STUDY OF *EPILOBIUM ANGUSTIFOLIUM* L. AMINO ACIDS CONTENT BY HPLC METHOD

Halyna Feshchenko, Oleksandra Oleshchuk, Liudmyla Slobodianiuk, Ivanna Milian

The use of plant raw materials is one of the areas of modern pharmaceutical science in the production of herbal drugs. The genus Epilobium counts more than 200 species, many species of which are used in traditional medicine. Among the Epilobium species, Epilobium angustifolium is one of the well-known medicinal plants which have been used worldwide in habitual medicine. There is insufficient information in the literature on the biologically active substances of Epilobium angustifolium L. The presence of three major polyphenol groups: phenolic acids, flavonoids, and ellagitannins were identified in E. angustifolium extracts. Traditionally, the infusion of leaves of this plant could be useful for headaches, cold and gastrointestinal disorder. The Epilobium angustifolium L. as an insufficiently studied plant is a promising object of study, including amino acids composition. To assess the relationship between the production of primary metabolites and their possible therapeutic properties, we analyzed the amino acid profile of the plant Epilobium angustifolium used in traditional medicine. The study of compounds generated by plants as a result of defense mechanisms permits an understanding of the molecular mechanism involved in their medicinal properties.

The aim. *Thus, the aim of the study was to conduct an HPLC analysis of the amino acids of E. angustifolium to establish the prospects for the use of the raw materials in medical and pharmaceutical practice. The results of the current study will be used in further breeding programs aimed to obtain an industrial form of E. angustifolium suitable for pharmaceutical and food applications.*

Materials and methods. *The determination of amino acids composition of Epilobium angustifolium was conducted using Agilent 1200 (Agilent Technologies, USA).*

Results. *The HPLC method identified sixteen free amino acids and seventeen bound amino acids in the Epilobium angustifolium herb. The studies have shown that Epilobium angustifolium L. herb is mainly composed of free amino acids such as L-phenylalanine (1.65 µg/mg), L-glutamic acid (1.51 µg/mg), L-arginine (1.24 µg/mg), L-alanine (0.98 µg/mg) and L-aspartic acid (0.57 µg/mg), which were presents in the greatest amount. The dominant bound amino acids in the studied raw material were L-glutamic acid, L-aspartic acid, L-leucine, and L-alanine, the content of which was 32.37 µg/mg, 10.59 µg/mg, 8.70 µg/mg, and 6.22 µg/mg respectively.*

Conclusions. *Using the HPLC method determined the amino acids in the herb of Epilobium angustifolium L. The concentrations of L-aspartic acid, L-glutamic acid, L-arginine, L-alanine and L-phenylalanine are predominate among free and bound amino acids in the Epilobium angustifolium L. herb. The result shows that Epilobium angustifolium L. is the source of amino acids, so the use of this plant raw material for new remedies is possible in the future*

Keywords: *Epilobium angustifolium L., herb, free amino acids, bound amino acids, HPLC*

1. Introduction

The use of medicinal plants in the prevention and treatment of different diseases is known since old times [1]. In past years, the search for plants with a long-standing history of use and minor side effects is of interest to our society [2]. The basic effect of using the plant is the regulation of metabolic disorders, as plant metabolites are close to the metabolites of the human body [3, 4]. The use of plant raw materials is one of the areas of modern pharmaceutical science in the production of herbal drugs [5].

The genus *Epilobium* counts more than 200 species, among which the most known are *E. angustifolium*, *E. parviflorum* and *E. hirsutum*, generally named Willow Herb, with mention to the willow-like nature of their leaves [6, 7]. Among the *Epilobium* species, *E. angustifo-*

lium is one of the well-known medicinal plants which have been used worldwide in habitual medicine [8, 9]. *Epilobium angustifolium* is called "fireweed" in North America, "maitohorsma" in Finland and "rosebay willow-herb" in Great Britain [10].

Plants belonging to the *Epilobium* genus are a rich source of secondary metabolites. A diverse of polyphenols present in rosebay willow-herb. Three major polyphenol groups have been identified in *E. angustifolium* extracts: phenolic acids, flavonoids, and ellagitannins. Flavonoids consist of flavonol aglycones (kaempferol, quercetin, and myricetin) and flavonoid glycosides, such as kaempferol-3-O-rhamnoside, kaempferol-3- O-arabinofuranoside and quercetin-3-O-galactoside [8, 9, 11]. From *Epilobium angustifolium* extracts several other

oligomeric tannins have been isolated, including oenothein A (trimer), and tellimagrandin I- based heptameric ellagitannins [9].

