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PHYTOCHEMICAL AND PHARMACOLOGICAL STUDY OF THE NORTHERN HIGHBUSH BLUEBERRY (VACCINIUM CORYMBOSUM L.) LEAVES DRY EXTRACT

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ABSTRACT

A study of qualitative phenolic composition in the dry extract from *Vaccinium corymbosum L.* leaves was conducted. Seven phenolic components were found, including two hydroxycinnamic acids and five flavonoids by HPLC. Among them chlorogenic acid (3,91 %) and rutin (1,67 %) were predominant, which also corresponds to the high content of total polyphenols (18,42 %) in the analysed herbal substance. Hypoglycemic and hypolipidemic effects of the dry extract from the northern highbush bilberry leaves were established too.

KEYWORDS: *blueberry leaves dry extract, phenolic compounds, pharmacological activity*.

INTRODUCTION

The fruits of blueberry (*Vaccinii fructus)* are widely used in medicine and pharmacy for a long time.[1] The name "golubika" in Ukrainian scientific terminology is used as one of the names of blueberries (*Vaccinium uliginosum L.*) - a wild plant, which belongs to the *Vaccinium* section of the *Vaccinium* genus and is found in all regions of the Northern Hemisphere including the territory of Ukraine (Polesie and Carpathians).[2,3] In the Ukrainian language, this plant species is found under different names, some of them are formed from the scientific Latin name - *Vaccinium corymbosum L.*, or represent the translation of the common name of this plant in English - highbush blueberry.

The products of blueberry are present only as supplements on the Ukrainian pharmaceutical market. Abroad, medicines with blueberry fruit extract are used for improving vision.[4] A decoction of blueberry leaves is used for diabetes, heart disease, anemia, gastritis and also as an astringent for colitis, enterocolitis and diarrhea due to content of tannins and flavonoids contained therein.[5]

Given the widespread prevalence of diabetes mellitus in Ukraine and the world, it is advisable to create a new product with hypoglycemic activity based on biologically active substances of the northern highbush blueberry leaves.

We have previously shown that ethanol at concentration of 70 % is the optimal extractant for biologicaly-active substances from common blueberry leaves, in particular for phenolic compounds.[6,7] Therefore, the aim of our further research was to study the chemical composition and some pharmacological activity of the dry extract from blueberry leaves obtained with 70 % ethanol.

MATERIALS AND METHODS

Plant material

Plant raw material, *Vaccinium corymbosum L.* leaves, were collected in growing season, June-July, 2020, from Kharkiv region, Ukraine. Plant raw material was air-dried in darkness at room temperature. Species identification was carried out by Prof. Gontova T.M. at the Department of Botany, the National University of Pharmacy, Ukraine.

Plant extract

0.5 kg of the blueberry leaves, crushed to a particle size of 1-2 mm, were placed in a flask, poured in 1.5 L of 70 % ethanol, and extracted overnight at room temperature. The extraction was repeated three times with new portions of the extractant (1.0 L). The final volume was combined, filtered and evaporated using a rotary vacuum evaporator to dry extract.

Spectrophotometrical analysis of leaves dry extract

Quantitative determination of total phenolic compounds, derivatives of hydroxycinnamic acids and flavonoids was carried out by spectrophotometric method. Optical density was measured on a Spekol 1500 spectrophotometer (Switzerland). The content of derivatives of hydroxycinnamic acids was determined in terms of chlorogenic acid at a wavelength of 327 nm, the content of flavonoids in terms of rutin - at a wavelength of 417 nm after the formation of a complex with aluminum chloride, the content of total phenolic compounds in terms of gallic acid - at a wavelength of 270 nm. For statistical reliability, the experiments were carried out at least three times.[8,9,10]

HPLC analysis of leaves dry extract

For hydrolysis, 300 μL of a 4 % alcoholic solution of the dry extract from the northern highbush blueberry leaves was placed in a 2 mL vial; 300 μL of a solution of 6 N hydrochloric acid in ethanol (1:1 by volume) was added. The sealed vial was kept in an oven at 100 °C for 1 hour. After cooling, the contents of the vial were centrifuged and transferred to the vial for analysis.

