CHEMICAL SCIENCES

AMINO ACID COMPOSITION OF THE STEM OF CISTANCHE TRIVALVIS, A PLANT FOUND AROUND THE KYZYLKUM DESERT

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Abstract

In this article, for the first time, using high-performance liquid chromatography, the amino acid composition and amino acid content in the underground part of the stem of the Cistanche trivalvis plant growing in the vicinity of the Kyzylkum desert of the Khorezm region of the Republic of Uzbekistan were studied, and the content of the following amino acids was determined: asparagine, serine, glutamine, histidine, lysine, alanine, alanine with valine, methionine, glutamate, tryptophan, phenylalanine, isoleucine, proline.

Keywords: Cistanche trivalvis, Kyzylkum desert of the Khorezm region, amino acids, Glutamine, Glycine, Asparagine.

Introduction Today, ensuring healthy nutrition of the population, eliminating the deficiency of various micro- and macroelements, vitamins and biologically active substances is one of the urgent problems in the world. As a result of modern lifestyle, environmental problems, malnutrition and stress factors, many metabolic disorders are observed in the human body. Therefore, the demand for biologically active food additives based on biologically active substances obtained from natural sources is increasing year by year. In recent years, the increased interest in plant-based biologically active additives compared to synthetic drugs is explained by their relative safety, good absorption by the body and fewer side effects with long-term use. In this regard, in-depth study of plants rich in medicinal and biologically active substances, determination of their chemical composition and assessment of the possibilities of practical application are of scientific and practical importance. Plants belonging to the Cistanche genus have long been used in Eastern medicine as medicinal plants with biologically active properties. The scientific literature notes that the representatives of this genus contain flavonoids, phenylethanoid glycosides, iridoids, polysaccharides, vitamins, and important macro- and microelements. These substances are important because they have antioxidant, adaptogenic, immunomodulatory, and metabolic support properties. However, the Cistanche trivalvis plant has been scientifically studied less than other Cistanche species, especially since systematic and comprehensive studies on its flavonoids, vitamins, proteins, amino acids, fats, carbohydrates, and macro- and microelements are insufficient. This does not allow us to fully assess the richness of this plant in biologically active substances and its potential practical significance. The issue of effective use of local plant raw materials found in Uzbekistan and the development of biologically active products that can replace imports is also relevant today. A thorough study of the chemical composition of Cistanche trivalvis allows us to evaluate this plant as a promising raw material for the development of biologically active food additives. In addition, the scientific results obtained will serve as a solid scientific basis for future research on the creation of biotechnological products based on this plant. Taking into account the above, this article, aimed at determining the chemical composition of Cistanche trivalvis, in particular the amount of amino acids, is relevant and scientifically and practically necessary.[1,2]

The aim of the study: To determine the amino acid composition and quantity of the stem part of Cistanche trivalvis, collected from the edges of the Kyzylkum desert in Khiva and Yangiarig districts of Uzbekistan (around Eshonrovot and Ulli Shurkul).

About the plant: Cistanche trivalvis is a chlorophyllless, obligate parasitic plant of the Orobanchaceae family, with pale yellow flowers on its stems, growing to a height of 20-50 cm (7.9-19.7 in). The stem is glabrous and yellow to purple-gray. The leaves are ovate-lanceolate, lanceolate, glabrous, and usually brown. The inflorescence is dense, more or less cylindrical. The corolla is broadly obconical, strongly curved, bright yellow, sometimes light purple, and naked. It is a parasitic plant distributed mainly in the Republic of Turkmenistan, Karakalpakstan, and Khorezm regions of Uzbekistan. Its main characteristic is that it absorbs its nutrients from the roots of host plants.[3]

Materials and Methods: The sample used was the underground stem of the Cistanche plant, collected in the Yangiarik district of the Khorezm region in May 2025. The plant sample was analyzed for qualitative and quantitative amino acid values using standard samples supplied by Sigma Aldrich (Germany). The determination was performed using a fluorescence detector (FLD) mounted on an Agilent 1260 II Infinity liquid

chromatography system manufactured by Agilent Technologies, USA. A Poroshell 120 EC-C18 column (150 mm × 4.6 mm × 4 µm), manufactured in the USA, served as the stationary phase. Pre-column derivatization was performed in an automated, programmable mode. For amino acid analysis, the mobile phases used were: Phase A—sodium dihydrogen phosphate solution (40 mM), pH 7.8, and Phase B—a mixture of acetonitrile, methanol, and water (in a ratio of 45:45:10), using a gradient mode.

Time | Phase A % (sodium dihydrogen phosphate solution, 40 mM, pH 7.8) | Phase B % (acetonitrile:methanol:water = 45:45:10).Table 1.

Table 1

Mobile phases for amino acid analysis

Time (min)	Phase A - sodium dihydrogen phosphate solution (40 mM), pH 7.8	Phase B - acetonitrile: methanol: water (45:45:10)
0.0	98	2
16	70	30
23	57	43
26	0	100
28	98	2
30	98	2

The flow rate was 1 ml/min, the thermostat temperature was 40°C, the injected sample volume was 5 µl, the analysis was carried out for 30 minutes, and the following chromatograms were obtained.

