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Comparative Analysis of the Elementary and Amino Acid Composition of *Nepeta olgae Regel L*. Plants Growing in Uzbekistan

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This article for the first time presents the results of the study of qualitative and quantitative elemental and amino acid composition of the aboveground part of the plant *Nepeta olgae Regel* (L.) taken in the territory of Chust and Kosonsai districts (from the slopes of Gova and Kosonsai mountains) of Namangan region during the period before and during flowering (May-June, 2021-2022).

The use of instrumental analysis of high-throughput energy dispersive X-ray fluorescence spectrometry, allowed to establish 20 mineral elements in the plant *Nepeta olgae Regel* (L.), among which to vital 9 elements and 3 to conditionally necessary.

The amino acid composition of the plant *Nepeta olgae Regel* (L.) was studied by high performance liquid chromatography (HPLC) and 17 compounds were identified. Of these, 8 were substitutable and 9 essential amino acids.

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1. INTRODUCTION

The main source for the search of new medicinal plants are the annals of folk medicine. From this point of view, of particular interest are plants of Lamiaceae family (lat. Lamiaceae). Uzbekistan is rich in such medicinal plants. Genus Nepeta L. is one of them.

The genus Nepeta L. (Lamiaceae) includes perennial or annual herbaceous, small shrubs, rarely trees. This genus has a wide distribution in temperate regions of Asia, Europe, and North Africa. *Nepeta olgae Regel* L. is a 30-80 cm tall perennial plant. The root is long and taprooted. The multi-branched, erect branches collected in caudex are covered with white, fluffy, dense hairs. Leaves are 1.3-4 cm long, broadly ovate, heart-shaped or rounded at the base. Leaf edges are finely dentate, the front side of the leaf is green, while the back side is covered with white hairs. The leaves are arranged on the stem in a supronate fashion, and the upper leaves are sessile. Deciduous leaves are 0.5-1 cm long; the lower leaves are ovate, the rest are ribbon-shaped. Inflorescences are 9-15 cm long, on pedicels, gathered in inflorescences, with 8-40 flowers. Petals are 1.5-2 times shorter than the calyx, lanceolate or needle-shaped, covered with white pubescence. The inflorescence is short, 1-2 mm long. Calyx leaves are 6-8 mm long, funnel-shaped, pubescent, lip 1.1-1.2 cm long, white, sometimes reddish, 2-2.5 mm long, 0.5-0.8 mm in diameter, inverted ellipsoidal in shape, 2n=18 chromosomes [1-4].

Some species of Nepeta L. are widely used in traditional medicine due to its diuretic, and also antispasmodic, anti-asthmatic, sedative and antiseptic properties [5-10].

Previously, we studied the composition of essential oils and antioxidant activity of *Nepeta olgae Regel* (L.) plant extracts growing in Uzbekistan, on the slopes of Gova and Kasansai mountains [11-15] in Namangan region.

Most medicinal plants (genus Nepeta L.) contain 1% essential oil, which has antioxidant activity and shows anti-ischemic effect in cerebral and cardiac ischemia [8-11].

Amino acids are one of the most important units in living organisms and plants. Amino acids perform a number of specific functions in the living organism: they are the building material of biologically important compounds, participate in the maintenance of nitrogen balance, have immune-active properties, and also have a hypolipidemic effect, etc. [6-10].

Natural amino acids have a higher physiological activity compared to their synthetic analogues, because in plant raw materials they are easily assimilated by the human body in complexes and in biologically accessible concentrations [7-9].

Amino acid preparations as effective drugs are prescribed for the treatment of many pathological processes. When there is a lack of amino acids, many vital processes in the body are slowed down. The study of the amino acid composition of plants is of scientific and practical importance [9-10].

Based on the above facts, the study of the content of free amino acids and macro- and microelement composition in plants is very relevant.

The purpose of the present study was a comparative analysis and study of the qualitative composition and quantitative content of amino acids and mineral components in the above-ground part of plants *Nepeta olgae Regel* (L.) taken in the territory of Chust and Kosonsai districts (from the slopes of Gova and Kosonsai mountains) of Namangan region before and during flowering.

