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THE STUDY OF THE AMINO ACID COMPOSITION OF THE ALCOHOLIC EXTRACT FROM BILBERRY LEAVES

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Key words: Ericaceae; bilberry; leaves; alcoholic extract; amino acid

The qualitative composition and the quantitative content of free and bound amino acids in the alcoholic extract of bilberry leaves have been determined by HPLC using a high performance liquid chromatograph by Agilent Technologies firm (model 1100) equipped with a flow-through vacuum degasser G1379A, a four-channel pump of the low pressure gradient G13111A, an automatic injector G1313A, a column thermostat G13116A, and a diode array detector G1316A. To conduct the analysis a "ZORBAX-XDB-C18" chromatographic column with the size of 4.6×50 mm filled with the octadecylsilyl sorbent and with the granule size of 1.8 μm was used. Identification of amino acids was performed by the retention time of standards. The content of bound amino acids was calculated by subtracting the content of free amino acids from their total content. As a result of the study 19 amino acids have been found and their quantitative content has been determined. Five amino acids are essential – threonine, methionine, isoleucine, leucine and arginine. The dominant compounds are glutamic acid, asparagine, serine, gamma-aminobutyric acid and leucine. The content of free amino acids is 34.75% of the total amino acids, and the content of the bound ones is 65.25%. Most amino acids in the extract studied are in a bound state; therefore, they will affect solubility, bioavailability and the total pharmacological effect.

In medical and pharmaceutical practice drugs based on bilberry (*Vaccinium myrtillus*) fruits are widely used. For example, such drugs as Strix, Optix, Visio Balance, Bilberry Forte, etc, which contain biologically active substances of bilberry, are represented at the pharmaceutical market of Ukraine; they are used in various eye disorders. In folk and scientific medicine the shoots and leaves of bilberry are used as a sugar-lowering remedy in the form of decoctions and are part of antidiabetic teas such as Arphasetinum and Myrphazinum, but there is no standardized drug based on extracts from this raw material at the market of Ukraine [2, 3, 6, 7, 8].

Previously, we reported on the qualitative and quantitative chemical determination in leaves and the extracts from bilberry leaves of some classes of BAS such as simple phenols, derivatives of hydroxycinnamic acids, flavonoids, polyphenolic compounds [2, 3, 7]. Continuing the study of BAS of bilberry leaves and products of their processing our attention is attracted to the fact that the amino acid composition has not been studied yet. Since amino acids can form salts and complex compounds with phenolic substances, their qualitative composition and the quantitative content will affect solubility, bioavailability and the total pharmacological effect of the extract. Therefore, it is expedient to study their composition in the alcoholic extract from bilberry leaves. The aim of our research was to study the amino acid composition of the alcoholic extract from bilberry leaves.

Materials and Methods

The object of our study was the liquid alcoholic extract from bilberry leaves obtained by extraction with 50% alcohol. This extract met the requirements of the general monograph "Extracts" of the SPhU [1] and was referred to liquid extracts.

The qualitative and quantitative analysis of free and bound amino acids in the extract of bilberry leaves was carried by a high performance liquid chromatograph by Agilent Technologies firm (model 1100) equipped with a flow-through vacuum degasser G1379A, a four-channel pump of the low pressure gradient G13111A, an automatic injector G1313A, a column thermostat G13116A, and a diode array detector G1316A. To conduct the analysis a "ZORBAX-XDB-C18" chromatographic column with the size of 4.6×50 mm filled with the octadecylsilyl sorbent and with the granule size of 1.8 μm was used.

The sample preparation to study the composition of free amino acids. In a 10 ml vial (A) add 0.3 ml of the extract. Then pour 3 ml of 0.1 N aqueous solution of hydrochloric acid containing 0.2% of β-mercaptoethanol into the vial. Close the vial hermetically and place in an ultrasonic bath for 2 h at the temperature of 50°C.

The sample preparation to study the total content of amino acids. In a vial (B) add 0.2 ml of the extract. Then pour 3 ml 6 N aqueous solution of hydrochloric acid containing 0.4% of β-mercaptoethanol into the vial. Close the vial hermetically and allow to stand for 24 h at the temperature of 110°C.

Centrifuge the vial with samples and filter. Into a 2 ml reaction vial collect 100 μl of filtrates from vial A and 20 μl from vial B and place in a vacuum desiccator at a temperature of 40-45ºC and pressure of 1.5 mm Hg to complete removal of hydrochloric acid. Then into the vial for analysis successively add 200 μl of 0.8 M borate buffer with pH 9.0, 200 μl of 20 mM solution of 9-fluorenylmethoxycarbonyl chloride in acetonitrile with an automatic injector, after a 10 min exposure into the reaction vial add 20 μl of 150 mM solution of amantadine hydrochloride in 50% water acetonitrile [4, 5].

The analysis was performed under the following chromatographic conditions: the eluent working pressure – 220-275 kPa; the column thermostat temperature – 50°C; the sample volume – 2 μL. There were the following parameters of detection: the scale of measurement – 1.0; the scan time – 0.5 s; detection wavelength – 265 nm.

Identification of amino acids was performed by the retention time of standards. The content of bound amino acids was calculated by subtracting the content of free amino acids from their total content. However, such amino acids as asparagine and glutamine are almost quantitatively transformed into aspartic and glutamic acids, respectively, in the process of acid hydrolysis. Under the same conditions cystine can be partially or completely decomposed into cysteine and cysteic acid. Therefore, it is useful to perform calculation of the content of such bound amino acids as asparagine and aspartic acid, glutamine and glutamic acid by their sum, respectively. The content of such bound amino acids as cystine and cysteine can be also calculated by their sum, but taking into account that 1 molecule of cystine breaks down into 1 molecule of cysteine and 1 molecule of cysteic acid.

