

Study of the amino acid composition of the herb *Campanula rotundifolia*
(*Campanula rotundifolia* L.)

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Study of amino acid composition of herba *Campanula rotundifolia* L.

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RESUME

The qualitative and quantitative amino acid composition of herb (*Campanula rotundifolia* L.) was studied. Authentically by HPLC revealed the presence of 16 amino acids, including 7 essential: threonine, valine, methionine, isoleucine, leucine, fenilallanin and lysine, 3 semi-essentials: tyrosine, histidine and arginine and 6 interchangeable: aspartic acid, serine, glutamic acid , proline, glycine and alanine. 100 mg of absolutely dry raw material contain 7.49 mg of amino acids. All amino acids were identified in this plant for the first time.

keywords:amino acid analyzer amino acid composition, *Campanula rotundifolia*.

SUMMARY

The qualitative and quantitative amino acid composition of the herb round-leaved bluebell (*Campanula rotundifolia* L.) was studied. The presence of 16 amino acids was reliably established by HPLC, among which 7 are essential: threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, 3 semi-essential: tyrosine, histidine, arginine and 6 non-essential: aspartic acid, serine, glutamic acid, proline, glycine , alanine. The amount of amino acids was 7.49 mg per 100 mg in terms of absolutely dry raw materials. All amino acids in this plant have been identified for the first time.

Keywords: amino acid analyzer, amino acid composition, round bell.

Currently, the search for new domestic sources of biologically active substances is one of the urgent problems of modern medicine. The value of amino acids for the body is determined primarily by the fact that they are used for the synthesis of proteins and peptides. In addition, a large number of non-peptide substances (special tissue proteins, enzymes, hormones, nucleic acids, etc.) that perform special functions are formed from amino acids. Amino acids are a natural universal regulator of metabolism in the human body.

In addition to the amino acids that make up proteins, living organisms have a constant reserve of "free" amino acids contained in tissues and cellular

juice. They are in dynamic equilibrium during numerous exchange reactions. Free amino acids in a living organism also perform specific tasks; Thus, aspartic acid is characterized by the presence of immunoactive properties. Proline promotes wound healing, it is one of the most important components of collagen, which is important for the normal functioning of human body tissues. Arginine and glycine have a pronounced hypolipidemic activity, arginine is able to enhance the release of various hormones from gland tissues (insulin), accelerate filtration through the kidneys and have an antioxidant effect. Isoleucine is necessary for the synthesis of hemoglobin [1, 2, 3]. There are a number of drugs based on amino acids: glycine, methionine, heprtral, cerebrolysin, and others [3].

Plant materials containing amino acids can be their source, due to the fact that plants contain a complex of biologically active substances in accessible concentrations and with high bioavailability. Amino acids are able to potentiate the action of other biologically active substances and ensure their better assimilation [4]. All of the above is evidence of the practical significance and relevance of the study of amino acids.

In this regard, the purpose of our study was to study the qualitative and quantitative composition of amino acids in the aerial part of the round-leaved bell.

The round-leaved bell is a perennial herbaceous plant 15–60 cm high, with a thin creeping rhizome. Stems numerous and densely leafy. Basal leaves and leaves of short non-flowering shoots are reniform or rounded heart-shaped. Stem leaves are lanceolate to narrowly linear. The flowers are collected in an apical spreading paniculate inflorescence. The fruit is a box [6].

The round-leaved bell is common in central Russia in all areas, like an ordinary plant. It grows in open forests, dry meadows, thickets of shrubs, on limestone and chalk outcrops [6]. In folk medicine, the round-leaved bell is used for diseases of the nervous system, respiratory failure, pain syndrome, tonsillitis, neoplasms, and also as a hemostatic. Fresh leaves of the bluebell, ground into a gruel, are used to treat skin diseases, which may be due to the participation of proline in the synthesis of skin collagen. The literature contains data on the use of bluebell as an antiepileptic agent, which may indicate the content of the sum of aspartic and glutamic acids, which are known for their use in diseases of the central nervous system [7,

Previously, nitrogen-containing compounds of the herb bellflower round-leaved Khidasheli V.D. were studied. They established the presence of choline and betaine in the herb of the plant [9]. However, there is no information on the amino acid composition of the bellflower in the literature.

