for extraction was used 50 % ethanol, which is the optimal extractant from an economic point of view and provides sufficient extraction of BAS.

Phenologlycoside (arbutin), 2 phenolic acids (gallic and ellagic), 6 flavonoids and 8 saponins were identified in the obtained extracts by HPLC and their quantitative content was determined. Among flavonoids, hyperoside and catechin were dominant, among saponins – ursolic acid, uvaol and lupeol. The content of all identified compounds in the modified extract was lower compared to the decoction-based extract. As for the identified phenolic compounds, they have previously been found in extracts from this raw material [8, 9, 26, 29], but their presence and quantity will be crucial in the future in the development of quality control methods. However, some saponins were first discovered, namely euscaphic and tormentic acids and erythrodiol [29, 31].

When dissolving the dry modified extract of *Arctostaphylos uva-ursi* in water, a clear dark brown solution is formed, in contrast to the original extract obtained with 50 % ethanol, in which there is both opalescence and a small amount of sediment. This suggests that the solubility of phenolic compounds increases with the addition of amino acids due to the formation of more hydrophilic conjugates and complexes. In addition, from the general UV spectra of the extracts (Fig. 5, 6) it can be seen that within the spectrum of aromatic groups there are hypo- and hyperchromic shifts, which also indicates the formation of conjugates and complexes. But these complexes are not stable, because by HPLC chromatographed in an acidic environment, they are not detected.

The content of hydroquinone-derived compounds (in terms of arbutin), hydroxycinnamic acids (in terms of chlorogenic acid), flavonoids (in terms of rutin) and the amount of phenolic compounds (in terms of gallic acid) was determined in the obtained extracts by spectrophotometry. As can be seen from the obtained results, the content of all these groups of BAS in the modified extract is lower than the extract from the decoction, while its anti-inflammatory and diuretic activity exceeds the extract from the decoction. This suggests that the addi-

tion of phenylalanine potentiates the action of phenolic compounds of *Arctostaphylos uva-ursi*.

According to the results of our study, a single intragastric administration of dry extract of *Arctostaphylos uva-ursi* leaves with phenylalanine led to an increase in diuretic activity in all selected test doses after 2 and 4 hours of observation. The most effective doses were doses of 75 mg/kg and 100 mg/kg, at the introduction of which the amount of urine excreted in experimental animals increased statistically significantly after 2 hours by 66 and 59 % ($p \leq 0.05$), respectively, and after 4 hours by 43 and 54 % ($p \leq 0.05$) compared with the control group. A single introduction of the dry extract from the decoction of *Arctostaphylos uva-ursi* leaves in 2 hours after the start of the experiment showed a significant increase in urination at doses of 50, 75 and 100 mg/kg compared with the control group, which was less than in animals receiving a solution of dry leaf *Arctostaphylos uva-ursi* extract with phenylalanine.

When studying the anti-inflammatory properties of the extracts, it was found that the dry extract from the leaves of *Arctostaphylos uva-ursi*, modified with phenylalanine shows the maximum antiexudative effect at a dose of 75 and 100 mg/kg by 65 and 68 % for 1 hour and 56 and 59 %, respectively, after 2 hours of observation. At the time of the final evaluation of the result, i.e. after 4 hours of the experiment, the antiexudative activity decreased in the groups of animals that received this extract at all doses and was several times lower than in the group of animals that received Diclofenac.

According to the results of the study of antiexudative activity, we can assume a probable mechanism of action of the studied dry extract of *Arctostaphylos uva-ursi* leaves with phenylalanine. The mechanism of anti-inflammatory activity of this extract is realized by the leukotriene pathway, where the greatest influence on the formation of edema have inflammatory mediators such as biogenic amines and kinins, and the peak activity of these mediators is observed from the first to the second hour.

