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Study of Amino-Acid Structure *Scutellaria Microdasys* Juz

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Abstract: For the first time qualitative and quantitative amino-acid structure *Scutellaria microdasys* Juz. is studied with use of a highly effective liquid chromatography. Presence 20 proteinogennic amino acids, including 10 irreplaceable is established. The sum of the interchangeable amino acids of the finely shelled skullcap is represented by uncharged, non-polar, aromatic, negatively charged aminocarboxylic acids. Essential amino acids are represented by uncharged, positively charged, nonpolar and aromatic aminocarboxylic acids.

Keywords: Scutellaria microdasys Juz., amino acids, amino-acid structure.

Introduction

The main structural unit of protein is amino acids. Since essential amino acids are not synthesized by animal and human cells, they enter the body from external sources as part of food proteins. A characteristic feature of plants is the ability to biosynthesize all proteinogenic amino acids. These amino acids can be divided into two groups [1]:

interchangeable, represented by negatively charged (aspartic acid, glutamic acid), uncharged (serine, glycine, asparagine, glutamine, cysteine), nonpolar (alanine, proline), aromatic aminocarboxylic acids (tyrosine);

irreplaceable, represented by nonpolar (valine, methionine, isoleucine, leucine), positively charged (arginine, histidine, lysine), aromatic (tryptophan, phenylalanine), (uncharged with amino carboxylic acids (threonine).

At the same time, plants are also distinguished by the synthesis of an extraordinary variety of amino acids that are not part of proteins, but are contained in plant cells and tissues in a free form. All of them are highly active substances and take part in many vital processes [2, 3].

Amino acid composition of *Scutellaria microdasys* Juz. (Шлемник мелкоопушенный) has not been studied practically, which served as the basis for this work.

Material and methods

The study was carried out on the basis of the Department of Inorganic Chemistry of Namangan State University (Namangan).

The paper used air-dry raw materials *Scutellaria microdasys* Juz., which is the biomass of a whole plant collected in places of natural growth on the territory of the Toshkent region in 2020. After harvesting, the vegetable raw materials were dried in air, under a canopy, at a temperature of 15-25°C for 25 days.

Sample preparation: 10 g of crushed raw materials were poured with 96% ethyl alcohol and extracted in a water bath at 80°C for 1 h. The resulting extraction was filtered, the raw materials were washed with 96% ethyl alcohol, the resulting eluates were combined, dealcoholized on a rotary evaporator to an aqueous residue and treated with chloroform three

times. The aqueous layer containing amino acids was separated and condensed under vacuum to a small volume. Qualitative reaction: when conducting a ninhydrin reaction with a part of the aqueous extraction of the finely furred skullcap, the appearance of red-purple staining was observed, which indicated the presence of amino acids [1,6].

Detection of amino acids: the method of ascending chromatography on Filtrak FH-4 paper (Germany) was used in comparison with standard samples in a 0.1-m hydrochloric acid solution at a temperature of 20-22°C in a darkened chromatographic chamber with three-fold transmission of the solution in a solvent system (BUV 4:1:2 or 4:1:5). The manifestation of amino acids on the chromatogram was carried out by treatment with 0.1% aqueous solution of ninhydrin, then the chromatogram was heated until colored spots appeared [1,6]. Amino acids on the chromatogram acquired a blue-violet staining with various shades. Amino acids with a higher Rf value were colored with pink, and those with a lower Rf value were colored with a blue tint.

Isolation of free amino acids. Determination of the qualitative composition and quantitative content of amino acids was carried out by high-performance liquid chromatography (HPLC). Precipitation of proteins and peptides of the aqueous extract of the samples was carried out in centrifuge cups. To do this, 1 ml (exact volume) was added to 1 ml of the test sample 20% THUK. After 10 minutes, the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. After separating 0.1 ml of the infusion fluid, it was dried lyophilically. The hydrolysate was evaporated, the dry residue was dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1) and dried. This operation was repeated twice to neutralize the acid. By reaction with phenylthioisocyanate, phenylthiocarbamyl derivatives (FTC) of amino acids were obtained by the method of Steven A., Cohen Daviel. Identification of amino acid derivatives was carried out by HPLC. HPLC conditions: Agilent Technologies 1200 chromatograph with DEAD detector, 75 x 4.6mm Discovery HS C18 columns. Solution A: 0.14M CH_3COONa + 0.05% TEA pH 6.4, B:CH_3CN. The flow rate is 1.2 ml/min, absorption is 269 nm. Gradient %B/min: 1-6%/0-2.5 min; 6-30%/2.51-40 min; 30-60%/40.1-45 min; 60-60%/45.1-50 min; 60-0%/50.1-55 min [4, 5, 7].

Results and discussion

Screening separation of amino acids by ascending chromatography on paper made it possible to obtain preliminary data on their qualitative composition. As a result, five substances were found in the aqueous extraction of the finely furred skullcap, which, according to the *Rf* values and the color of the developed spots in comparison with reliable samples, were attributed to asparagine, aspartic acid, tyrosine, isoleucine and tryptophan (Table 1).

Table 1 Results of screening detection of amino acids in *Scutellaria microdasys* Juz. by the method of ascending chromatography on paper.