The biomass of the leaves of *Epilobium angustifolium* contains organic acids, tannins, anthocyanins, carotenoids, flavonoids (quercetin, kaempferol, myricetin), ascorbic acid, pectins, polysaccharides, lignin, coumarins, tannins, sterols, triterpenes, carotene, simple phenols and polyphenolic compounds, glycosides [12, 13].

The quinic acid esters have been detected in *Epilobium angustifolium* using the HPLC–DAD–MS method [14]. Also, cholesterol, campesterol, stigmasterol, β-sitosterolandits glycosides, and esters have been reported from Epilobium angustifolium [10].

Studies by Ukrainian scientists have also shown the presence of amino acids in the aerial part and rhizomes of fireweed. L-alanine and L-phenylalanine were dominant amino acids in the *E. angustifolium* [15].

Fireweed (*Epilobium angustifolium* L.) herb is a well-known plant due to its anti-inflammatory, antibacterial [6], antioxidant, and analgesic properties [16–18]. Traditionally, the infusion of leaves of this plant could be useful for headaches, cold and gastrointestinal disorders [16]. It is also used locally as an antiseptic for wounds and different skin diseases [19, 20].

Finnish scientists provide interesting data on the high antimicrobial activity of fireweed honey against *Streptococcus pneumoniae*, *S. pyogenes*, *Staphylococcus aureus*, and methicillin-resistant *S. aureus* [21].

Epilobium angustifolium infusions due to their astringent and emollient properties were commended by the American herbal lists in the 19th and early 20th century as a highly effective agent to treat gastrointestinal diseases such as dysentery and diarrhea of different aetiologies as well as other bowel and intestinal disorders associated with infection, irritation, and inflammation. Fireweed herb is also used to treat whooping cough, asthma and hiccough [10].

To assess the relationship between the production of primary metabolites and their possible therapeutic properties, we analyzed the amino acid profile of the plant *Epilobium angustifolium* used in traditional medicine. The study of compounds generated by plants as a result of defense mechanisms permits an understanding of the molecular mechanism involved in their medicinal properties. Thus, the aim of our research was to identify and determine the quantitative content of amino acids in *E. angustifolium* L. to establish the prospects for the use of the raw materials in medical and pharmaceutical practice. The results of the current study will be used in further breeding programs aimed to obtain an industrial form of *E. angustifolium* suitable for pharmaceutical and food applications.

2. Planning (methodology) of research

In Fig. 1, *a* representation of the design of the experiment planning process is shown.
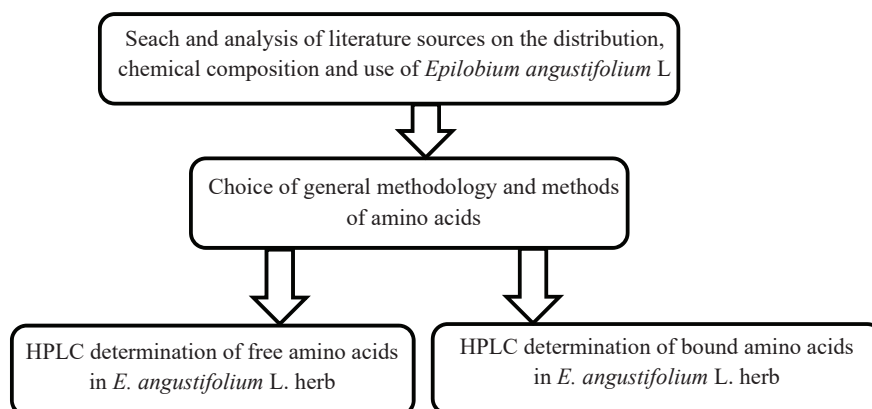

Fig. 1. Design of the experiment

3. Material and method
3. 1. Plant materials

Epilobium angustifolium L. herb was selected as the object of the study. The aboveground part of the studied plant was collected in Ternopil region (Ukraine) in the period of flowering in July 2017. The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine). The voucher specimens of herbal raw materials have been deposited in the departmental herbarium for future records. The study plant material was dried using the conventional oven with forced air circulation method and stored in paper bags in a dry place.