From Table 6 it is seen that the dry extract from the leaves of *Arctostaphylos uva-ursi*, modified with phenylalanine showed activity against *St. aureus*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, *B. subtilis* and *C. albicans* at the level of the extract from the decoction of the leaves of *Arctostaphylos uva-ursi*, however the activity of the decoction is slightly higher, but it is more active against *Pseudomonas aeruginosa* ATCC 27853. The obtained extracts show a wider range of antimicrobial activity in comparison with the reference drug Chlorophyllipt. In our opinion, tannins are responsible for the antimicrobial activity in *Arctostaphylos uva-ursi* extracts under experimental conditions [9], and they are worse extracted by 50 % ethanol and additionally their concentration decreases slightly when phenylalanine is added, which explains the greater antimicrobial action of the decoction. Previously, the activity of extracts of *Arctostaphylos uva-ursi* obtained with 95 % ethanol against 25 strains of microorganisms was studied [30], for the most part the spectrum of activity of extracts obtained with 50 % ethanol is similar, but additional activity was detected against *C. albicans*.

Study limitations. When studying the extracts by HPLC, the number of standard substances was limited, so not all substances in the extracts could be identified. Only archival strains of microorganisms were used in the study, while it would be interesting to test the activity against clinical strains obtained from real patients.

The prospects for the further research. To create new drugs, based on phytochemical studies, it is necessary to develop and validate quality control methods for the obtained modified extract from the leaves of *Arctostaphylos uva-ursi*.

The obtained modified extract is a promising substance for the creation of new drugs in various dosage forms, so the development of solid dosage forms with the extract is a promising area of development of this topic.

Given the chemical composition of the obtained modified extract and the data of literature sources [28], it is possible that it will have hypoglycemic and hypolipidemic action, and be a promising agent for the correction of metabolic syndrome.

6. Conclusions

The chemical composition, diuretic, antimicrobial and anti-inflammatory activity of dry extract of *Arctostaphylos uva-ursi* leaves modified with phenylalanine were determined.

Phenologlycoside (arbutin), 2 phenolic acids (gallic and ellagic), 6 flavonoids and 8 saponins were identified in the obtained extracts by HPLC and their quantitative content was determined. The content of hydroquinone-derived compounds (in terms of arbutin, 6.98±0.05; 5.06±0.04 %, respectively), hydroxycinnamic acids (in terms of chlorogenic acid 2.88±0.02) was determined by the method of spectrophotometry in the obtained extracts; 3.15±0.02 %, respectively), flavonoids (in terms of rutin 4.30±0.06; 3.05±0.03 %, respectively) and the sum of phenolic compounds (in terms of gallic acid 17.68±0.09; 19.80±0.06 %, respectively). Phenylalanine-modified dry extract has the maximum anti-exudative effect at a dose of 50 mg/kg, reducing edema by 57 %. The maximum diuretic effect of the extract was at a dose of 75 mg/kg, and after 2 hours the diuresis was greater by 60 % compared with the control rats.

The obtained dry extract is characterized by better solubility, pharmacotechnological characteristics and pharmacodynamics, so it is a promising substance for the creation of new drugs in various dosage forms.

Conflict of interests

The authors declare there is no conflict of interests.

Funding

The research was funded by the Ministry of Health Care of Ukraine at the expense of the State Budget in the framework # 2301020 "Scientific and scientific- technical activity in the field of health protection" on the topic "Modern approaches to the creation of new medicines for a correction of metabolic syndrome".

References

1. Kotvitska, A. A., Kubarieva, I. V., Lekhmak, I. B., Havrysh, N. B., Zhirova, I. V. (2019). The international experience study of organization of medical and pharmaceutical care to the population in emergency situations. Journal of Advanced Pharmacy Education and Research, 9 (3), 7–12.

2. Iermolenko, T., Zupanets, I., Sakharova, T. (2013). Pharmacological study of new urolitholytic drug Flarosukcin in experimental immature rats. International Journal of Pharmaceutical Sciences Review and Research, 21 (2), 258–263.

3. Plekhanov, A., Dambaev, A. (2016). Urinary tract infections: epidemiology, etiology, pathogenesis, risk factors, diagnosis (review). Acta Biomedica Scientifica, 1 (1), 70–74. doi: http://doi.org/10.12737/21490

4. Mashkovskii, M. D. (2010). Lekarstvennye sredstva. Moscow: OOO «Izd-vo Novaia Volna», 1216.

5. Derzhavna Farmakopeia Ukrainy. Vol. 1 (2015). Kharkiv: Derzhavne pidpryiemstvo «Ukrainskyi naukovyi farmakopeinyi tsentr yakosti likarskykh zasobiv», 1128.

