

Determination of amino acids of some plants from *Gentianaceae* family

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Abstract

Medicines from plants are widely used in the complex treatment of different diseases every day. Therefore, theoretical and practical interest is the in-depth study of the herb of perspective plants like *Centaurium erythraea* Rafn. and *Gentiana cruciata* L. These plants have a long history of usage and interest of people. The aim of the study was to determine the content of amino acids in these plants. The amino acids composition and content in the herb of study species of the family *Gentianaceae* determined by the HPLC method. The results of the research revealed that the raw material of *Centaurium erythraea* Rafn. and *Gentiana cruciata* L. contains free and bound amino acids. Sixteen free and seventeen bound amino acids were identified in the herb of *Centaurium erythraea* Rafn. The herb of *Gentiana cruciata* L. contained thirteen free and fifteen bound amino acids. L-glutamic acid, L-arginine, L-aspartic acid, and L-cystine were predominant of *Centaurium erythraea* Rafn. herb. Amino acids L-lysine, L-serine, L-aspartic acid, and L-phenylalanine were present in the herb of *Gentiana cruciata* L. in the greatest amount. The metabolic processes in which these amino acids are involved connected to the medicinal properties of the study plants according to their use in official or nontraditional medicine.

Keywords

Centaurium erythraea Rafn., *Gentiana cruciata* L., herb, amino acids, HPLC

Introduction

The use of medicinal plants with therapeutic properties is as old as human civilization. Herbal, mineral and animal products have been the main source of drugs for the treatment and prevention of various diseases for a long time (Rates 2001; Budniak et al. 2020; Marchyshyn et al. 2021b). The use of plant raw materials is one of the areas of modern pharmaceutical science in the production of herbal medicines (Slobodianiuk et al. 2020). The main effect of using the medicinal plant is the regulation of metabolic disorders, as plant metabolites are close to the metabolites of the human body (Darzuli et al. 2019; Slobodianiuk et al. 2021a). Nowadays, the search for plants with a history of constant use and small side effects is of interest to our

society (Huzio et al. 2020; Kurylo et al. 2020; Darzuli et al. 2021).

Species belonging to the *Gentianaceae* family are important because of their pharmacological use, namely the formation of very rich and specific secondary metabolites (Rybczyński and Wójcik 2019). The *Gentianaceae* family consists of 99 genera and about 1736 species, and it is distributed worldwide (Tomiczak et al. 2019). Many *Gentians* species are widespread in the Pyrenees, Himalayas and alpine mountains throughout Eurasia. The *Gentianaceae* family includes the following genera: *Centaurium* Hill., *Gentiana* L., *Swertia* L., *Menyanthes* L., etc (Budniak et al. 2021a). Plants belonging to these genera are a source of important pharmacologically active phytochemicals that have antimicrobial, antipyretic, anti-inflammatory, cyto-

and hepatoprotective, stomachic, analgesic, and gastro-protective properties (Singh 2008; Tomiczak 2016). Many species of *Gentianaceae* family are very widely used for pharmaceutical purposes, namely *Centaurium erythraea* Rafn. and *Gentiana cruciata* L.

Centaurium erythraea Rafn., commonly known as centaury, is a pharmacologically important medicinal plant from the *Gentianaceae* family. Centaury is a biennial, sometimes annual, herb that grows in humid and semi-arid areas throughout the northern hemisphere (Guedes et al. 2019; Simonovic et al. 2021). *Centaurium erythraea* Rafn., an official plant that is part of the Ukrainian Pharmacopoeia, the European Pharmacopoeia, the British Herbal Pharmacopoeia, and many others (Stoiko and Kurylo 2018).

The aboveground parts of common centaury are a abundant source of various bioactive specialized metabolites, including phenolic compounds, terpenoids, steroids and alkaloids (Šiler and Mišić 2016). The most abundant bioactive specialized metabolites, secoiridoids and xanthones, exhibit antidiabetic, antioxidative, hepatoprotective, antibacterial and antifungal activities, and are commonly used for gastrointestinal disorders treating, appetite stimulation, digestion issues regulation, against fever, anemia, anorexia, rheumatism, hypertension and many other conditions. The prevailing monoterpenoids of the iridoid type in *Centaurium erythraea* Rafn. are secoiridoid glycosides sweroside, gentiopicrin and swertiamarin (Filipovic et al. 2019).

