Study of the Amino Acid Composition of Extracts from Narrow-Tubed Skullcap Plants

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Abstract: A study and comparative analysis of the amino acid composition (free and bound amino acids) of the aerial parts and roots of the narrow-tubed skullcap plant were conducted using modern physicochemical methods, specifically thin-layer chromatography (TLC). A method for determining and separating amino acids in extracts from medicinal plant raw materials was proposed, demonstrated using the aerial parts of the narrow-tubed skullcap plant. The results obtained can serve as an additional quality criterion for raw materials, as any deviations from technological regulations during harvesting, drying, storage, and the preparation of medicinal forms affect the chromatographic profile.

Keywords: amino acids, aerial part of narrow-tubed skullcap, dried, thin-layer chromatography.

**INTRODUCTION**

Investigations into the chemical composition and pharmacological characteristics of plant raw materials (PRM), comprehensive phytopreparations, and specific chemicals extracted from plants facilitate the creation of novel, highly efficacious pharmaceutical products and uncover new manufacturing sources [1–2]. Recent research indicates that plants comprise roughly 30% amino acids (AAs), existing in either free or bound states, as determined by protein content analysis.

The widespread occurrence of AAs in plants and their high biological activity contribute to the effective action of PRM and the drugs derived from them. Furthermore, AAs as part of accompanying substances enhance absorption efficiency [3-4].

Thin-layer chromatography (TLC) is currently used in pharmaceutical analysis to assess the authenticity and purity of substances. TLC, offering all the advantages of chromatographic methods, is widely applied due to its rapidity, accessibility, sufficient sensitivity, selectivity, low cost, and ease of analysis. Recently, publications have emerged indicating the potential for quantitative analysis of TLC data using specialized software [5-7].

Spectral analysis methods, such as spectrophotometry in the UV and visible regions and photoelectric colorimetry, have also found wide application. However, these methods have several drawbacks, including the complexity and duration of analyses, instability of colored products in chromatic reactions, and insufficient sensitivity and selectivity for amino acid determination in the simultaneous presence of others without prior separation. The occurrence of molecular diffusion and the brief exposure period are likely to blame for this [8-11].

Quantitative analysis of process products. Chromatographic analysis of watery products was carried out on a gas

chromatograph "Crystal 5000" with a capillary flame ionization detector. Chromatographic analysis of gas manufactures was carried out on a gas chromatograph "Crystal 5000.02".

**METHOD OF RESEARCH**

The object of the study was narrow-tubed skullcap plants collected for analysis. To remove excess moisture and prevent the decomposition of organic compounds, the plants were dried in drying cabinets at temperatures of 40–60 °C until the residual moisture content was no more than 20%, preserving the amino acid structure.

The dried plant raw material (PRM) was ground to a powdery state to increase the contact area with the extractant. A laboratory cyclone-type mill, TAGLER LMC-5 "Cyclone," was used for this process.

Defatting of the PRM to remove lipids was performed using the organic solvent hexane. The extraction was repeated several times until the fats were completely removed. Laboratory-grade hexane ("Pure Hexane") was used for the extraction process.

Extraction of Total Amino Acids from Narrow-Tubed Skullcap Leaves

A 100 mL conical flask was filled with around 2.5 g of crushed plant material (weighed exactly) that could fit through a screen with 0.5 mm apertures. The powdered raw material and water were combined together in a 1:10 ratio. Then, 25 mL of pure water was poured to the substance, taking into account how much water it could soak up.

For two hours, the flask was heated in a boiling water bath and attached to a reflux condenser. The mixture was heated to a temperature of 40–60 °C (occasionally up to 80 °C to enhance extraction).

To improve the efficiency of the extraction process, the water was slightly acidified to a pH of 4–6 using weak acetic acid. The mixture was periodically shaken to wash particles of raw material off the flask walls. After heating, the flask and its contents were cooled to room temperature.

The extract was put through multiple layers of gauze, which squeezed the raw material particles, and then put into a 25 mL volumetric flask. The mixture was further filtered to remove solid residues using filter paper designed for general filtration with a pore size of approximately 11 mm (Whatman Grade).