The study of the qualitative composition and quantitative content of phenolic compounds in the dry extract of the northern highbush blueberry leaves, before and after hydrolysis, was carried out by high performance liquid chromatography (HPLC) using an Agilent Technologies chromatograph (model 1100), which is equipped with a G1379A flow-through vacuum degasser, a four-channel low gradient pump, pressure G13111A, automatic injector G1313A, column thermostat G13116A and diode-matrix detector G1316A. For the analysis, a 2.1 × 150 mm chromatographic column was used, which was filled with octadecylsilyl sorbent with a grain size of 3.5 µm "ZORBAX SB-C18". The analysis was carried out under the following conditions: thermostat temperature - 35 ° C; the flow rate of the mobile phase - 0.25 mL / min; mobile phase - solution A (0.1 % H_3PO4, 180 μL/L triethylamine, 3 mL/L tetrahydrofuran in water) and solution B (MeOH) in the ratio 90:10 (first 8 min), 70:30 (between 8^{th} and 24^{th} min), and after the 24th min only solution B was used; the working pressure of the eluent 240-300 kPa. The following detection parameters were used in the analysis: measurement scale - 1.0; scanning time - 0.5 s; spectrum acquisition parameters - each peak at 190–600 nm. Phenolic compounds were identified by the retention time of standards for hydroxycinnamic acids and flavonoids and their spectral characteristics.[11,12]

Evaluation of Hypoglycemic and Hypolipidemic activity

To study the hypoglycemic activity of dry extract from the blueberry leaves, insulin resistance was modeled by keeping animals on a diet enriched with fructose (60.3% fructose, 18.3% protein, 5.2% fat).[13,14] The experiment was carried out on 18-month-old male Wistar rats and they were divided into three groups: 1) intact animals, which were kept on a standard diet of the vivarium of the National University of Pharmacy (NUPh); 2) animals that were kept on a fructose diet for six weeks; 3) animals that were kept on a fructose diet for four weeks and an additional two weeks on this diet with daily administration of dry extract from blueberry leaves at a dose of 2.5 mg per 100g of body weight. The animals were decapitated under chlorazole-urethane anesthesia. The object of the study was blood serum.

The content of glucose, insulin, and triacylglycerols (TAG) in serum was determined using standard kits from Felicity Diagnostics (Ukraine) and Lachema (Czech Republic). The concentrations of α- (lipoprotein) cholesterol (α-CS) and β-(lipoprotein) cholesterol (β-CS) were determined using standard enzymatic cholesterol oxidase kits from Boehringer Mannheim GmbH Diagnostics (Germany), after separating the lipoprotein fractions by turbidimetric method.[15] The content of arginine and citrulline was determined by photometric methods using standard reagent kits. To assess the level of endogenous nitrogen monoxide (NO), the content of nitrites and nitrates was determined spectrophotometrically using the Griss reagent.[14,16]

RESULTS AND DISCUSSION

In the dry extract from the leaves of the northern highbush blueberry were identified two hydroxycinnamic acids and five flavonoids (three glycosides and two aglycons) by HPLC method (Table 1). Only three component were viewed before hydrolysis - chlorogenic acid (3.91 %) rutin (1.67 %) and kemferol-3-O-glycoside (0.7 %). After acid hydrolysis all seven components were established as caffeic acid (1.34 %) and quercetin-3-O-glycoside (0.99 %) predominate among all phenols.

Table 1: HPLC phenolic composition of dry extract of the northern highbush blueberry leaves.

