

1. Introduction

Amino acids are the substances of primary synthesis contained in the top and underground organs of almost all flowering plant. They are synthesized from simple inorganic compounds and take part in the synthesis of protein, coenzymes, flavonoids, steroidal compounds, polyphenols, complex carbohydrates, lipids, vitamins and pigments. They are contained in plants in the form of easily digested complexes and in bioavailable concentrations. That's why they have higher bioactivity in comparison with synthetic analogues.

Up to date 300 herbal amino acids are known, 20 of which are the components of structural proteins and enzymes. Recent researches have shown that there are near 30 % of amino acids from total concentration of organic compounds in plants. Abundance of amino acids in plants and their biological activity facilitate effective action on the organism of both drug raw materials and medicinal preparation from it.

Based on the above stated, looking for new sources of easily digested amino acids among underexplored medicinal plants of Ukraine is actual. Ukraine possesses a sufficient raw material base.

Therefore, the purpose of the work was to determine the qualitative composition and quantitative content of amino acids in *Thalictrum foetidum* L. herb as a promising herbal substance for use in medicine and pharmacy [1, 2].

2. Methods

The object of this study was herbal raw material – *Thalictrum foetidum* L. herb, prepared on the territory of Ukraine. The analysis of amino acid composition has been performed by the high performance liquid chromatography method. High Performance Liquid Chromatograph Agilent 1200 device (Agilent technologies, USA) was employed during this study.

Preparation of analytical test

Principle:

The method is based on the extraction of free amino acids from the herbal raw material and on the acid hydrolysis of the herbal medicinal products followed by the analysis of hy-

STUDY OF AMINO ACID COMPOSITION IN A *THALICTRUM FOETIDUM* L. HERB

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Abstract: The submitted article is concerned with the study of amino acid composition in a *Thalictrum foetidum* L. herb. Investigations have been performed by the high performance liquid chromatography method based on the extraction of free amino acids from the herbal raw material and on the acid hydrolysis of the herbal medicinal products followed by analysis of hydrolizates using the HPLC with precolumn derivatization with 9-fluorenylmethoxycarbonyl chloride (FMOC) and o-phthalaldehyde (OPA) attended by the detection with fluorescence detector.

Amino acid identification has been performed by the comparison of retention times with the mixture of standard amino acids (Agilent 5061-3334). The content of bound amino acids has been determined by the subtraction of free amino acids composition from their total composition.

As a result of study 15 essential and non-essential amino acids have been found. It is important to note that L-Serine, L-Histidine, Glycine and L-Phenylalanine are not in a free state in *Thalictrum foetidum* L. herb. The quantity of L-Glutamic and L-Aspartic acids is 17.47 and 13.41 µg/mg, respectively (common amino acids). The content of abovementioned acids in a bound state is 15.66 and 9.84 µg/mg, respectively. As for content of other amino acids which were found in *Thalictrum foetidum* L. herb, it has to be said about their practically the same composition which is in the range from 4 to 5 µg/mg. Content of L-Histidine (1.80 µg/mg in general and bound state) and L-Tyrosine (1.76 µg/mg in general state and 0.08 µg/mg in bound state) is minimum in this herb.

It has been established that amino acids are in a free and bound state in the investigated herbal raw material. The content of bound amino acids is higher than general content. Among the amino acids, which are in the overwhelming majority in the studied herb, it stands to mention L-Glutamic and L-Aspartic. These amino acids play an important role in supporting the physiological processes of the body, especially nervous system.

In consideration of experimental data to be noticed is that *Thalictrum foetidum* L. is a promising plant for creation of phytotherapeutic medications with neurally mediated action.

Keywords: *Thalictrum foetidum* L., herb, biologically active substances, amino acids, high performance liquid chromatography.

drolyzates using the HPLC with precolumn derivatization with 9-fluorenylmethoxycarbonyl chloride (FMOC) and o-phthalaldehyde (OPA) attended by the detection with fluorescence detector.

The equipment and chromatographic conditions

Chromatographic separation was performed using liquid chromatograph Agilent 1200 device (Agilent technologies, USA). Column is Zorbax AAA with a length of 150 mm, internal diameter 4.6 mm, sorbent particle size is µm. Mobile phase A is 40 mM Na₂HPO₄, pH 7.8; B – ACN:MeOH: water (45:45:10, v/v/v). Separation mode is gradient with the constant flow rate 1.5 ml/min. Oven temperature is 40 °C. Precolumn derivatization was performed in the automatic programming mode using FMOC reagent (Agilent 5061-3337) and OPA reagent (Agilent 5061-3335). Detection of the derivatized amino acids was performed with fluorescence detector.