Centaurium erythraea is a plant used in traditional medicine for some cardiovascular disorders, namely hypertension (Chda et al. 2020). It has been used in traditional human medicine as a gastric, digestive, sedative, antipyretic and depurative agent (Mihaylova et al. 2019). Centaury is traditionally given as a bitter tonic to increase gastric secretions, to relieve dyspeptic discomfort and loss of appetite. *Centaurium erythraea* is part of some cosmetics and toiletries due to its soothing and astringent properties for allergies (Stoiko et al. 2017).

Some *Gentiana* species are endangered plants. They include *Gentiana cruciata* L. But modern biotechnological methods offer alternative approaches to traditional cultivation methods, leading to rapid micropropagation of *Gentiana cruciata* L (Budniak et al. 2021a). The *Gentiana cruciata* herb and roots are a source of secoiridoid glycosides, such as sweroside, swertiamarin and gentiopicoside (Szucs et al. 2002; Tomiczak 2020). Some pharmacological applications have been identified in *Gentiana cruciata* L., namely anticholinesterase, antigenotoxic, antioxidant and antimicrobial activities (Olennikov et al. 2019). Its root has been used in folk medicine for stomachic and sedative effects. In addition, *Gentiana cruciata* roots stimulate production of white blood cells (Hayta et al. 2011).

However, few studies have focused on plants' primary metabolites and the relationship with their therapeutic properties. It was observed that the experimental content of amino acids in *Centaurium erythraea* Rafn. and

Gentiana cruciata L. is absent. Studies of the compounds formed by plants as a result of defense mechanisms allow us to understand the molecular mechanism involved in their medicinal properties. Thus, the aim of our study was to determine the amino acid content of *Centaurium erythraea* Rafn. and *Gentiana cruciata* L.

Material and method

Plant materials

Centaurium erythraea Rafn. herb was collected in Ukraine, on the outskirts of Zboriv (Ternopil region) during the flowering period in July 2017. The herb of *Gentiana cruciata* L. was collected in Western Ukraine, at the territory of Volove, Ternopil region during the flowering period in 2017. The herbs of the study objects was authenticated by professor Svitlana Marchyshyn (TNMU, Ternopil, Ukraine). A vouchers specimens of *Centaurium erythraea* Rafn. no. 135 and *Gentiana cruciata* L. no. 133 are kept at the Department of Pharmacognosy and Medical Botany, TNMU, Ternopil, Ukraine. The study plants materials were dried using the conventional method and stored in paper bags in a dry place (Husak et al. 2018; Marchyshyn et al. 2021a; Slobodianiuk et al. 2021b).

Chemicals and standards

Only reagents of known analytical grade and distilled water or demineralized water or equivalent purity water are used. Standards of amino acids, including L-aspartic acid, L-histidine, L-arginine, L-lysine, L-alanine, L-proline, L-isoleucine, L-tyrosine, L-valine, L-glutamic acid, L-cystine, L-methionine, L-serine, L-threonine, L-leucine, L-phenylalanine, Glycine all these amino acid standards were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA), were of analytical grade (> 99% purity).

Derivatizing agents o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) were purchased in Merck. Acetonitrile (ACN) and hydrochloric acid (HCl) were from Sigma-Aldrich.

Sample preparation, HPLC determination of amino acids

The amino acids composition of *Centaurium erythraea* Rafn. and *Gentiana cruciata* L. herbs are determined by HPLC method with a pre-column derivatization FMOC and OPA.

Reference solutions of free amino acids have been made with distilled water at 0.03 M concentrations of each (weighed with analytical accuracy), stored in the refrigerator and further diluted before use, in every second day.