MATERIALS AND RESEARCH METHODS The object of the study was crushed, air-dry round-leaved bluebell (*Campanula rotundifolia* L.), harvested in the Kursk region during the period of mass flowering in 2015.

Qualitative analysis of amino acids was carried out in aqueous extract using a ninhydrin sample and thin layer chromatography.

For quality definitions and chromatographic research amino acids, a sample of 5.0 g of crushed air-dry raw material was poured into 50 ml of purified water and heated in a water bath under reflux for 1 hour. The resulting extract was filtered, and the raw material was refilled with 50 ml of water, and the operation was repeated. Extraction was carried out three times and the obtained extracts were combined, evaporated under vacuum to 25 ml and used for qualitative reactions and chromatographic analysis.

A qualitative reaction with ninhydrin was carried out by mixing equal volumes of the test solution and 0.1% freshly prepared ninhydrin solution and gently heated. In the presence of amino acids, a red-violet color should appear [4, 5, 10].

Chromatography was carried out in thin layers of the sorbent. 0.03–0.05 ml of the extract was applied to the prepared Sorbfil plate PTSH-P-A 10X20 and chromatography was carried out in a solvent system: 96% ethyl alcohol, concentrated ammonia in a ratio (16:4.5), in parallel with known samples amino acids. After passage, the chromatogram was dried in air, treated with a 0.2% alcohol solution of ninhydrin, and heated in an oven at a temperature of 100–105°C for several minutes. Amino acids appeared as red-violet spots [4].

Further, to determine the total amino acid composition, an amino acid analyzer was used - an automated liquid chromatograph (AAA 400) as follows: an accurate sample of the raw material (0.2 g) was introduced into a flask with a thin section, 20 ml of a solution of 6 M hydrochloric acid was added, tightly closed and thermostated at temperature 110°C for 23 hours. At the end of the hydrolysis, the flask was cooled to room temperature, the acid extract was filtered, and evaporated to dryness using a rotary evaporator. To the dry residue was added 5 ml of water and the procedure was repeated twice to remove residual hydrochloric acid.

To the residue evaporated to dryness was added 50 ml of loading buffer (pH - 2.2), which was prepared as follows: weighed 14 g of citric acid, 11.5 g of sodium chloride, 0.1 g of sodium azide, 5 ml of thiodiglycol, the volume was brought up to the mark with distilled water. The resulting and filtered solution was added to the prepared ion-exchange column.

Amino acid analysis was carried out under the following conditions: the flow of buffer solutions was 0.3 ml/min, the flow rate of the ninhydrin reagent was 0.2 ml/min, detection was carried out in the UV regions of 440 and 570 nm, and the temperature of the reactor thermostat was 121°C [11].

RESULTS OF THE STUDY

Qualitative reactions and thin-layer chromatography compared with known samples revealed the presence of 11 free amino acids in the herb round-leaved bell, of which 4 are essential (lysine, phenylalanine, threonine, leucine) and 7 are non-essential (aspartic acid, serine, glutamic acid, glycine, arginine, proline, alanine).

Next, we used a more complex and modern method of analysis, the HPLC method, which was performed on an automated liquid chromatograph AAA 400 from INGOS - Laboratory Instruments (Czech Republic), to determine the qualitative and

quantitative total content amino acids. Received range amino acids are presented in table. one.

As a result of the determination of amino acids by HPLC in the sample after hydrolysis with hydrochloric acid, the presence of 16 amino acids was established, which are 7 essential (threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine) and 9 non-essential amino acids (aspartic acid, serine, glutamic acid, proline, glycine, alanine, tyrosine, histidine, arginine). The results of the analysis of the amino acid composition of the herb round-leaved bell after hydrolysis are presented in Table. 2.