3. 2. Chemicals and standards

Standards of amino acids were of analytical grade (>99 % purity). The chemicals were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA) and were: 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 [22, 23]. Derivatizing agents o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) were purchased in Merck. Acetonitrile (ACN) and hydrochloric acid (HCl) were from Sigma-Aldrich.

3. 3. Sample preparation, HPLC determination of amino acids

The amino acids composition of *Epilobium angustifolium* L. is determined by HPLC method with a pre-column derivatization OPA and FMOC. The research was performed on the basis of the Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv, Ukraine.

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 Na2HPO4, pH 7,8; mobile phase B – CH3CN:CH3OH:H2O (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. For the extraction of free amino acids of powdered the raw material (to the 130 mg of *E. angustifolium* 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 h. Extraction of bound and free amino acids was performed by adding 2 ml of water solution of 6 N hydrochloric acid to the powdered of the raw material (to the 132 mg of *E. angustifolium* L.). Hydrolysis was conducted for 24 hours in a thermostat at 110 °C [24, 25].

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 of the peak area of the amino acids [26]. The content of bound amino acids was determined by subtracting the content of free amino acids from their total content.

3. 4. 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 μmol/ml range, and the linearity range for which the correlation coefficient that characterizes the regression line $R2$ was obtained, was examined visually.

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. All statistical tests were performed at a confidence level of 95 % and $k=2$.

4. Results

HPLC method represents an effective, comprehensive and quantitative technique for the analysis of amino acids. Thus, the qualitative composition and quantitative content of amino acids in *Epilobium angustifolium* L. were determined by this method. Table 1 presents the amino acids composition of the herb of *Epilobium angustifolium* L. The HPLC method identified sixteen free amino acids (Fig. 2) and seventeen bound amino acids (Fig. 3) in the raw material.
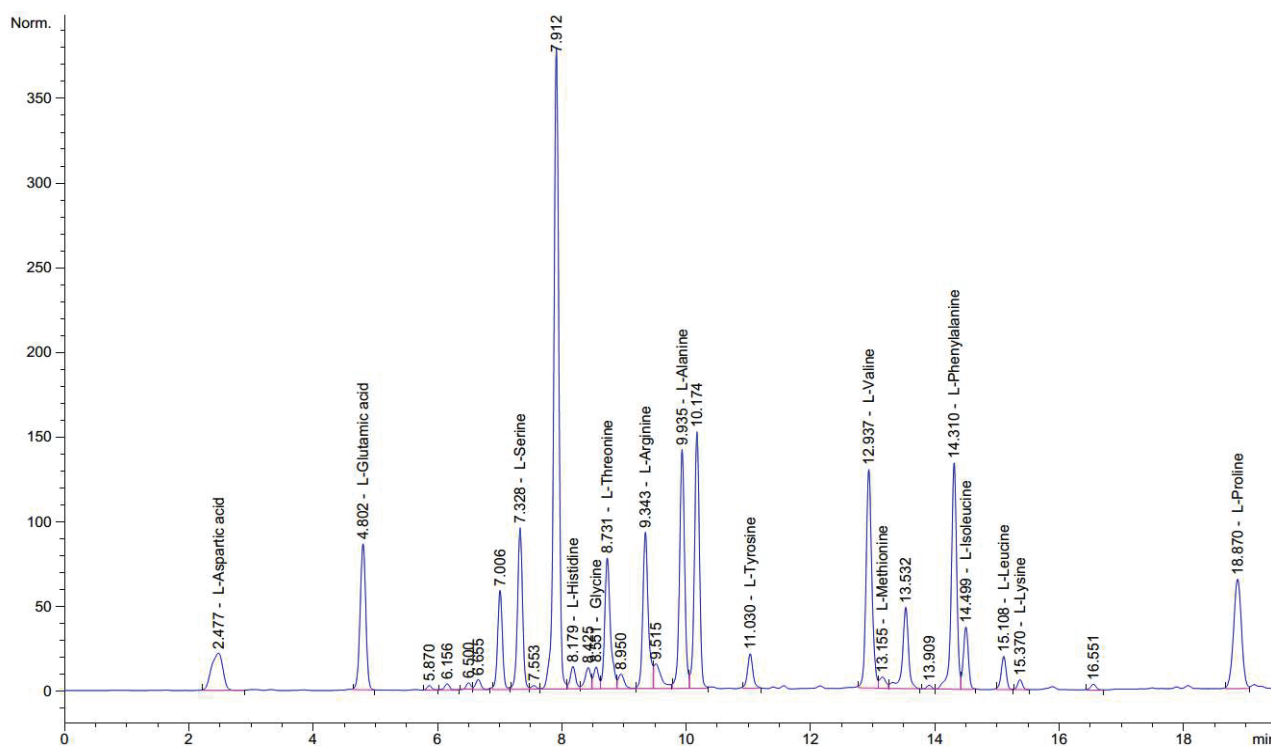