2. MATERIALS AND METHODS

The material for the study was air-dried shredded grass *Nepeta olgae Regel*(L.), harvested, on the territory of Chust district from the slopes of Gova and Kosonsai mountains of Namangan region in the period before and mass flowering of the plant (in May-June, 2021-2022).

To determine the ash content, the plant was crushed and held at 600˚C for 3 hours. The ash remaining after calcination was cooled and weighed on analytical scales. For the qualitative and quantitative content of minerals of *Nepeta olgae Regel*(L.) plants, the method of atomic adsorption spectral analysis was used. Elemental analysis was carried out in the "Central

Laboratory" of the State Committee for Geology of the Republic of Uzbekistan. High-performance energy dispersive X-ray fluorescence spectrometer from Japan, Rigaku NEX CG EDXRF Analyzer Polarization in set - 9022 19 000 0 was used for ash analysis.

Biological substances of *Nepeta olgae Regel* (L.) plants were extracted by extraction in various solvents.

The total amount of extractive (biologically active) substances was determined by the method of subtraction according to the formula: X = (A-V)-100/m.

Where A - mass of transparent extract, V - mass of solvent, m - mass of raw material in grams, X - yield in % of total extractive substances.

Extracts were identified by chemical test, thin layer chromatography.

Precipitation of proteins and peptides of the aqueous extract was performed in centrifuge beakers. For this purpose, 1 ml (exact volume) of 20% trichloroacetic acid CCl_3COOH (TCA) was added to 1 ml of the test sample. After 10 min, the precipitate was separated by centrifugation at 8000 rpm for 15 min. After separating 0.1 ml above the precipitate, the liquid was lyophilically dried. The hydrolysate was evaporated, the dry residue was dissolved in triethylamine-acetonitrile-water mixture (1:7:1), and dried. This operation was repeated twice to neutralize the acid. Reaction with phenylthioisocyanate produced phenylthiocarbamyl derivatives (PTC) of amino acids by the method of Steven A., Cohen Daviel [16]. The amino acid derivatives were identified by HPLC.

HPLC conditions: Agilent Technologies 1200 chromatograph with DAD detector, 75x4.6 mm Discovery HS C_{18} column. Solution A: 0.14 M CH_3COONa + 0.05% TEA pH 6.4, B:CH_3CN. Flow rate 1.2 ml/min, absorbance 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.

3. RESULTS AND DISCUSSION

Nepeta L. is rich in secondary metabolites such as amino acids, macro and trace elements, monoterpenes, sesquiterpenes, diterpenes, triterpenes, flavonoids, and other phenolic compounds [6-18].

One of the main indicators for the commodity grower is the amount of ash and extractive substances in the plant. On this basis, we determined the total ash content of plants *Nepeta olgae Regel* (L.) based on the requirements of State Pharmacopoeia (GF) GOST 22839, GOST 24027.2 [19]. For this purpose above-ground part of medicinal plant *Nepeta olgae Regel* (L.) was taken in the territory of Namangan region during flowering period (May-June, 2021-2022). The received results are presented in the Table 1.

The results of Table 1 show that the ash content of the studied plants is from 6.65% to 8.2%, which corresponds to the requirements of GF.

To determine the amount of biologically active substances in the plant the amount of extractive substances was determined. Extracts of *Nepeta olgae Regel* (L.) plants were isolated with various solvents. The results are presented in Table 2.

From the results of the experiment (Table 2), it was clear that the use of ethanol as a solvent resulted in the highest yield.

The extracts were identified by chemical test, thin-layer chromatography, and HPLC: To 3 ml of the extract/fraction, 1 ml of 10% NaOH was added. And the yellow staining observed in each extract/fraction tested indicated the presence of flavonoids. Chemical analysis of *Nepeta olgae Regel* (L.) leaf extracts revealed the presence of flavonoids, coumarins, and glycosides in extracts with methanol, and the absence of alkaloids and tannins.