HPLC analysis of amino acids was conducted using Agilent 1200 (Agilent Technologies, USA). Samples were analyzed using a column length Zorbax AAA – 150 mm, inner diameter – 4.6 mm, the diameter of sorbent grain

3 μ (Hypersil ODS (prepared by BST, Budapest, Hungary)). Mobile phase A – 40 mM Na_2HPO_4 , pH 7.8; mobile phase B – $\text{CH}_3\text{CN}:\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (45:45:10, v/v/v). Gradient separation regime with a constant mobile flow rate of 1.5 mL/min. The temperature of the thermostat column is 40 °C.

The pre-column derivatization was conducted with a help of an automatic programmable regulations using OPA reagent and FMOC reagent. Identification of derivatized amino acids was done by a fluorescence detector (Jambor and Molnar-Perl 2009; Vons et al. 2018; Slobodianiuk et al. 2019). For the extraction of free amino acids of powdered the raw material (to the 130 mg of *Centaurium erythraea* Rafn.; to the 131 mg of *Gentiana cruciata* L.), put in a test flask, 0.1 mol/L water solution of hydrochloric acid was added. The extraction was performed in the ultrasonic water bath at 50 °C for 3 hours. Extraction of bound and free amino acids was performed by adding 2 mL of a water solution of 6 M hydrochloric acid to the powdered of the raw material (to the 131 mg of *Centaurium erythraea* Rafn.; to the 132 mg of *Gentiana cruciata* L.). Hydrolysis was conducted for 24 hours in a thermostat at 110 °C (Slobodianiuk et al. 2021d).

0.5 mL of centrifuged extract was vaporized on a rotary evaporator and then rinse three times with purified water to eliminate hydrochloric acid. The product received was resuspended in 0.5 mL water and filtered through membrane filters from restored cellulose with pores of 0.2 μm . Before recording the samples into the chromatographic column in the automatic software mode, fluorescence derivative amino acids were obtained.

Identification of amino acids was performed according to their hold-up time (using standards as a reference) at 265 nm. The quantitative content of amino acids is calculated from the value of the peak area of the amino acids (Slobodianiuk et al. 2021c).

Validation of the method

The validation method and the analysis procedure of the amino acid content were performed according to validation guides for EURACHEM analytical methods.

To evaluate the sensitivity and linearity of the signal in relation to the concentration, 8 linear calibrations were generated for each amino acid. The calibration curves of each amino acid were plotted in the 0.625–5 $\mu\text{mol}/\text{mL}$ range, and the linearity range for which the correlation coefficient that characterizes the regression line R^2 was obtained, was examined visually.

The mass spectrometer operated in automatic scanning mode (SCAN). The performance parameters of the reference amino acid method, concentrations, limit of detection (LOD), limit of quantification (LOQ) and calibration curves were statistically calculated using Statistica v 10.0 (StatSoft Inc.) program (Budniak et al. 2021b) and are shown in Table 1. All statistical tests were performed at a confidence level of 95% and $k = 2$.

Table 1. Performance parameters of the amino acid determination method.

Amino acid	Correlation coefficient R^2	Limit of detection LOD, $\mu\text{mol}/\text{mL}$	Limit of quantification LOQ, $\mu\text{mol}/\text{mL}$	Retention time
L-aspartic acid	0.9999	0.005437	0.01779	2.46
L-glutamic acid	0.9997	0.001589	0.005342	4.78
L-serine	0.9999	0.004365	0.014549	7.35
L-histidine	0.9989	0.001235	0.005238	8.19
Glycine	0.9994	0.002345	0.004895	8.58
L-threonine	0.9996	0.01817	0.060565	8.75
L-arginine	0.9998	0.010724	0.035745	9.41
L-alanine	0.9987	0.003456	0.013567	9.97
L-tyrosine	0.9996	0.004678	0.014356	11.06
L-cystine	0.9995	0.001592	0.005308	12.19
L-valine	0.9999	0.002622	0.00874	12.96
L-methionine	0.9996	0.01785	0.06543	13.15
L-phenylalanine	0.9995	0.004532	0.01356	14.33
L-isoleucine	0.9999	0.01235	0.05426	14.51
L-leucine	0.9989	0.002897	0.018652	15.12
L-lysine	0.9999	0.096521	0.321737	15.39
L-proline	0.9998	0.003978	0.013261	18.91

Results and discussion

The amino acids profiles of the herb of *Gentiana cruciata* L. and *Centaurium erythraea* Rafn. were determined using HPLC method (Figs 1–4; Table 2).

