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STUDY OF THE AMINO ACID COMPOSITION OF PLANT RAW MATERIAL AND LYOPHILIZED EXTRACT OF COMMON THYME (*THYMUS VULGARIS* L.) FROM SOUTHERN UKRAINE

Actuality. *The search for new sources of biologically active compounds, particularly amino acids, is an important task for modern pharmacy due to their diverse effects on the human body. One of the promising sources is medicinal plants, among which Thymus vulgaris L., cultivated in Ukraine and having a sufficient raw material base, is of particular interest. Plants of the genus Thymus L. are traditionally used as anti-inflammatory, wound-healing, and antimicrobial agents. However, the modern scientific literature lacks data on the amino acid composition analysis of this species.*

The purpose of the work *was to investigate the amino acid composition of the herb and lyophilized extract of Thymus vulgaris L. as a promising source of biologically active compounds.*

Materials and methods. *The plant material of Thymus vulgaris L. (flowering tops up to 15 cm long) was collected in the Zaporizhzhia region during the flowering period (June–August 2023). To confirm the qualitative composition and determine the quantitative content of biologically active amino acids, both protein-bound and free, the method proposed by Stein and Moore was applied using a high-performance liquid chromatography system AAA-881 (Czech Republic) with certified reference standards. Free amino acids were determined without protein hydrolysis using the standard addition method.*

Research results. *The obtained data confirmed the presence of 17 amino acids in the herb of Thymus vulgaris L. and 15 amino acids in the lyophilized extract, including seven essential ones (methionine, valine, leucine, lysine, isoleucine, phenylalanine, and threonine). The highest concentrations were recorded for glutamic and aspartic acids, leucine, phenylalanine, alanine, proline, serine, lysine, valine, arginine, and histidine.*

Conclusions. *The amino acid profile analysis confirmed that the herb of Thymus vulgaris L. represents a promising plant material and a valuable natural source of amino acids for the development of complex water-soluble phytopharmaceutical preparations.*

Key words: *amino acids, herb, lyophilized extract, Thymus vulgaris L., high-performance liquid chromatography (HPLC).*

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ДОСЛІДЖЕННЯ АМІНОКИСЛОТНОГО СКЛАДУ РОСЛИННОЇ СИРОВИНИ ТА ЛІОФІЛІЗОВАНОГО ЕКСТРАКТУ ЧЕБРЕЦЮ ЗВИЧАЙНОГО (*THYMUS VULGARIS* L.) ПІВДНЯ УКРАЇНИ

Актуальність. *Пошук нових джерел біологічно активних речовин, зокрема амінокислот, є важливим завданням для сучасної фармації через їхню різнобічну дію на організм. Одним із перспективних джерел є лікарські рослини, серед них – Thymus vulgaris L, який культивується в Україні і має достатню сировинну базу. Рослини роду Thymus L. використовуються в традиційній медицині як протизапальний, ранозагоювальний та антимікробний засіб. У сучасній фаховій літературі немає даних про проведення амінокислотного аналізу досліджуваного виду.*

Мета роботи *– дослідження амінокислотного складу трави та ліофілізованого екстракту чебрецю звичайного (Thymus vulgaris L.) як перспективного джерела біологічно активних речовин.*

Матеріал і методи. *Об'єктом дослідження вибрано рослинну сировину чебрецю звичайного (верхівки завдовжки до 15 см), заготовлену в Запорізькій області впродовж періоду цвітіння (червень – серпень 2023 р).*

Для підтвердження якісного та визначення кількісного складу біологічно активних зв'язаних у складі білка, а також вільних амінокислот використовували методику, запропоновану Штейном і Муром, на високоефективному рідинному хроматографі моделі AAA 881 (Чехія) з використанням стандартних зразків.

Вільні амінокислоти визначали без гідролізу білкових сполук методом стандартних додавань.