6. Parejo, I., Viladomat, F., Bastida, J., Codina, C. (2001). A single extraction step in the quantitative analysis of arbutin in bearberry (Arctostaphylos uva-ursi) leaves by high-performance liquid chromatography. Phytochemical Analysis, 12 (5), 336–339. doi: http://doi.org/10.1002/pca.602

7. Radulović, N., Blagojević, P., Palić, R. (2010). Comparative Study of the Leaf Volatiles of Arctostaphylos uva-ursi (L.) Spreng. and Vaccinium vitis-idaea L. (Ericaceae). Molecules, 15 (9), 6168–6185. doi: http://doi.org/10.3390/molecules15096168

8. Linderborg, K., Laaksonen, O., Kallio, H., Yang, B. (2011). Flavonoids, sugars and fruit acids of alpine bearberry (Arctostaphylos alpina) from Finnish Lapland. Food Research International, 44 (7), 2027–2033. doi: http://doi.org/10.1016/j.foodres.2010.10.036

9. Amarowicz, R., Pegg, R. B., Kosińska, A. (2009). SE-HPLC separation of myosin complex with tannins from bearberries (Arctostaphylos uva-ursi L. Sprengel) leaves – a short report. Czech Journal of Food Sciences, 27 (5), 386–391. doi: http://doi.org/10.17221/234/2008-cjfs

10. Amarowicz, R., Pegg, R. B. (2013). Inhibition of proliferation of human carcinoma cell lines by phenolic compounds from a bearberry-leaf crude extract and its fractions. Journal of Functional Foods, 5 (2), 660–667. doi: http://doi.org/10.1016/j.jff.2013.01.009

11. Dmytriievska, I. D., Danylov, S. A., Dmytriievskyi, D. I. (2011). Dotsilnist stvorennia ta rozrobka tekhnolohii novoi likarskoi formy roslynnykh preparativ – sukha nastoika. Ukrainskyi zhurnal klinichnoi ta laboratornoi medytsyny, 6 (2), 4–8.

12. Koshevoi, O. N. (2011). Amino-acid and monosaccharide compositions of Salvia officinalis leaves. Chemistry of Natural Compounds, 47 (3), 492–493. doi: http://doi.org/10.1007/s10600-011-9976-3

13. Dobrochaeva, D. N., Kotov, M. I., Prokudin, Iu. N., Barbarich, A. I. (1999). Opredelitel vysshikh rastenii Ukrainy. Kyiv: Naukova dumka, 548.

14. Myha, M., Koshovyi, O., Gamulya, O., Ilina, T., Borodina, N., Vlasova, I. (2020). Phytochemical study of Salvia grandiflora and Salvia officinalis leaves for establishing prospects for use in medical and pharmaceutical practice. ScienceRise: Pharmaceutical Science, 1 (23), 23–28. doi: http://doi.org/10.15587/2519-4852.2020.197299

15. Starchenko, G., Hrytsyk, A., Raal, A., Koshovyi, O. (2020). Phytochemical Profile and Pharmacological Activities of Water and Hydroethanolic Dry Extracts of Calluna vulgaris (L.) Hull. Herb. Plants, 9 (6), 751. doi: http://doi.org/10.3390/plants9060751

16. Ilina, T., Kashpur, N., Granica, S., Bazylko, A., Shinkovenko, I., Kovalyova, A. et. al. (2019). Phytochemical Profiles and In Vitro Immunomodulatory Activity of Ethanolic Extracts from Galium aparine L. Plants, 8 (12), 541. doi: http://doi.org/10.3390/plants8120541

17. Gontova, T., Ilyinska, N., Golembiovska, O. (2016). A study of the component composition of phenolic compounds obtained from Dahlia varieties Ken's Flame herb. Der Pharma Chemica, 8 (18), 455–459.