The resulting filtrate was purified from soluble impurities using high-quality activated carbon (Sigma-Aldrich Activated Carbon), which is suitable for analytical and laboratory research.

To concentrate the extract, the filtrate was evaporated using a Heidolph Hei-VAP vacuum rotary evaporator, reducing its volume and increasing the concentration of amino acids.

Extraction of Total Amino Acids from Narrow-Tubed Skullcap Roots

Approximately 10.0 g of crushed plant material (precisely weighed), with particle sizes passing through a sieve with 0.5 mm openings, was placed in a 100 mL conical flask. The powdered raw material was mixed with water at a ratio of 1:10. Then, 100 mL of purified water was added, accounting for the water absorption coefficient of the material.

The flask was connected to a reflux condenser and heated in a boiling water bath for 2 hours. The mixture was heated to a temperature of 40–60 °C (occasionally up to 80 °C to enhance extraction). To improve the extraction efficiency, the water was slightly acidified to a pH of 4–6 using weak acetic acid. The mixture was periodically shaken to remove particles of raw material from the flask walls. After heating, the flask and its contents were cooled to room temperature.

The extract was filtered through several layers of gauze, squeezing the raw material particles, and transferred into a 25 mL volumetric flask.

After extraction, the mixture was cooled and filtered using filter paper to remove solid residues. General-purpose filter paper with a pore size of approximately⁓11 mm (Whatman Grade) was used. The resulting filtrate was purified from soluble impurities using high-quality activated carbon for analytical and laboratory research (Sigma-Aldrich Activated Carbon).

To concentrate the extract, the filtrate was evaporated using a Hei-VAP vacuum rotary evaporator, reducing the volume and increasing the concentration of amino acids.

Preliminary qualitative analysis confirmed the presence of amino acids in the studied extracts.

Reaction with Ninhydrin. Ninhydrin (Reingold's reagent) reacts with amino acids, producing a blue coloration, which is characteristic of amino acids containing a free amine group. Ninhydrin (2,2-dichloro-1,3-dicarbonyl-1,3-dihydroxybenzophenone) reacts with the amine groups of amino acids, forming a complex that oxidizes the amino acid, leading to the formation of several products, including dipeptides, peptides, and larger molecules when there is an abundance of amino acids. During the reaction of amino acids with ninhydrin, the main products formed are: The ninhydrin-amino acid complex: This is the primary compound formed in the reaction between amino acids and ninhydrin. The complex is characterized by a blue color, which serves as an indicator for the presence of amino acids.

The reaction with ninhydrin can be written as:

R-NH2 + Ninhydrin → [Ninhydrin-Amine Complex] → Blue coloration.

Amino Acid + Ninhydrin → Oxidized Product + Carbon Dioxide + Blue

Example with Glycine (amino acid):

Glycine + Ninhydrin → Oxidized Product + Blue Complex

To separate and identify amino acids from the total extract, thin-layer chromatography (TLC) was used. This method is based on differences in the movement speed of amino acid molecules over an adsorbent layer under the influence of a mobile phase. The stationary phase used was a silica gel-coated plate.

A solvent mixture of butanol: acetic acid: water in a ratio of 4:1:1 was used as the mobile phase. Small volumes of amino acid solutions and total amino acid extracts were spotted onto the starting line of the TLC plate, 1–2 cm from the lower edge. The plate was left to dry completely.

The plate was then placed in a chamber containing the mobile phase, ensuring that the starting line was above the level of the solvent. The mobile phase ascended the plate due to capillary action, separating the amino acids based on their different adsorption and solubility properties. After the separation was complete, the plate was removed from the chamber and dried. To visualize the amino acids, the plate was treated with ninhydrin and heated. This resulted in the formation of colored spots—purple, blue, and yellowish. The UV spectra of the amino acids extracted from the narrow-tubed skullcap plants were then studied.