Table 1. Mass and quantity of ash

No	Plant names		Weight of plants (grams)	Amount of ash (grams)	% indicator
1	*Nepeta olgae Regel* (*L.*) (above ground part)	Slopes Gova	63,49	4,22	6,65
		Slopes of Mt. Kosonzaya	60,14	4,31	7,16
2	*Nepeta olgae Regel* (*L.*) (roots)	Slopes Gova	55,83	4,19	7,5
		Slopes of Mt. Kosonzaya	54,51	4,47	8.2

Table 2. Amount of biologically active substances above ground parts (dry weight, grams) of the plant *Nepeta olgae Regel* (L)

No	Name of the plant and where it grows	Solvents			
		Ethanol, 70 %	Methanol,	Chloroform	Ethylacetate
1	*Nepeta olgae Regel (L.)* from the slopes of Mount Gova	65,0	17,24	10,12	21,01
2	*Nepeta olgae Regel (L.)* from the slopes of Mount Kosonzaya	61,8	18,14	10,09	22,32

Analysis by thin-layer chromatography was performed on a Merck silica gel-coated plate. A solvent mixture of chloroform:methanol in a ratio of 20:1 was used, where the separated components were determined in a wide range of Rf values. The components were visualized in ultraviolet/visible light (254 and 366 nm) and sprayed with the following reagents to detect stains of different groups: Dragendorf's reagent for alkaloids, potassium hydroxide solution in methanol for coumarins, alcoholic aluminum chloride solution for flavonoids, and sulfuric acid for steroids and terpenes.

Thin-layer chromatographic analysis of the 70% methanol extract yielded three spots with Rf values of 0.24, 0.47, and 0.67. Three spots gave a positive test for flavones and flavonols, but negative for isoflavones, anthocyanins, flavonones, and leucoanotacans when sprayed with conc. H_2SO_4 and a 10% - NaOH solution, respectively.

Phenolic compounds in the plant *Nepeta olgae Regel* (L.) were first identified by HPLC (extractant-alcohol, methyl 70%) extracts, where rutin, quercetin, kaempferol and myricetin (flavanoids), sitosterol, daucosterol, coumaric acid, gallic acid, caffeic acid and ascorbic acid dominate. It is shown that plants *Nepeta olgae Regel* (L.) are valuable sources of phenolic compounds and can be recommended as promising for practical application.

Nepeta olgae Regel (L.) (63.49 g of the above-ground part from the slopes of Mount Gowa and 60.14 g from the slopes of Mount Kosonsaya, 55.83 g of the roots from the slopes of Mount Gowa and 54.51 g from the slopes of Mount Kosonsaya) were taken for elemental analysis. The extracted mass was heated in a muffle furnace at 6000 C. The ash composition was checked by elemental analysis at the atomic absorption analysis unit (elemental ash analysis was conducted in the "Central laboratory" of the State Committee for Geology of the Republic of Uzbekistan).

As a result of elemental analysis of plant ash composition we determined the content of 20 macro- and microelements.

As a result of studying elemental composition of plants *Nepeta olgae Regel* (L.) the following data were obtained, which are presented in Table 3.

It was found that the plant *Nepeta olgae Regel* (L.) is rich in mineral elements, of which 9 elements are vital (essential) and 3 are conditionally essential (conditionally essential). Contained bioelements in the order of their quantitative content form the following series.

1. Roots - the slopes of Mount Gov: Si=Ca>K>Mg>Al=Na>P>Fe>Ti>Mn>Ba>Sr >B>Zr=V=Cu>Be.
2. Roots are the slopes of Mount Kosonsai: Si>Ca>K>Mg>Al=Na>P>Fe>Ti>Mn>Ba>Sr >B>Zr=V=Cu>Be.
3. The above-ground part is the slopes of Mount Gova: K>Si>Al>Ca>Mg>P>Fe>Na>Zr>Ti=Sr>Mn >Ba>Zn>B>Cu>Be>Cr=V>Pb.
4. The above-ground part is the slopes of Mount Kasansaya: K>Si>Al>Ca=Mg>P>Fe>Na>Zr>Ti=Sr=Mn >Ba>Zn>B>Cu>Be>Cr=V>Pb.

The study of accumulated minerals revealed that silicon, calcium, potassium, magnesium, sodium, aluminum, phosphorus, iron are accumulated in significant amounts in the roots. Potassium, silicon, aluminum, calcium, magnesium, phosphorus, iron, sodium are accumulated in the above-ground parts from the slopes of the Gowa and Kasansay mountains.