Sixteen free amino acids were determined in the *Centaurium erythraea* Rafn. herb. Among them, seven amino acids are essential namely L-histidine, L-threonine, L-valine, L-phenylalanine, L-isoleucine, L-leucine, and L-lysine. One acid (L-arginine) is a semi-essential amino acid. The other seven amino acids are nonessential amino acids.

Free L-glutamic acid, L-arginine and L-aspartic acid were present in the herb of *Centaurium erythraea* Rafn. in the greatest amount, their content was 1.56 $\mu\text{g}/\text{mg}$ (1.29% from total content amino acids), 1.16 $\mu\text{g}/\text{mg}$ (0.96%) and 0.92 $\mu\text{g}/\text{mg}$ (0.76%), respectively. L-glutamic acid participates in many metabolic reactions, is a source of glucose, and holds its level in the blood (Okasha et al. 2019). Also, this acid is used as a conjugate because its effectiveness of anticancer medicine is better and reductions of its toxicity in attitude to normal cells. The synthetic amides of this acid show activity concerning Ehrlich ascites carcinoma (Dutta et al. 2013; Slobodianiuk et al. 2021). L-arginine is used by all cells and constitutes 5–7% of the total amino acids in normal human nutrition. This amino acid is used by the body for tissue repairing, protein synthesis, immune cell function, and the urea cycle. Also, L-arginine is converted to citrulline and operates as a vasodilator (Pahlavani et al. 2014). L-aspartic acid is an amino acid present in endocrine glands, nervous tissues, and used as a stock for the food and pharmaceutical industries (D'aniello et al. 2000; Slobodianiuk et al. 2021).

Seventeen bound amino acids were identified of the *Centaurium erythraea* Rafn. herb. Eight amino acids are essential like L-lysine, L-phenylalanine, L-valine, L-methionine, L-isoleucine, L-histidine, L-leucine, L-threonine, and one acid, L-arginine is a semi-essential amino acid.