The extracted amino acids were prepared as aqueous solutions, and the UV spectrum was recorded using the SF-4A spectrophotometer, developed in the Soviet Union. The measurement range of the SF-4A spectrometer covers from 190 nm to 900 nm, allowing for the study of both ultraviolet and visible regions of the spectrum.

The following results were obtained: The spectrum exhibited a main peak around 210–215 nm, along with some weak peaks in the 240–280 nm range, which is associated with the presence of aromatic groups or transitions in the amide part of the molecule. This suggests the presence of arginine. The spectrum showed weak absorption in the UV region, with a maximum absorption around 220–230 nm. This corresponds to proline, an amino acid with a cyclic structure that lacks aromatic rings.

The spectrum displayed weak absorption in the 190–210 nm range, which is due to its amino acid structure. Glycine, like proline, does not have aromatic rings. The absorption in the UV region, around 210–220 nm, is attributed to the presence of carboxyl and amino groups, as well as peaks arising from amide bonds. Glutamic acid may exhibit such a spectrum. The spectrum shows absorption in the 210–230 nm range, with no significant peaks in the UV region. This is characteristic of valine, which has a hydrophobic side chain and, like other simple amino acids, lacks aromatic rings. The spectrum typically resembles those of other alanine-like amino acids, with maximum peaks in the 210–230 nm range. Methionine, which contains a carbon-sulfur methyl group, shows this characteristic pattern.

All the amino acids studied typically exhibited absorption in the ultraviolet spectrum between 190 and 280 nm. However, according to the literature, the primary classes of water-soluble biologically active substances (BAS) in the studied plant raw materials are flavonoids, tannins, organic acids, chlorophylls, sterols, lignans, and mineral salts. Therefore, it is hypothesized that the observed coloration is due to the presence of amino acids.

**RESEARCH RESULTS**

The first step was to look at the free amino acids (AA) in the plant raw material (PRM) that had been removed with water. As the literature advised, a 0.2% solution of ninhydrin in acetone was used as a reagent to discover amino acid zones. When ninhydrin meets amino acids, it creates an ammonium salt of diketohydridenketohydraminamine, which stays blue-violet.

The experiment tested more than fifteen eluating methods for amino acid extraction (1, 6, 7, 8) across a wide range of polarities, as the scientific literature advised. The n-butanol-ice acetic acid-water system (4:1:1) with a polarity of 5.69 had the best separation and quality of the chromatographic zone. The height of the eluent flow required to be at least 13 cm.

The obtained extracts from the studied plant raw material (PRM) were applied to the starting line of chromatographic plates "Silufol" (10x15 cm) in volumes of 10 and 5 mL. The resulting chromatograms are shown in Figure 1.

For each chromatographic zone, the values of R*f* (retention factor), distribution coefficients (K), and sorption selectivity (L), height equivalent to a theoretical plate (H), and the number of theoretical plates (N) were calculated (Table 1). In the case of studying the amino acid composition and isolating individual components using thin-layer chromatography (TLC), sorption selectivity is the key characteristic of process efficiency. The separation of two adjacent zones is considered complete when R*f* (18).

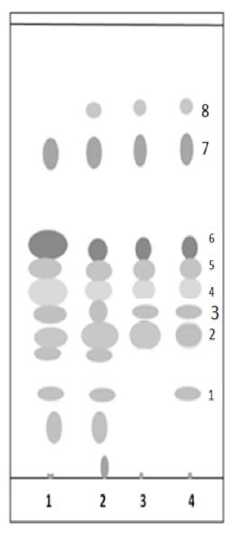
The identification of zones on the chromatograms was performed by comparing them with reliable standard samples (0.1% aqueous solutions of amino acids: arginine, glycine, glutamic acid, proline, phenylalanine, methionine, valine, leucine), as shown in Table 1.