Table 3. Macro- and micronutrient composition of plants *Nepeta olgae Regel (L.)*

№	Elemental composi-tion	Roots				Above ground part			
		The slopes of the mountain Govy		The slopes of mount Kosonzaya		The slopes of the mountain Govy		The slopes of mount Kosonzaya	
		%	mg/g	%	mg/g	%	mg/g	%	mg/g
1	Si**	15	150	13	130	15	150	12	120
2	Al	4	40	4	40	8	80	5	50
3	Ca*	15	150	14	140	20	200	22	220
4	Na*	4	40	4	40	6	60	5	50
5	K*	10	100	8	80	10	100	7	70
6	Fe*	1	10	1,2	12	1	10	1	10
7	Mg*	5	50	4,6	46	5	50	4	40
8	P*	1,5	15	1,7	177	4	40	2	20
9	Ba	0,06	0,6	0,04	0,4	0,06	0,6	0,03	0,3
10	Sr**	0,03	0,3	0,02	0,2	0,03	0,3	0,03	0,3
11	B	0,01	0,1	0,01	0,1	0,02	0,2	0,02	0,2
12	Mn*	0,1	1	0,1	1	0,06	0,6	0,03	0,3
13	V	0,004	0,04	0,005	0,05	0,006	0,06	0,002	0,02
14	Ti**	0,3	3	0,2	2	0,3	3	0,3	3
15	Cr	0	0	0	0	0,001	0,01	0,001	0.01
16	Cu*	0,004	0,04	0,005	0,05	0,005	0,05	0,003	0,03
17	Pb	0	0	0	0	0,004	0,04	0,005	0,05
18	Zn*	0	0	0	0	0,002	0,02	0,001	0,01
19	Be	0,0002	0,002	0,0002	0,002	0,0002	0,002	0,0002	0,002
20	Zr	0,006	0,06	0,005	0,05	0,01	0,1	0,01	0,1

** - essential elements,*
*** - conditionally essential elements*

The content of silicon and calcium in the roots of plants from the slopes of the Gowa and Kasansay mountains is at the same level (150 mg/g). Calcium is characterized by its participation in the formation and maintenance of functionality of the musculoskeletal system, participation in the formation and transmission of nerve impulses, activation of some enzymatic systems, and the release of hormones. The biological role of silicon in the human body is not fully elucidated, but it is believed that it is necessary for the mineralization of bone tissue, synthesis of collagen, has a positive effect on the skin, hair and nails, contributes to the prevention of atherosclerosis and Alzheimer's disease. Silicon functions are also associated with the formation of connective tissue, its role is to participate in chemical reactions of bonding small subunits of fibrous tissues of the body, collagen and elastin, together giving them elasticity and strength [20].

The highest content is noted for potassium ≈200-220.00 mg/g, in the above-ground part of the plant from the slopes of Gowa and Kasansay mountains, which is an integral component in the passage of nerve impulses in the living organism, control of muscle contraction, including the heart, and the maintenance of blood pressure in a normal state [21].

The proportion of magnesium was ≈60 mg/g; in the organism it acts as a cofactor in more than 300 enzymatic reactions. Magnesium is involved in protein biosynthesis, fatty acid oxidation, energy production, and glucose metabolism [22].

The content of the toxic element lead in the plant *Nepeta olgae Regel (L.)* was 0.002 mg/g. The given value does not exceed the limit of maximum allowable concentration (C_{mac} = 0,01mg/g) that in its turn corresponds to the requirements of SanPiN 2.3.2.1078-01 from 2002 regarding allowable quantitative content of heavy metals in tea [SanPiN 2.3.2.1078-01., 2002].

The established mineral composition allows the use of this species in the treatment of mineral deficiency in the body.

Next, we studied free amino acids in the above-ground part of aqueous extracts of the plant.

Amino acids are immunomodulators and antidepressants (glutamine, tyrosine), reduce

triglycerides in blood (lysine), etc. It is also important that amino acids are actively involved in the synthesis and utilization of vitamins. Such amino acids include valine, lysine (involved in the formation of carnitine), tryptophan (involved in the formation and utilization of B complex vitamins), glutamine (involved in the synthesis of riboflamines, folic acid). The use of herbal supplements usually does not cause side effects.