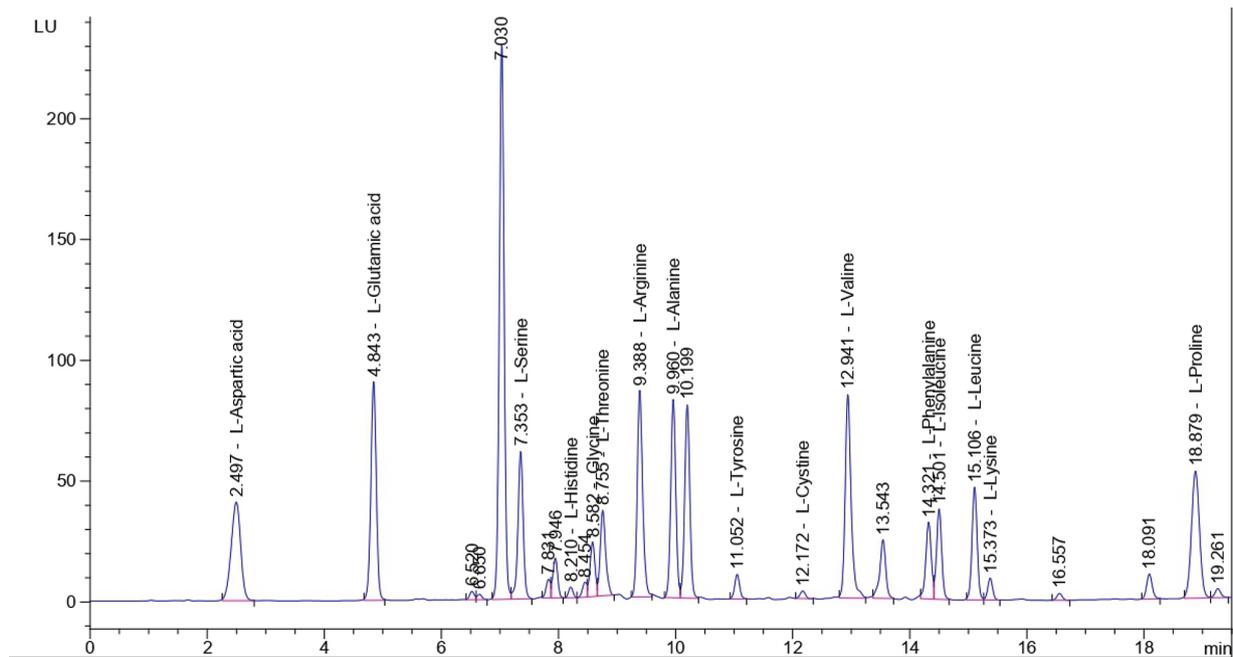


Figure 1. HPLC chromatogram of free amino acids of *Centaurium erythraea* Rafn.

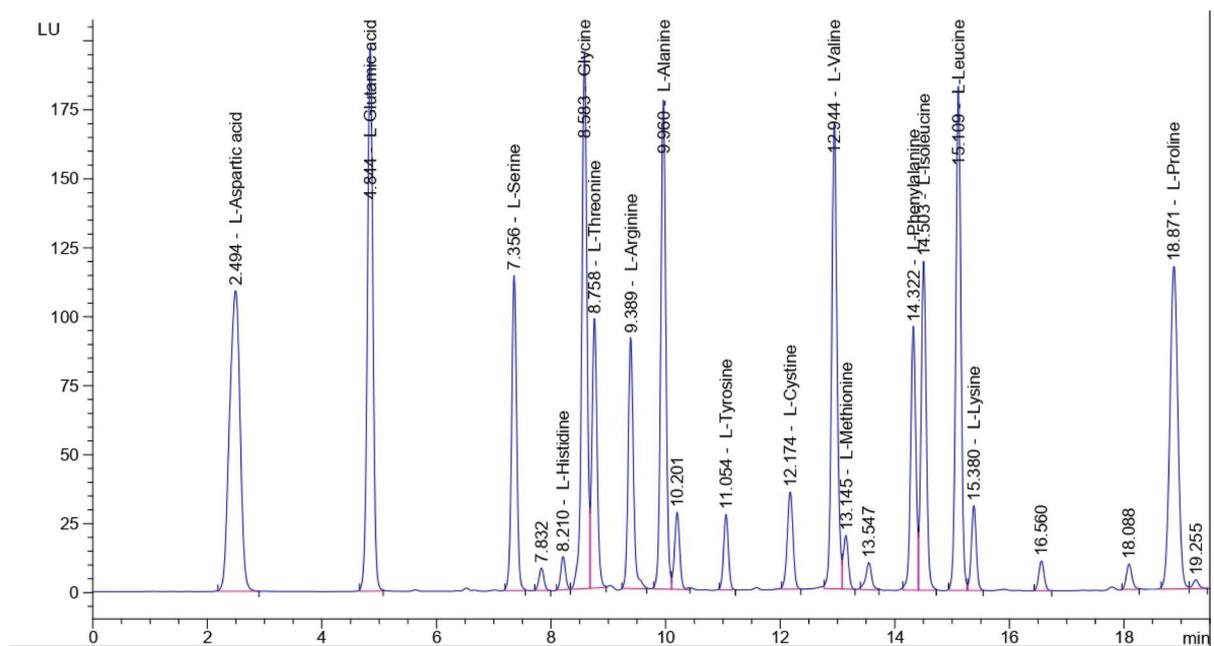


Figure 2. HPLC chromatogram of bound amino acids of *Centaurium erythraea* Rafn.