On the chromatograms of the extracts from the aerial part, zones of replaceable amino acids (AAs) were found with R*f* values of 0.20 ± 0.02 (arginine), 0.30 ± 0.02 (proline), 0.35 ± 0.04 (glycine), 0.42 ± 0.02 (glutamic acid); and of essential AAs with R*f* values of 0.46 ± 0.02 (valine), 0.56 ± 0.06 (methionine), and 0.63 ± 0.02 (leucine). There were also unidentified zones of AAs with R*f* values of 0.14 ± 0.02 and 0.25 ± 0.02.

It was established that the composition of free AAs in extracts from dried roots is somewhat different. On the chromatograms of the extracts, common zones of replaceable AAs with R*f* values of 0.35 ± 0.04 (glycine), 0.30 ± 0.02 (proline), 0.42 ± 0.02 (glutamic acid); and of essential AAs with R*f* values of 0.46 ± 0.02 (valine), 0.56 ± 0.06 (methionine), 0.63 ± 0.02 (leucine), and 0.72 ± 0.02 (phenylalanine) were identified.

Additional characteristic zones of unidentified AAs were found in the root extracts, with R*f* values of 0.06 ± 0.005, 0.14 ± 0.02, and 0.25 ± 0.02, as well as an arginine zone with R*f* = 0.20 ± 0.02, which was absent in the chromatogram of the aerial part extract. The root part contains AAs both in free and bound forms (protein compounds).

The results of the calculation of the sorption selectivity indicate satisfactory separation of chromatographic zones on the chromatogram and justify the use of this methodology for the studied plant species (Table 1,2).



**FIGURE 1.** shows the chromatogram views of extracts:

1. From the aerial part (N) of Scutellaria baicalensis (sample volume 10 mL);

2. From the leaves (L) of Scutellaria baicalensis (sample volume 5 mL);

3. From the roots (K) of Scutellaria baicalensis (sample volume 10 mL);

4. A mixture of 0.1% aqueous solutions of standard AA samples (sample volume 5 mL) after development with a 2% ninhydrin solution in acetone.

The identified amino acids are as follows: 1. Arginine; 2. Proline; 3. Glycine; 4. Glutamic acid; 5. Valine; 6. Methionine; 7. Leucine; 8. Phenylalanine.

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**TABLE 1.** Identification of chromatographic zones of free amino acids on chromatograms. Extract from the aerial part of Scutellaria baicalensis

|  |  |  |  |
| --- | --- | --- | --- |
| **№ zone** | **R*f*** | **Color in Visible Light** | **Amino Acid Identification** |
| 1 | 0,142 | Pink | Unidentified |
| 2 | 0,197 | Pink | Arginine |
| 3 | 0,244 | Pink | Unidentified |

**TABLE 2.** Identification of chromatographic zones of free amino acids on chromatograms. Extract from the roots of Scutellaria baicalensis.

|  |  |  |  |
| --- | --- | --- | --- |
| **№ zone** | **R*f*** | **Color in Visible Light** | **Amino Acid Identification** |
| 1 | 0,063 | Pink | Unidentified |
| 2 | 0,143 | Pink | Unidentified |
| 3 | 0,198 | Pink | Arginine |
| 4 | 0,270 | Pink | Unidentified |
| 5 | 0.317 | Orange | Proline |
| 6 | 0,357 | Pink | Glycine |
| 7 | 0,400 | Crimson | Glutamic Acid |
| 8 | 0,440 | Lavender | Valine |
| 9 | 0,600 | Pink | Methionine |
| 10 | 0,650 | Рink-Lavender | Leucine |
| 11 | 0,720 | Pink | Phenylalanine |

**CONCLUSIONS**

The studied Scutellaria baicalensis plant contains predominantly asparagine, glutamic acid, arginine, leucine, alanine, serine, and threonine. Overall, the aerial part of the plant is a richer source of amino acids compared to the roots.

In the aerial parts of Scutellaria baicalensis, compared to the roots, amino acids such as arginine, histidine, tyrosine, and serine are present in larger quantities, which may indicate their preferential accumulation in specific plant organs.

1. The amino acid composition (free and bound amino acids) of the aerial parts and roots of Scutellaria baicalensis was studied using modern physicochemical methods (TLC).