To study the amino acid composition in the period before and during flowering of *Nepeta olgae Regel (L.*) plants, aqueous extracts were taken. Qualitative reactions were performed to confirm the presence of amino acids [23].

For this purpose, 50 ml of the aqueous extract was evaporated under vacuum to 25 ml. After that, 10 ml of the extract was taken for analysis and mixed with an equal volume of 0.1 % freshly prepared ninhydrin solution and gently heated. When cooled, red-violet staining was observed, indicating the presence of amino acids. Also qualitative detection of amino acids was performed by TLC on "Silufol" plates in solvent systems n-butanol-acetic acid-water in the ratio 12:3:5. Chromatograms were developed with 0.25 % alcoholic solution of ninhydrin. Amino acids appeared as red-violet spots.

Precipitation of proteins and peptides from the aqueous extract of the samples was performed in centrifuge beakers. For this purpose, trichloroacetic acid (TCA) was added to the sample. The precipitate was separated by centrifugation from the supernatant, and the precipitate was lyophilically dried. The hydrolysate was evaporated, the dry residue was dissolved in a triethylamine-acetonitrile-water mixture (1:7:1), and dried. Reaction with phenylthioisocyanate produced phenylthiocarbamyl derivatives (PTC) of amino acids by the method of Steven A., Cohen Daviel [24].

PTC-amino acids were identified by HPLC, the results of which are presented in Table 4.

The data obtained (Table 4) show that 17 amino acids were found in the plant *Nepeta olgae Regel* (L.); from the slope of Mount Gowa during the period before flowering, free amino acids in aqueous extracts are higher than from the slope of Mount Kosonsaya during the flowering period. Among them, the most predominant amino acids are: cysteine (Mount Gowa slope 115.0656 mg/g, Mount Kasansaya slope 118.11243 mg/g in the pre-bloom period), in the blooming period (80.59836 mg/g from Mount Gowa slope and 76.210036 mg/g from Mount Kasansaya slope) respectively. Glutamic acid from the slopes of Mount Gowa and Mount Kasansaya during the pre-bloom period (18.24874 mg/g and 17.342114 mg/g), and from the slopes of Mount Gowa and Mount Kasansaya during the bloom period (4.180328 mg/g, 4.335472 mg/g), respectively, related to the substituted amino acids. Ten compounds were classified as substitutable amino acids and their proportion was 73 % and 74 % before flowering and 72 % and 69 % during flowering from Gowa and Kasansaya slopes, respectively, of the total amount of amino acids contained. As for the essential amino acids, which are of special pharmacological value, 8 compounds which preserve immune functions were identified with a content of was 15 % before flowering, 20 % in the flowering period from the slopes of Gowa and Kasansaya mountains respectively. The amino acid of scientific interest is valine, which is a branched-chain amino acid (4.6 % before and during flowering on the slopes of Gowa and Kosonzaya, respectively), which is not synthesized in the body and is an essential amino acid with a branched-chain.

An aqueous extract of plants from the slopes of the Gowa and Kasansaya mountains in the period before flowering is rich in the following amino acids: cis, glu, hys, arg, pro, val, gli, asp, sul, ala. Extracts of *Nepeta olgae Regel* (L.). plants collected from the slopes of the Gowa Mountains and from the slopes of Mount Kosonzaya during the flowering period are rich in cis, glu, val, gli, pro, arg, ser, tiro, ala.

It should be noted that free amino acids are rich in extracts of plants from the slopes of Mount Gowa before flowering. The content of free amino acids asp and glu from the slopes of Mount Gowa and from the slopes of Mount Kosonsai during the flowering period was significantly reduced to 4-5 times less than during the period before flowering. Also, the essential amino acids were 36.6% before flowering on the slopes of Mount Hova and 35.0% on the slopes of Mount Kosonsaya, on the slopes of Mount Hova during flowering -67.6% and on the slopes of Mount Kosonsaya was -44.8%.

The health organization has determined the human body's daily requirement for amino acids to ensure its balance.