As a result of the study, it was found that 11 amino acids were determined in the herb of the bellflower by TLC, 16 amino acids were determined by HPLC, of which 7 are essential. The total content of amino acids in the herb round-leaved bell is 7.49 mg per 100 mg in terms of absolutely dry raw materials. The predominant amino acids are: glutamic acid (20.56%), aspartic acid (11.34%), leucine (7.61%), lysine (6.94%).

Table 1

Amino acid spectrum after acid hydrolysis of the aerial part of the bluebell round-leaved

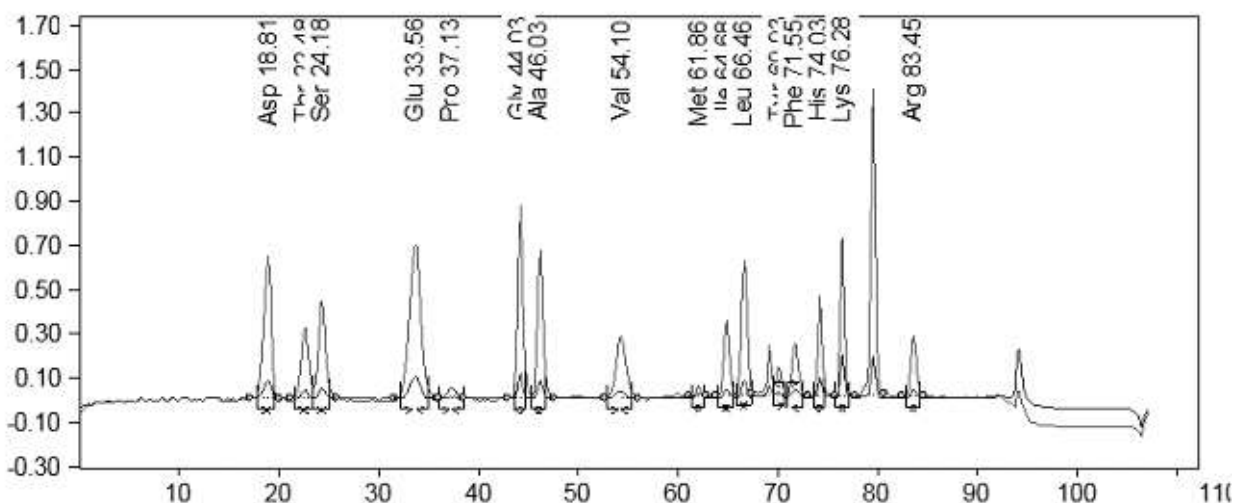


table 2

The content of amino acids after hydrolysis in the aerial part of the bell round-leaved

No.	Name amino acids	Content amino acids in relation to sum amino acids %, To
one	Aspartic acid	11.34
2	Threonine*	4.93
3	Serene	5.34
4	Glutamic acid	20.56
5	Proline	5.20
6	Glycine	5.07
7	Alanine	4.54
eight	Valine*	5.74
9	Methionine*	0.53
10	Isoleucine*	4.54
eleven	Leucine*	7.61
12	Tyrosine	2.40
thirteen	Phenylalanine*	4.80
14	Histidine	4.40
15	Lysine*	6.94
sixteen	Arginine	6.00
	The amount of amino acids in mg in terms of 100 mg of absolutely dry raw materials	7.49

Note: * - essential amino acids

DISCUSSIONS AND CONCLUSIONS

1. The qualitative composition of free amino acids of bluebell herb has been studied round-leaved by TLC, while 11 amino acids were identified, among which 4 are essential.

2. In the herb bellflower round-leaved method of highly efficient Liquid chromatography after hydrolysis with hydrochloric acid identified 16 amino acids, among which 7 are essential, among which glutamic acid, aspartic acid, leucine, lysine predominate.

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