№	Name	Retention time, (min.)	Quantitative content (mg / 100 g)	
			before hydrolysis	after hydrolysis
1.	Chlorogenic acid	13.14	3907,6 ± 0,15	523,4 ± 0,23
2.	Caffeic acid	17.22	nd	1343,6 ± 0,09
3.	Rutin	19.70	1660,4 ± 0,11	204,2 ± 0,21
4.	Quercetin-3-O-glucoside	21.17	nd	987,1 ± 0,12
5.	Kaempferol-3-O-glucoside	22.82	699,8 ± 0,18	488,8 ± 0,10
6.	Quercetin	23.27	nd	382,1 ± 0,08
7.	Kaempferol	25.18	nd	88,9 ± 0,17

[1]*nd-not detected*

Similar results are shown from Ştefãnescu et al. (2020),[17] in their work on *V. corimbosum.* Flavonoid and phenolic acid profile confirm quantity and quality of phenolics in northern highbush blueberry leaves as some other compounds were established from these authors.

The results of the spectrophotometric quantitative determination of the main groups of phenols in dry extract from the leaves of the northern highbush blueberry are shown in Table 2.

Table 2: The quantitative content of the main groups phenols in blueberry leaves dry extract.

Phenolic group	Spectrophotometric method	Content (%)
Total phenolic compounds	(in terms of gallic acid)	18.42 ± 0.97
Hydroxycinnamic acids	(in terms of chlorogenic acid)	2.92 ± 0.12
Flavonoids	(in terms of rutin)	3.03 ± 0.11

Expected in the spectrophotometrical analysis total phenolics were at a higher concentration (18.42%) corresponding with significant flavonoids and phenolic content (3.03% and 2.92% respectively). Thus phytochemical methods and analysis results could be used in further standardization of this herbal substance.

Our results showed again similarity with results established by Ştefãnescu et al. (2020),[17] concerning quantity of main group phenols in leaves from *V. corymbosum.* The main reason for diversity in phenolics quantity~~,~~ authors explain by the differences in the geographical areas and habitat as well as different growing season of all these plant samples which is valid and also applies to our study.

The results from conducting pharmacological experiment are shown in Table 3.

Table 3: The content of glucose, insulin, TAG, α- and β-cholesterol in the blood serum of rats with the introduction of dry extract against the background of a high-fructose diet (M ± m, n = 6).

Indicator	Intact	Diet	Diet + Blueberry dry extract
Glucose, mmol/L	4,4 ± 0,09	14,2 ± 0,19*	9,1 ± 0,10*#
Insulin, pg/ml	1199 ± 25	3005 ± 48*	2347 ± 21*#
Triacylglycerols, mmol /L	0,78 ± 0,03	2,26 ± 0,06*	1,85 ± 0,09*#
α-cholesterol, mmol /L	1,31 ± 0,03	0,69 ± 0,03*	0,79 ± 0,02*
β-cholesterol, mmol /L	2,73 ± 0,06	3,56 ± 0,06*	3,44 ± 0,14*

* - *deviation is significant regarding intact (p ≤ 0.05),*

 # - deviation is significant regarding "Diet" ($p \leq 0.05$).

Keeping rats on a fructose-fortified diet caused an almost three-fold increase in serum glucose levels. At the same time, hyperinsulinemia is observed, which, with simultaneous hyperglycemia, indicates cell insensitivity to insulin, that is, the development of insulin resistance. The increase in the concentration of TAG, which is achieved after six weeks of the experiment, is a consequence of the mobilization of fat from adipose tissue and an increase in endogenous synthesis of TAG and very low density lipoproteins by the liver due to the weakening of the inhibitory effect of insulin on lipolysis.

Hypoglycemic activity of fruits from *V. corymbosum*, was investigated by Huang et al., (2018).[18] They claim that blueberry extract may exert hypoglycemic properties through the synergistic effects of consisting in them phenolics. All data was confirmed with our result concerning hypoglycemic activity but in in-vitro conditions.

CONCLUSION

As a result of phytochemical study of the phenolic composition of the dry extract from the northern highbush blueberry leaves by HPLC, 7 compounds of phenolic nature were identified, in particular, derivatives of hydroxycinnamic acids and flavonoids and at the same time was proven its hypoglycemic and hypolipidemic capacity. Although there is a need for further investigations in order to define the hypoglycemic mechanisms of blueberry extract, the prospects for creating a new drug with hypoglycemic activity have been shown.

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