The other amino acids are nonessential amino acids. L-methionine was found only in bound form. Among the bound amino acids L-cystine, L-glutamic acid, L-aspartic acid were present in the *Centaurium erythraea* Rafn. herb in the greatest amount, their content were 17.30 $\mu\text{g}/\text{mg}$ (14.29%), 16.00 $\mu\text{g}/\text{mg}$ (13.22%), 12.99 $\mu\text{g}/\text{mg}$ (10.73%), respectively. L-cystine ensures elasticity of keratin, and therefore, it is a part of complexes of vitamins for an advance of appearance of the hair, and skin, different additives, and shampoos. L-cystine is also used in complex therapy for the treatment of bronchitis, Alzheimer's disease, diabetes, as well at joint diseases (Blazheyevskiy et al. 2020).

The herb of *Gentiana cruciata* L. contained thirteen free amino acids and fifteen bound amino acids. L-phenylalanine dominates among free amino acids in the studied raw material, its content was 0.18 $\mu\text{g}/\text{mg}$ (0.11%). L-phenylalanine is one of several essential amino acids. Human hemoglobin is one of the richest sources of phenylalanine, it's content 9.6% by weight (Britannica, The Editors of Encyclopaedia 2018). Bound L-lysine, L-serine, and L-aspartic acid were present of the herb in *Gentiana cruciata* L. in the greatest amount, which was 19.61 $\mu\text{g}/\text{mg}$ (11.81%), 19.28 $\mu\text{g}/\text{mg}$ (11.61%), and 18.28 $\mu\text{g}/\text{mg}$ (11.01%), respectively. L-lysine plays a great role in the output of carnitine,