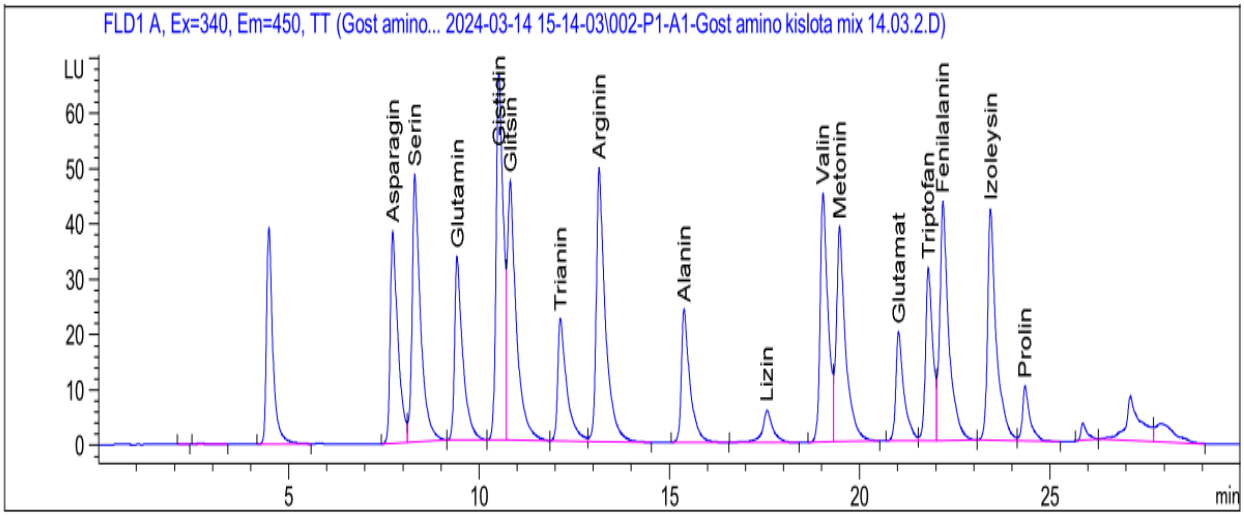

Figure 1*. Chromatogram obtained from standard samples of 16 different amino acids*

Hydrolysis of the amino acids contained in the sample was carried out as follows:First, all samples were ground to a size of 1 mm. A 5.0 g sample was ground and weighed to the nearest 0.001 mg using an analytical balance (FA220 4N). Next, the sample was placed in a 200 ml flask with a reflux condenser, 50 ml of hydrochloric acid (6 N) was added, and the flask was placed in a thermostat at 110°C. Hydrolysis was carried out for 24 hours with magnetic stirring. After hydrolysis was complete, the solution was cooled to room temperature, 10 ml was removed, and centrifuged for 10 minutes at 12,000 rpm. Then, 5 ml of the centrifugate was removed and neutralized with 6 N sodium hydroxide.

After this, 1 ml of the solution was filtered through a filter with a pore size of 0.45 µm, placed in a vial and the following results were obtained [4,5,6,7].

Results and Discussion: The results obtained are presented in Table 2.

Table 2.

Composition and quantity of amino acids in the underground parts of the stem of the plant Cistanche trivalvis, growing in the Khorezm region

№	Name of indicators	Underground stem parts Cistanche trivalvis (mg/g)	№	Name of indicators	Underground stem parts Cistanche trivalvis (mg/g)
1.	Asparagine	0.12	9.	Lysine	3.70
2.	Serin	1.57	10.	Valin	1.48
3.	Glutamine	0.55	11.	Methionine	0.62
4.	Histidine	0.44	12.	Glutamate	0.33
5.	Glycine	5.80	13.	Tryptophan	0.20
6.	Threonine	2.85	14.	Phenylalanine	0.17
7.	Arginine	1.76	15.	Isoleucine	1.15
8.	Alanin	0.77	16.	Proline	1.24

The obtained results were converted from mg/L to mg/g using the following formula:

$$M_{(A\min\ \ oacid)} = \frac{C * V}{m * 1000}$$

Where:

$M_{\text{amino acid}}$ —amino acid content (mg/g),

C —is the result obtained by HPLC (mg/l),

V —is the total volume of the solution used for hydrolysis (ml),

m— is the sample mass (g).

As a result of the conducted research, it was determined that 16 types of amino acids were present in the underground stem parts of the Cistanche trivalvis plant growing in the Khorezm region. Among the identified amino acids, the highest quantitative indicator was glycine, its amount was 5.80 mg/g. It was also noted that lysine — 3.70 mg/g, threonine — 2.85 mg/g, arginine — 1.76 mg/g, serine — 1.57 mg/g and valine — 1.48 mg/g.

The results of the analysis show that the underground stem parts of the Cistanche trivalvis plant are a natural raw material rich in biologically active amino acids. In particular, the high content of glycine indicates that this plant can have a positive effect on the functioning of the nervous system, participate in metabolism, and play an important role in antioxidant defense mechanisms.The relatively high content of lysine is also important. Lysine is one of the important amino acids involved in the processes of protein biosynthesis, immune system activity, and the formation of enzymes and hormones in the body. Threonine is involved in protein metabolism, liver function, and immunological processes. Arginine plays an important biological role in vascular function, nitric oxide metabolism, and regeneration processes.In addition, the presence of important non-essential amino acids such as valine, methionine, tryptophan, phenylalanine, and isoleucine in the plant allows us to evaluate the Cistanche trivalvis plant as a promising raw material for the production of biologically active food additives.The total amount of amino acids was calculated as follows: Total amino acid content = 22.95 mg/g.

This indicator indicates that the underground stem parts of Cistanche trivalvis contain a sufficient amount of amino acid complex. In particular, the relatively high content of biologically active amino acids such as glycine, lysine and threonine justifies the potential use of this plant in the pharmaceutical, food biotechnology and production of biologically active additives.

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