№	Substance	Meaning *Rf*	Staining of spots after development	A reliable sample	Meaning *Rf*
1	Substance I	0,13	Blue-purple	Asparagine	0,12
2	Substance II	0,22	Blue-purple	Aspartic acid	0,23
3	Substance III	0,44	Blue-purple	Tyrosine	0,45
4	Substance IV	0,66	Blue-purple	Isoleucine	0,67
5	Substance V	0,49	Blue-purple	Tryptophan	0,50

To clarify the data on the qualitative composition and quantitative content of amino acids in *Scutellaria microdasys* Juz. we conducted a study of samples obtained from the helmet on an amino acid analyzer chromatograph Agilent Technologies 1200. This made it possible to detect and identify 10 interchangeable and 10 essential amino acids in them. The sum of the interchangeable amino acids of *Scutellaria microdasys* Juz. It is represented by negatively

charged, uncharged nonpolar (aliphatic) and aromatic amino acids (Table 2). Essential amino acids consist of nonpolar, uncharged, positive charged and aromatic carboxylic acids (Table 3)

Table 2 The content of interchangeable amino acids in *Scutellaria microdasys* Juz. determined by HPLC

Amino Acid	mg/g of the analyzed sample	Amino Acid	mg/g of the analyzed sample
Uncharged aminocarboxylic		Nonpolar aminocarboxylic	
Serin	0,445836	Alanin	1,274978
Glycine	0,447473	Proline	0,458561
Asparagine	0,434454	Sum of acids	1,733549
Glutamine	1,875966	Negatively charged aminocarboxylic	
Cysteine	0,649784	Aspartic acid	2,25426
Sum of acids	3,853513	Glutamic acid	1,47809
Aromatic aminocarboxylic		Sum of acids:3,73235	
Tyrosine	0,634297		
The sum of all interchangeable aminocarboxylic acids: 9,953699			

Table 3 The content of essential amino acids in *Scutellaria microdasys* Juz. determined by HPLC

Amino Acid	mg/g of the analyzed sample	Amino Acid	mg/g of the analyzed sample
Nonpolar aminocarboxylic		Positive charged aminocarboxylic	
Valin	0,634258	Arginine	2,011095
Methionine	0,779303	Histidine	0,122831
Isoleucine	0,583498	Lysine	0,022843
Leucine	0,544777	Sum of acids	2,156769
Sum of acids	2,241836	Aromatic aminocarboxylic	
Uncharged aminocarboxylic		Tryptophan	0,052358
Threonine	0,495688	Phenylalanine	0,471425
Sum of acids	0,495688	Sum of acids	0,523783
Sum of essential acids: 5,418076			
Total amount:15.371767			

Among the identified amino acids in the analyzed samples from raw materials *Scutellaria microdasys* Juz. in terms of the total quantitative content, replaceable uncharged aminocarboxylic acids are in the lead (3,853513 mg/g). Replaceable negatively charged aminocarboxylic acids are contained in a slightly smaller amount (3,73235mg/g). The following are arranged in descending order: irreplaceable non-polar aminocarboxylic (2.241836 mg/g), irreplaceable positive charged aminocarboxylic (2.156769 mg/g), replaceable non-polar aminocarboxylic (1.733549 mg/g), replaceable aromatic aminocarboxylic (0.634297mg/g), irreplaceable aromatic aminocarboxylic (0.523783 mg/g) and irreplaceable uncharged aminocarboxylic acids. Of the individual amino acids in the total amount identified, aspartic acid and arginine, glutamic acid, glutamine alanine cysteine, valine, tyrosine predominated (Tables 4, 5).

Table 4 The content of individual interchangeable amino acids in *Scutellaria microdasys* Juz. determined by HPLC (% of the total amount of identified amino acids)

Amino Acid	%	Amino Acid	%
Uncharged aminocarboxylic		Nonpolar aminocarboxylic	
Serin	2,900	Alanin	8,294
Glycine	2,911	Proline	2,983
Asparagine	2,826	Sum of acids	11,277
Glutamine	12,204	Negatively charged aminocarboxylic	
Cysteine	4,227	Aspartic acid	14,665
Sum of acids	25,069	Glutamic acid	9,616
Aromatico aminocarbossilico		Sum of acids	24,281
Tyrosine	4,126		
The sum of all interchangeable amino carboxylic acids is 64,753			

The total amount of identified amino acids is 100%

Table 5 The content of individual essential amino acids in *Scutellaria microdasys* Juz. determined by HPLC (% of the total amount of identified amino acids)

Amino Acid	%	Amino Acid	%
Negatively charged aminocarboxylic		Positive charged aminocarboxylic	
Valin	4,127	Arginine	12,083
Methionine	5,07	Histidine	0,799
Isoleucine	3,796	Lysine	0,148
Leucine	3,544	Sum of acids	14,031
Sum of acids	14,584	Aromatic aminocarboxylic	
Uncharged aminocarboxylic		Tryptophan	0,341
Threonine 3,225		Phenylalanine	3,067
		Sum of acids	3,407
Sum of essential acids: 35,247			

The total amount of identified amino acids is 100%

Conclusion

Qualitative and quantitative analysis of amino acids of *Scutellaria microdasys* Juz was carried out for the first time. An Agilent Technologies chromatograph is used on an amino acid analyzer, as a result of which the presence and content of 20 proteinogenic amino acids, including 10 essential ones, represented by negatively charged, uncharged, nonpolar, aromatic (replaceable) and positive charged, nonpolar, uncharged, aromatic aminomonocarboxylic acids (irreplaceable) are established.

The data obtained allow us to characterize *Scutellaria microdasys* Juz. as a full-fledged source of a complex of proteinogenic amino acids.

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