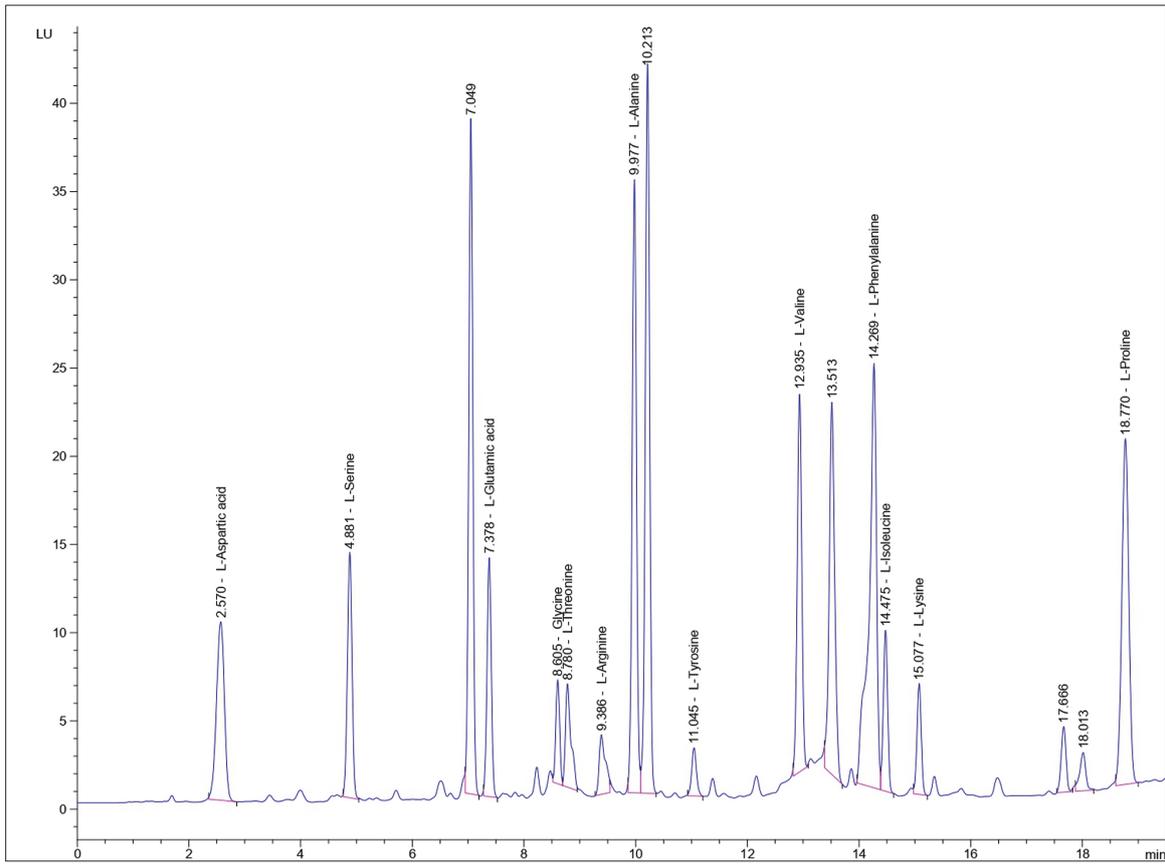


Figure 3. HPLC chromatogram of free amino acids of *Gentiana cruciata* L.

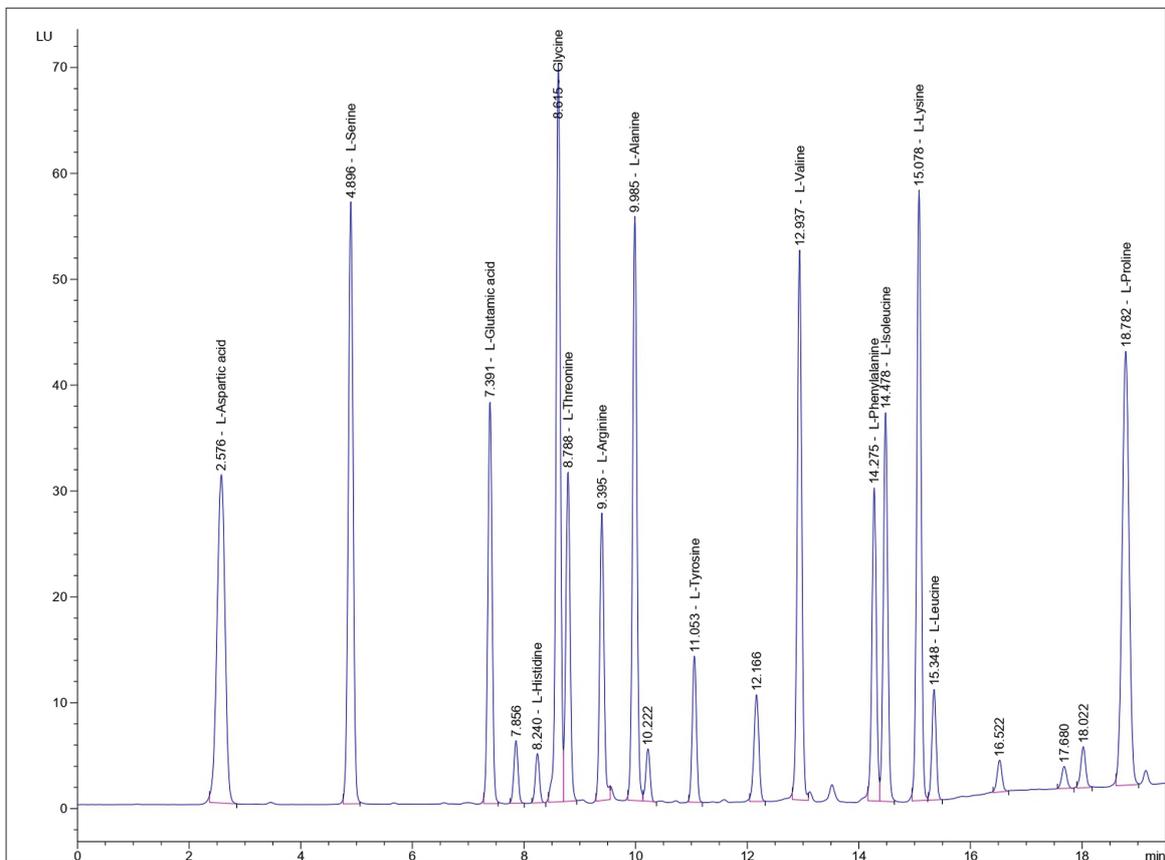


Figure 4. HPLC chromatogram of bound amino acids of *Gentiana cruciata* L.