18. Koshovyi, O. N., Vovk, G. V., Akhmedov, E. Yu., Komissarenko, A. N. (2015). The study of the chemical composition and pharmacological activity of Salvia officinalis leaves extracts getting by complex processing. Azerbaijan Pharmaceutical and Pharmacotherapy Journal, 15 (1), 30–34.

19. Shinkovenko, I. L., Kashpur, N. V., Ilyina, T. V., Kovalyova, A. M., Goryacha, O. V., Koshovyi, O. M. et. al. (2018). The immunomodulatory activity of the extracts and complexes of biologically active compounds of Galium verum L. herb. Ceska a Slovenska Farmacie, 67 (1), 25–29.

20. Mykhailenko, O., Gudžinskas, Z., Kovalyov, V., Desenko, V., Ivanauskas, L., Bezruk, I., Georgiyants, V. (2020). Effect of ecological factors on the accumulation of phenolic compounds in Iris species from Latvia, Lithuania and Ukraine. Phytochemical Analysis, 31 (5), 545–563. doi: http://doi.org/10.1002/pca.2918

21. Zdoryk, O. A., Khokhlova, K. O., Georgiyants, V., Vyshnevska, L. I. (2014). Investigation of Physical and Chemical Stability of Ointment with Herbals. International Journal of Pharmaceutical Compounding, 18 (3), 248–252.

22. Berkhin, E. B. (1977). Metody izucheniia deistviia novykh khimicheskikh soedinenii na funktsiiu pochek. Khimiko-farmatsevticheskii zhurnal, 11 (5), 3–11.

23. Stefanov, O. V. (Ed.) (2001). Doklinichni doslidzhennia likarskykh zasobiv. Kyiv, 528.

24. Beaux, D., Fleurentin1, J., Mortier F. (1998). Effect of Extracts of Orthosiphon stamineus Benth, Hieracium pilosella L., Sambucus nigra L. and Arctostaphylos uva-ursi (L.) Spreng. In Rats. Phytotherapy research, 12 (7), 498–501. doi: http://doi.org/10.1002/(sici)1099-1573(199811)12:7<498::aid-ptr343>3.0.co;2-2

25. Koshevoy, N., Zabolotny, A., Koshevaya, I., Kostenko, E. M., Rozhnova, T. (2019). Research of moisture-meter device for bulk and liquid materials. 2019 XXIX International Scientific Symposium "Metrology and Metrology Assurance" (MMA). doi: http://doi.org/10.1109/mma.2019.8935983

26. Kurkin, V. A., Riazanova, T. K., Platonov, I. A., Pavlova, L. V. (2015). Quantitative determination of arbutin in the leaves of arctostaphylos uva-ursi (L.) Spreng. Chemistry of plant raw material, 1, 95–100.

27. Migas, P., Krauze-Baranowska, M. (2015). The significance of arbutin and its derivatives in therapy and cosmetics. Phytochemistry Letters, 13, 35–40. doi: http://doi.org/10.1016/j.phytol.2015.05.015

28. Zagayko, A. L., Kolisnyk, T. Y., Chumak, O. I., Ruban, O. A., Koshovyi, O. M. (2018). Evaluation of anti-obesity and lipid-lowering properties of Vaccinium myrtillus leaves powder extract in a hamster model. Journal of Basic and Clinical Physiology and Pharmacology, 29 (6), 697–703. doi: http://doi.org/10.1515/jbcpp-2017-0161

29. Kutsik, R. V., Zuzuk, B. M., Nedostup, A. T., Petsko, T. (2003). Toloknianka obyknovennaia Arctostaphylos uva-ursi (L.) Spreng. (Analiticheskii obzor). Provizor, 18, 27–31.

30. Dykes, G. A., Amarowicz, R., Pegg, R. B. (2003). Enhancement of nisin antibacterial activity by a bearberry (Arctostaphylos uva-ursi) leaf extract. Food Microbiology, 20 (2), 211–216. doi: http://doi.org/10.1016/s0740-0020(02)00107-7

31. Jahodár, L., Grygarová, V., Budésinský, M. (1988). Triterpenoids of Arctostaphylos uva-ursi roots. Pharmazie, 43 (6), 442–443.

Received date 11.11.2020
Accepted date 22.12.2020
Published date 30.12.2020

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