Results and Discussion

The results of determination of the qualitative composition and the quantitative content of free and bound amino acids in the alcoholic extract from bilberry leaves are given in Table.

As a result of the study of the amino acid composition of the alcoholic extract from bilberry leaves 19 amino acids have been found. Among them there are 17 free and 17 bound amino acids, five of them are essential – threonine, methionine, isoleucine, leucine and arginine. The content of free amino acids is 34.75% of the total amino acids, and the content of the bound ones is 65.25%.

Table

The amino acid composition of the alcoholic extract from bilberry leaves

Amino acid	The content of amino acids (mg per L of the liquid extract)	
	Free	Bound
Aspartic acid	2.3	15.8
Glutamic acid	3.3	21.9
Glutamine	6.9	0.0
Asparagine	18.5	0.0
Serine	4.8	18.4
Arginine	10.9	11.8
Glycine	2.4	4.3
Threonine	8.4	8.6
Alanine	5.3	12.3
Proline	11.7	13.1
Gamma-aminobutyric acid	19.9	26.7
Phenylalanine	8.3	9.1
Methionine	5.0	34.7
Isoleucine	6.3	8.5
Leucine	8.7	22.7
Histidine	3.7	5.3
Cysteine	0.0	12.9
Monoethanolamine	4.4	12.1
Tyrosine	0.0	7.0

CONCLUSIONS

The amino acid composition of the alcoholic extract from bilberry leaves has been studied. It has been found that the dominant compounds are glutamic acid, asparagine, serine, gamma-aminobutyric acid and leucine. Most amino acids in the extract from bilberry leaves are in a bound state; therefore, they will affect solubility, bioavailability and the total pharmacological effect. This fact should be considered in the technological process and its standardization.

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ДОСЛІДЖЕННЯ АМІНОКИСЛОТНОГО СКЛАДУ СПИРТОВОГО ЕКСТРАКТУ З ЛИСТЯ ЧОРНИЦІ ЗВИЧАЙНОЇ

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***Ключові слова**: Вересові; чорниця звичайна; листя; спиртовий екстракт; амінокислота*

Вивчено якісний склад і кількісний вміст вільних та зв'язаних амінокислот у спиртовому екстракті з листя чорниці звичайної методом високоефективної рідинної хроматографії за допомогою високоефективного рідинного хроматографа фірми Agilent Technologies (модель 1100), укомплектованого проточним вакуумним дегазатором G1379A, 4-и канальним насосом градієнта низького тиску G13111A, автоматичним інжектором G1313A, термостатом колонок G13116A, діодноматричним детектором G1316A. Для проведення аналізу була використана хроматографічна колонка розміром 4,6×50 мм, заповнена октадецилсилільним сорбентом, зернення 1,8 мкм, «ZORBAX-XDB-C18». Ідентифікацію амінокислот проводили за часом утримання стандартів. Розрахунок вмісту зв'язаних амінокислот проводився шляхом віднімання вмісту вільних амінокислот від їх загального вмісту. У результаті дослідження було виявлено 19 амінокислот та визначено їх кількісний вміст. П'ять амінокислот є незамінними – треонін, метіонін, ізолейцин, лейцин та аргінін. Домінуючими сполуками є глутамінова кислота, аспарагін, серин, гамма-аміномасляна кислота та лейцин. Вміст вільних амінокислот складає 34,75% від суми усіх амінокислот, а вміст зв'язаних – 65,25%. Більшість амінокислот у досліджуваному екстракті знаходиться у зв'язному стані, тому вони будуть впливати на розчинність, біодоступність та загальний фармакотерапевтичний ефект екстракту.

ИССЛЕДОВАНИЕ АМИНОКИСЛОТНОГО СОСТАВА СПИРТОВОГО ЭКСТРАКТА ИЗ ЛИСТЬЕВ ЧЕРНИКИ ОБЫЧНОЙ

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***Ключевые слова:** Вересковые; черника обыкновенная; листья; спиртовый экстракт; аминокислота*

Изучен качественный состав и количественное содержание свободных и связанных аминокислот в спиртовом экстракте из листьев черники обыкновенной методом высокоэффективной жидкостной хроматографии с помощью высокоэффективного жидкостного хроматографа фирмы Agilent Technologies (модель 1100), укомплектованного проточным вакуумным дегазатором G1379A, 4-х канальным насосом градиента низкого давления G13111A, автоматическим инжектором G1313A, термостатом колонок G13116A, диодноматричным детектором G1316A. Для проведения анализа была использована хроматографическая колонка размером 4,6×50 мм, заполненная октадецилсилильным сорбентом, зернение – 1,8 мкм, «ZORBAX-XDB-C18». В результате исследования было выявлено 19 аминокислот и определено их количественное содержание. Идентификацию аминокислот проводили по времени удержания стандартов. Расчет содержания связанных аминокислот проводился путем вычитания содержания свободных аминокислот от их общего количества. Пять обнаруженных аминокислот являются незаменимыми – треонин, метионин, изолейцин, лейцин и аргинин. Доминирующими соединениями являются глутаминовая кислота, аспарагин, серин, гамма-аминомасляная кислота и лейцин. Содержание свободных аминокислот составляет 34,75% от суммы всех аминокислот, а содержание связанных – 65,25%. Большинство аминокислот в исследуемом экстракте находится в связанном состоянии, поэтому они оказывают влияние на растворимость, биодоступность и общий фармакотерапевтический эффект.