Table 2. The content of the amino acids in the herb of *Gentiana cruciata* L. and *Centaurium erythraea* Rafn.

The name of the amino acid	The content of the amino acid							
	<i>Centaurium erythraea</i> Rafn. herb				<i>Gentiana cruciata</i> L. herb			
	Free		Bound		Free		Bound	
	$\mu\text{g}/\text{mg}$	%	$\mu\text{g}/\text{mg}$	%	$\mu\text{g}/\text{mg}$	%	$\mu\text{g}/\text{mg}$	%
L-aspartic acid	0.92±0.02	0.76	12.99±0.2	10.73	0.08±0.002	0.05	18.28±0.3	11.01
L-glutamic acid	1.56±0.05	1.29	16.0±0.1	13.22	0.06±0.001	0.04	12.13±0.4	7.31
L-serine	0.54±0.01	0.45	4.40±0.01	3.64	0.06±0.002	0.04	19.28±0.4	11.61
L-histidine	0.13±0.004	0.11	1.83±0.05	1.52	—	—	4.48±0.03	2.70
Glycine	0.13±0.005	0.11	5.68±0.06	4.69	0.01±0.0004	0.01	11.18±0.3	6.73
L-threonine	0.37±0.01	0.31	4.52±0.1	3.73	0.03±0.0009	0.02	8.64±0.2	5.20
L-alanine	0.57±0.02	0.47	5.80±0.1	4.79	0.09±0.004	0.05	10.62±0.3	6.40
L-tyrosine	0.15±0.004	0.12	1.78±0.06	1.47	0.02±0.0007	0.01	5.20±0.07	3.13
L-valine	0.58±0.01	0.48	4.77±0.07	3.94	0.05±0.001	0.03	8.62±0.2	5.19
L-methionine	—	—	1.03±0.03	0.85	—	—	—	—
L-phenylalanine	0.41±0.01	0.34	5.48±0.05	4.52	0.18±0.007	0.11	9.38±0.2	5.64
L-isoleucine	0.34±0.01	0.28	5.21±0.07	4.30	0.03±0.001	0.02	8.93±0.3	5.37
L-leucine	0.43±0.01	0.35	8.16±0.05	6.74	—	—	9.30±0.1	5.60
L-lysine	0.46±0.02	0.38	7.53±0.06	6.22	0.03±0.0008	0.02	19.61±0.4	11.81
L-proline	0.51±0.01	0.42	5.21±0.05	4.30	0.07±0.002	0.04	10.65±0.3	6.41
L-arginine	1.16±0.03	0.96	4.86±0.05	4.01	0.02±0.0007	0.01	9.04±0.1	5.44
L-cystine	0.28±0.01	0.24	17.30±0.3	14.29	—	—	—	—

Note: — — not found.

a nourisher responsible for converted fatty acids into energy and helps to reduce cholesterol (Ghoreyshi et al. 2019; Tufarelli et al. 2020; Slobodianiuk et al. 2021). L-serine plays a major role in cellular proliferation, brain development and function, and necessary for specific functions in the central nervous system (Koning et al. 2003).

Conclusions

The amino acids, present in the herb of *Centaurium erythraea* Rafn. and *Gentiana cruciata* L., were studied by HPLC analysis. The results revealed that the raw material of the study species plants contains significant amounts of free and bound amino acids. Sixteen free and seventeen

bound amino acids were identified of the *Centaurium erythraea* Rafn. herb. The content of L-glutamic and L-aspartic acids are high in the herb of *Centaurium erythraea* Rafn. and *Gentiana cruciata* L. allowing these amino acids to be considered differential markers of the study species plants. Also, the dominant free amino acid in the *Centaurium erythraea* Rafn herb is L-arginine (0.96% from total content amino acids); among the bound amino acids is L-cystine (14.29%). Among bound amino acids in the herb of *Gentiana cruciata* L., the dominant ones are L-lysine (11.81%) and L-serine (11.61%). Among free amino acids, essential amino acid phenylalanine (0.11%) prevails. Character metabolic processes in which these amino acids participate are associated with the therapeutic properties of research plants.

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