Identification of components

Amino acid identification has been performed by the comparison of retention times with the mixture of standard amino acids (Agilent 5061-3334). The content of bound amino acids has been determined by the subtraction of free amino acids composition from their total composition [3, 4].

3. Results

HPLC-chromatograms obtained at the determination of amino acids in *Thalictrum foetidum* L. herb are given on the Fig. 1. Identified free, common and bound amino acids are listed in Table 1.

As a result of study 15 essential and non-essential amino acids have been found. It is important to note that L-Serine, L-Histidine, Glycine and L-Phenylalanine are not in a free state in *Thalictrum*

foetidum L. herb. L-Glutamic and L-Aspartic acids are characterized with the highest content: 17.47 and 13.41 µg/mg, respectively (common amino acids) In the bound state these amino acids have the important role in the physiological processes of the body and their content in this state is 15.66 and 9.84 µg/mg, respectively.

Regarding the content of other amino acids which were found in *Thalictrum foetidum* L. herb, it has to be said about their practically the same composition which is in the range

from 4 to 5 µg/mg. The content of L-Histidine (1.80 µg/mg in general and bound state) and L-Tyrosine (1.76 µg/mg in general state and 0.08 µg/mg in bound state) is minimum in this herb.

In that way, L-Glutamic is closely connected with protein, carbohydrate, lipid metabolism and with other types of metabolism. More than 20 % of protein nitrogen present in the form of glutamic acid and its amide, 60 % of carbon of L-Glutamic can be included in glycogen, 20–30 % – in fatty acids. L-Glutamic

binds toxic byproducts in brain tissue [5, 6]. This is a single amino acid which is oxidized in the tissues of cerebrum and serves as energy source for neuron activity. It takes part in the synthesis of acetylcholine. Furthermore L-Glutamic undergoes the reverse transformation into glutamine under the existence of tissue glutaminase, at the binding ammonia forms. Above-mentioned properties of glutamic acid explain its beneficial effect at some central nervous system diseases [7, 8].