Table 4. Content of free amino acids in aqueous extracts of *Nepeda olgae Regel (L.)*

Name of amino acids	*Nepeda O.* protein hydrolysis in the period before flowering		*Nepeda O.* protein hydrolysis during flowering	
	The slopes of the mountain Gova	The slopes of Mount Kosonzaya	The slopes of the mountain Govy	The slopes of Mount Kosonzaya
	Concentration mg/g			
Asparagine acid*	11,65804	10,85601	2,624647	3,122211
Glutamic acid	18,24874	17,342114	4,180328	4,335472
Serine	10,27902	11,23102	7,662628	6,262641
Glycine*	12,14171	10,94811	9,672638	8,234413
Asparagine	0	0	0	0
Glutamine*	0	0	0	0
Cysteine*	115,0656	118,11243	80,59836	76,210036
Threonine*	3,394428	2,314318	2,668622	2,221034
Argenine***	14,54696	13,65882	8,233917	6,846953
Alanine*	9,039418	8,336422	6,963536	5,233821
Proline*	14,37114	12,67003	9,575243	10,677843
Tyrosine*	7,181934	8,210331	7,293257	7,369522
Valine**	12,22028	10,227782	10,20219	10,492130
Methionine**	3,00758	4,660883	2,298603	2,132410
Isoleucine**	6,124535	5,426778	6,682156	5,292142
Leucine**	7,266769	7,390112	6,869699	5,165212
histidine***	15,89016	14,69321	15,17128	13,202235
Tryptophan**	0	0	0	0
Phenylalanine**	5,512743	5,5524771	6,272755	4,631728
Lysine**	4,525551	5,122221	5,497996	4,384912
Total	270,4746	266,7531	192,4679	175,8147
Σ*	197,9856	197,7065	138,7728	121,4460
Σ**	42,0519	40,6945	40,4920	34,31957
Σ***	30,4371	28,35203	23,4052	20,04188
Σ*/ Σ**+ Σ***	2,73125	2,86338	1,47792	2,23404

** - replaceable*
*** - indispensable (not synthesized in the body)*
**** - conditionally essential*

The highest requirement is for glutamic acid 6,000 mg/day, the lowest for histidine 2,000 mg/day.

Today, experts believe that a person needs to consume more substitutable acids - 56.9 g per day. And the emphasis should be on glutamine, asparagine, and serine. Only 22.6 g of essential amino acides are needed per day, of which the most important are leucine, phenylalanine, and lysine. Phenylalanine participates in the formation of collagen in the body, improves memory, attention, and mood. It is also essential for immune function.

Valine refers to BCAAs (branched-chain amioasids). BCAAs include three amino acids, val, leu, and il. These amino acids are metabolized in the muscle, not in the liver. In fact, up to 90% of amino acids can be absorbed by BCAAs during the first three hours after a meal. Muscle hunger for BCAAs is why they are successfully added to athletes' diets [25].

The above confirms the importance of discovering plants that contain many branched amino acids and have both medicinal and nutritional value.

Proline is the main component of collagen and in the presence of vitamin C helps wound healing, promotes good joint function, participates in the formation of biologically active peptides and can be used in the treatment of injuries.

The first amino acid in the plant *Nepeta olgae Regel* (L.) is cysteine. Cysteine aids in digestion by participating in the process of reamination. It

helps neutralize some toxic substances and protects the body from the damaging effects of radiation. One of the most powerful antioxidants, its antioxidant effect is enhanced by the simultaneous intake of vitamin C and selenium [25].

4. CONCLUSIONS

The content of macro- and microelements in plants *Nepeta olgae Regel* (L.) was studied and the content of 20 macro- and microelements was noted. Large amounts of Si, Ca, K, Mg, Na, Al, P, Fe were found in plant roots, and K, Si, Al, Ca, Mg, P, Fe, Na in the above-ground part. Toxic elements such as Pb are not detected in the roots of plants, and in the above-ground part Pb is present in insignificant amounts.

The amino acid composition of *Nepeta olgae Regel* (L.) was studied for the first time. In them 17 amino acids, including 9 essential, of which 7 essential and 2 conditionally essential amino acids were found. The total content of amino acids was found to be 270.4746 mg/g before flowering and 192.4679 mg/g during flowering. Cysteine, glutamic acid, histidine, proline, arginine, glycine, valine, aspartic acid, serine and alanine prevail in the plant. Aspartic acid, glutamine, and tryptophan were found to be absent from the plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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