2. Using the parameters of chromatographic separation efficiency in a thin layer of sorbent, the optimal system for identifying and separating free amino acids through TLC was justified for extracts from Scutellaria baicalensis (with examples from both the aerial parts and roots).

3. The chromatographic profile of amino acids from the extracts can be used to assess the quality of the raw material. The results obtained can serve as an additional quality criterion for the plant material, as any deviations from the technological regulations during harvesting, WDdrying, and storage of the raw material, as well as the extraction process, can affect the chromatographic profile.

**REFERENCES**

1. Muradov, R., Fayzullaev, N., Ergashev, I., & Norkulov, U. (2023). The study of the phenolic substances of the aerial part of the plant “Kokamaron” (Scutellaria Leptosiphon Juz.). E3S Web of Conferences, 431, 01061. <https://doi.org/10.1051/e3sconf/202343101061>
2. Patent No. 2095808 (Russia). "Method for the Separation and Detection of Amino Acids." I.I. Malakhova, V.D. Krasikov, B.V. Tyaglov, E.V. Degterev. 1997.
3. Patent No. 2078342 (Russia). "Method for the Separation of Amino Acids in Biological Fluids." B.V. Tyaglov, E.V. Degterev, P.P. Malakhova, et al. 1997.
4. Hernández-Orte, P., Ibarz, M. J., Cacho, J., & Ferreira, V. (2003). Amino acid determination in grape juices and wines by HPLC using a modification of the 6-Aminoquinolyl-N-Hydroxysuccinimidyl carbamate (AQC) method. Chromatographia, 58(1–2), 29–35. <https://doi.org/10.1365/s10337-003-0002-1>
5. Trineeva, O. V., Sinkevich, A. V., & Slivkin, A. I. (2015). Study of the amino acid composition of plant extracts objects. Khimija Rastitel’nogo Syr’ja, (2), 191–198. <https://doi.org/10.14258/jcprm.201502292>
6. Shukurov, J., & Fayzullaev, N. (2024). Direct synthesis of dimethyl ether from synthesis gas. AIP Conference Proceedings, 3045, 060042. <https://doi.org/10.1063/5.0197641>
7. Mahajan, P. S., Nikam, M. D., Khedkar, V., Jha, P., Badadhe, P. V., & Gill, C. H. (2016). An Organocatalyzed Efficient One‐pot Synthesis, Biological Evaluation, and Molecular Docking Studies of 4,4′‐(Arylmethylene)bis‐(3‐methyl‐1‐phenyl‐1H‐pyrazol‐5‐ols). Journal of Heterocyclic Chemistry, 54(2), 1109–1120. <https://doi.org/10.1002/jhet.2681>
8. Trineeva, O. V. (2021). Development of theoretical approaches to determination of the main groups of biologically active substances of medicinal plant raw materials by TLC method. Drug Development & Registration, 10(2), 69–79. <https://doi.org/10.33380/2305-2066-2021-10-2-69-79>
9. Shukurov, J. (2025). Modeling the production of dimethyl ether from natural gas. AIP Conference Proceedings, 3304, 040062. <https://doi.org/10.1063/5.0269234>
10. Yu, X., Li, J., Peng, R., Zhang, X., Yue, W., Wang, Y., Lan, Y., & Wang, Y. (2025). Flos lonicerae and Baikal skullcap Extracts Improved Laying Performance of Aged Hens Partly by Modulating Antioxidant Capacity, Immune Function, Cecal Microbiota and Ovarian Metabolites. Animals, 15(19), 2882. <https://doi.org/10.3390/ani15192882>
11. Wang, Y., Yang, R., Fu, Z., Ma, Z., & Bai, Z. (2024). The Photoperiod Significantly Influences the Growth Rate, Digestive Efficiency, Immune Response, and Antioxidant Activities in the Juvenile Scalloped Spiny Lobster (Panulirus homarus). Journal of Marine Science and Engineering, 12(3), 389. <https://doi.org/10.3390/jmse12030389>