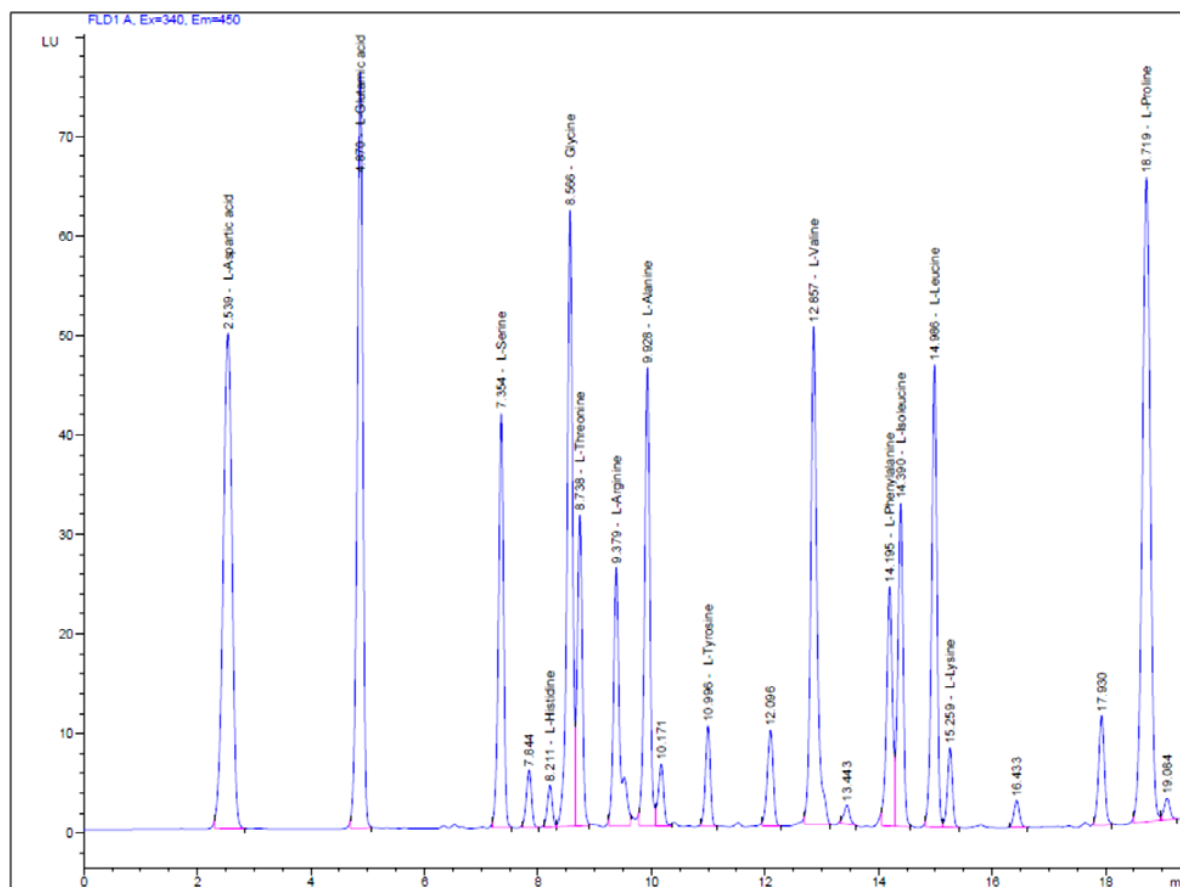


Fig. 1. HPLC-chromatogram, obtained at the determination of amino acids in *Thalictrum foetidum* L. herb

Table 1
Amino acid composition of *Thalictrum foetidum* L. herb

No.	Free amino acids			Common amino acids			Bound amino acids
	RetTime, [min]	Name	Amount, mkg/mg	RetTime, [min]	Name	Amount, mkg/mg	Amount, mkg/mg
1	2.562	L-Aspartic	3.57	2.539	L-Aspartic	13.41	9.84
2	4.915	L-Glutamic	1.82	4.87	L-Glutamic	17.47	15.66
3	7.35	L-Serine	0.00	7.354	L-Serine	4.63	4.63
4	8.208	L-Histidine	0.00	8.211	L-Histidine	1.80	1.80
5	8.562	Glycine	0.00	8.566	Glycine	4.85	4.85
6	8.813	L-Threonine	0.07	8.738	L-Threonine	3.91	3.84
7	9.384	L-Arginine	0.66	9.379	L-Arginine	5.10	4.44
8	10.001	L-Alanine	0.68	9.928	L-Alanine	4.38	3.70
9	10.999	L-Tyrosine	1.68	10.996	L-Tyrosine	1.76	0.08
10	12.814	L-Valine	0.03	12.857	L-Valine	4.37	4.34
11	14.184	L-Phenylalanine	0.00	14.195	L-Phenylalanine	4.08	4.08
12	14.451	L-Isoleucine	0.80	14.39	L-Isoleucine	3.87	3.08
13	14.978	L-Leucine	0.00	14.986	L-Leucine	5.52	5.52
14	15.207	L-Lysine	2.14	15.259	L-Lysine	3.95	1.81
15	18.811	L-Proline	6.58	18.719	L-Proline	9.15	2.57

L-Aspartic plays an important role in the correct function of nervous and endocrine systems and stimulated hormone production (growth hormone, testosterone, progesterone). It is contained in proteins and acts as an excitatory neurotransmitter of the central nervous system. L-Aspartic acid is an important at the process of formation of other amino acids such as asparagine, methionine, isoleucine, arginine, threonine and lysine; it helps at chronic-fatigue build. This amino acid is an important at the transport of minerals, necessary for formation and function of DNA and RNA; it fortifies the immune system, stimulating the antibody response and immunoglobulins; amino acid has the positive influence on the work of the central nervous system, it keeps mental alertness and intensifies brain functions; promotes elimination of toxins as well ammonia, which negatively affects the function of brain, nervous system and liver. Under stress the organism needs additional doses of amino acids. Besides this L-Aspartic acid is an effective medication used to treat depression; it facilitates conversion of carbohydrates to energy [9, 10].

Regarding the content of other amino acids which were found in *Thalictrum foetidum* L. herb, it has to be said about

their practically the same composition which is in the range from 4 to 5 µg/mg. Content of L-Histidine (1.80 µg/mg in general and bound state) and L-Tyrosine (1.76 µg/mg in general state and 0.08 µg/mg in bound state) is minimum in this herb.

4. Discussion

As a result of study 15 essential and non-essential amino acids have been found in a *Thalictrum foetidum* L. herb using the high performance liquid chromatography method. It has been established that amino acids in the composition of the investigated herbal raw material are in free and bound state. It has to be noted that their content in bound state is the highest. Among the amino acids, which are in the *overwhelming majority* in the studied herb, it stands to mention L-Glutamic and L-Aspartic. These amino acids play an important role in supporting the physiological processes of the body, especially nervous system.

In the consideration of experimental data to be noticed is that *Thalictrum foetidum* L. is a promising plant for creation of phytotherapeutic medications with neurally mediated action.

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