Fig. 2. HPLC chromatogram of free amino acids of *Epilobium angustifolium* L.
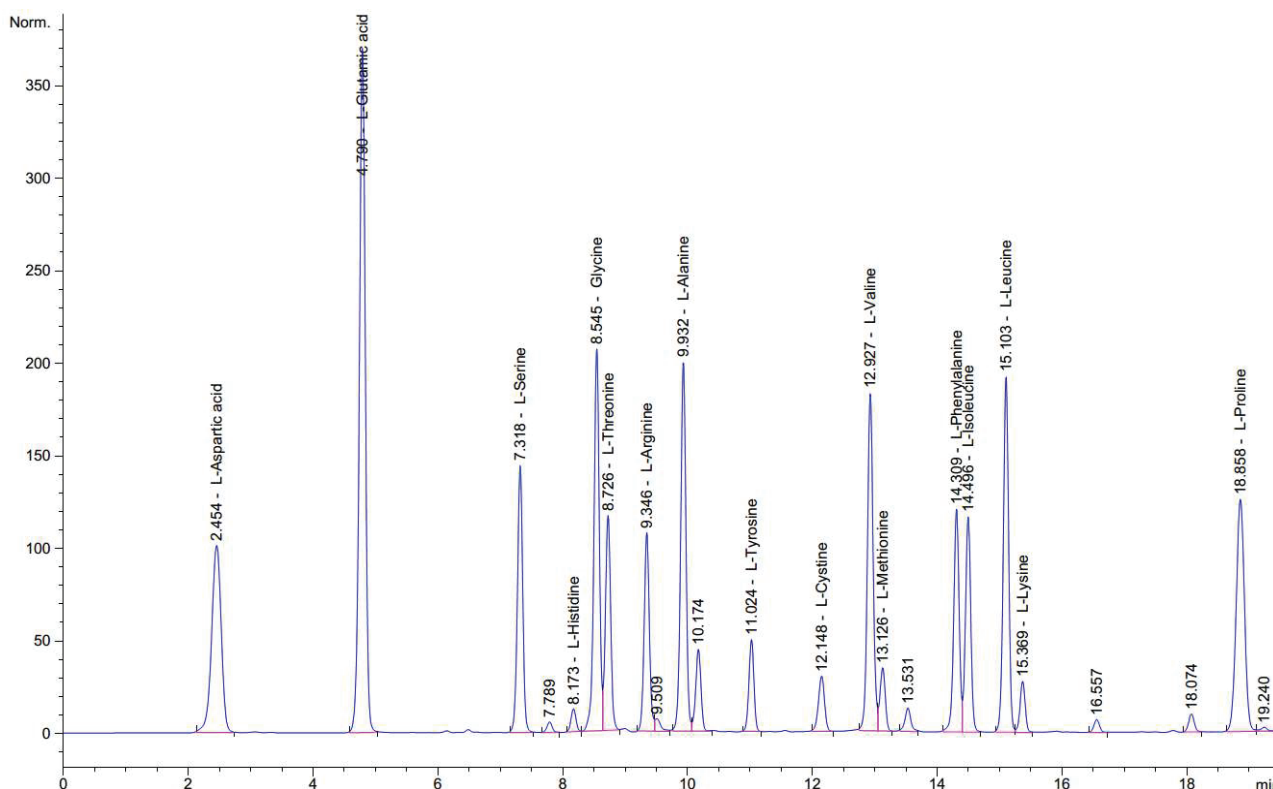
Fig. 3. HPLC chromatogram of total amino acids of *Epilobium angustifolium* L.

Table 1
The content of the amino acids composition in *Epilobium angustifolium* L. herb

The name of the amino acid	The content of the amino acid, µg/mg		
	Epilobium angustifolium L. herb		
	Free	Bound	Total
L-aspartic acid	0.57±0.02	10.59±0.18	11.15±0.16
L-glutamic acid	1.51±0.06	32.37±0.21	33.88±0.24
L-serine	0.79±0.03	5.46±0.13	6.25±0.15
L-hystidine	0.49±0.02	1.64±0.05	2.13±0.06
Glycine	0.07±0.01	6.02±0.11	6.09±0.14
L-treonin	0.84±0.03	4.93±0.08	5.77±0.11
L-arginine	1.24±0.05	5.60±0.06	6.85±0.13
L-alanine	0.98±0.03	6.22±0.07	7.20±0.09
L-tyrosine	0.30±0.02	3.16±0.10	3.47±0.06
L-cystine	–	16.56±0.18	16.56±0.18
L-valine	0.83±0.04	4.95±0.05	5.78±0.05
L-methionine	0.09±0.01	1.74±0.04	1.83±0.03
L-phenylalanine	1.65±0.06	5.81±0.07	7.45±0.08
L-isoleucine	0.32±0.02	5.01±0.04	5.33±0.05
L-leucine	0.17±0.01	8.70±0.12	8.87±0.10
L-lysine	0.24±0.02	5.96±0.09	6.20±0.08
L-proline	0.59±0.02	5.41±0.08	6.01±0.07

The studies have shown that *Epilobium angustifolium* L. herb is mainly composed of free amino acids such as L-phenylalanine (1.65 µg/mg), L-glutamic acid (1.51 µg/mg), L-arginine (1.24 µg/mg), L-alanine (0.98 µg/mg) and L-aspartic acid (0.57 µg/mg), which were presents in the greatest amount (Fig. 2, Table 1). The dominant bound amino acids in the studied raw material were L-glutamic acid, L-aspartic acid, L-leucine, and L-alanine, the content of which was 32.37 µg/mg, 10.59 µg/mg, 8.70 µg/mg, and 6.22 µg/mg respectively (Fig. 3, Table 1). However, L-cystine (16.56 µg/mg) was found only in bound form.

5. Discussion

Amino acids are essential in the synthesis of proteins and precursors in the formation of secondary metabolism molecules [27]. Also, amino acids participate in various physiological processes such as skeletal muscle function, synthesis of hormones, and antioxidant capacity [28, 29].

The dominant components of amino acids from *Epilobium angustifolium* L. herb with respect to total content were L-aspartic acid, L-glutamic acid, L-arginine, L-alanine, and L-phenylalanine. L-aspartic acid provides for a specific type of amine transamination in the liver and takes a part in the control of the metabolic functions of the brain and central nervous system cells [30]. Glutamic acid is an exciting neurotransmitter for the central nervous system, the spinal cord, and the brain. L-glutamic acid serves as fuel for the brain and helps to correct the physiological imbalances in the body [31]. For example, L-arginine supplementation improves the function of the intestinal barrier and vascular development [32, 33], L-alanine is used to treat muscle degeneration [34]. L-phenylalanine is used by the brain to produce norepinephrine, which transmits signals between nerve cells. In addition, it promotes vitality and alertness, reduces pain, and regulates human mood. This acid is also used in the treatment of depression, migraine, painful menstruation, and Parkinson's disease [35, 36].

The amino acids profile plays an important role in chemical properties, so it is useful information for further research.

Study limitations. The investigation needs additional study of amino acids of the *Epilobium angustifolium* L. herb in various phases of vegetation. For the statistical significance of the study, it would be advisable to explore wild samples of raw materials from various regions of Ukraine.

Prospects for further research. The obtained results might be used in the standardization and quality assurance of new remedies containing *Epilobium angustifolium* L. herb.

6. Conclusion

Using the HPLC method determined the amino acids in the herb of *Epilobium angustifolium* L. The concentrations of L-aspartic acid, L-glutamic acid, L-arginine, L-alanine and L-phenylalanine are predominate among free and bound amino acids in the *Epilobium angustifolium* L. herb. Specific metabolic processes in which these amino acids take part may be related to the medicinal properties of plants as per their use in traditional medicine and, therefore may alleviate the understanding of their useful properties. Nevertheless, further studies are required to determine and isolate compounds responsible for the special medicinal properties of the plant.

Conflict of interests

The authors declare that they have no conflicts of interest.

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