Результати дослідження. *Отримані дані свідчать про наявність 17 амінокислот у траві Thymus vulgaris L. і ліофілізованому екстракті 15 амінокислот, 7 з яких є незамінними (метіонін, валін, лейцин, лізин, ізолейцин, фенілаланін, треонін). У найбільш високих концентраціях установлено: глютамінову і аспарагінову кислоти, лейцин, фенілаланін, аланін, пролін, серін, лізин, валін, аргінін, гістидін.*

Висновок. *Проведений амінокислотний аналіз підтверджує, що трава чебрецю звичайного (Thymus vulgaris L.) є перспективною сировиною та цінним природним джерелом амінокислот для розроблення комплексних водорозчинних фітопрепаратів.*

Ключові слова: *амінокислоти, трава, ліофілізований екстракт, Thymus vulgaris L., метод високоефективної рідинної хроматографії (ВЕРХ).*

Introduction. The genus Thymus L. (thyme) of the Lamiaceae family comprises more than 350 species in the modern world flora, of which up to 50 occur in Ukraine (Mazulin, 2023). The plants are distributed throughout Europe, North Africa, and the Mediterranean region. Species of the genus are characterized by a great diversity of biological forms, successfully adapting to the conditions of highly varied natural biocenoses or being cultivated in specialized agricultural facilities (Stahl-Biskup 2002; Morales, 2002).

As well known, the pharmacopoeial species include the herb of creeping thyme (Thymus serpyllum L.) and a mixture of plant material from common thyme (Thymus vulgaris L.) and Spanish white thyme (Thymus zygis L.).

Pharmacognostic research on Thymus species of the Ukrainian flora and the development of new domestic phytomedicines based on them is a relevant task. Its practical significance lies in the rational use, harvesting, and phytochemical analysis of plant raw materials for both the pharmaceutical industry and medicine.

Throughout history, the aerial parts of Thymus L. species have been highly valued; they have been widely used as herbal teas, seasonings, and spices, as well as for various medicinal purposes – as analgesics, and for radiculitis and neuritis in the form of aromatic baths and compresses (Nikolić, 2013; Carović-Stanko, 2016). Infusions and complex phytopreparations are prescribed as expectorant, antimicrobial, and sedative remedies in acute and chronic bronchopulmonary diseases. A pronounced anti-inflammatory, antioxidant, antimicrobial, wound-healing, and detoxifying effect of the infusion (1:10), tincture, and extracts from the plant herb has been established, which is attributed to the presence of flavonoids, hydroxycinnamic acids, and essential oil (Waheed, 2024; Hammoudi, 2022; Kowalczyk, 2020; Harna, 2016).

In modern medicine, complex phytopreparations from the herb of Thymus L. species are widely used due to their anti-inflammatory, wound-healing, and antimicrobial properties. Currently, a number of countries produce modern phytomedicines from the herb or essential oil of Thymus species.

In the pharmaceutical market of Ukraine, the following medicinal products are available: Altemix Broncho Syrup (LLC «PC Zdorovye»), Bronchipret tablets (Bionorica SE, Germany), Bronchostop Cough Lozenges (Kwizda Pharma GmbH, Austria), Pectolvan Phyto Iceland Moss Extract, and Echinasal Syrup (Herbapol S.A., Poland) (tabletki.ua, 2025).

Thymus vulgaris L. (common thyme) is a small, semi-shrub plant up to 25 cm tall with a well-developed taproot system. The stem is typically erect or ascending, quadrangular, highly branched, and woody in the lower part. The herbaceous branches are slender and covered with characteristic grayish pubescence. The leaves are small, opposite, entire, shortly petiolate, usually elongate-lanceolate, and gray-green in color. Numerous essential-oil glands are clearly visible on both surfaces. The flowers are small, bilabiate, and pentamerous. The calyx is green; the corolla is pale lilac, lilac-pink, or white. The inflorescences are spike-like. The fruit is a cenobium. In Ukraine, flowering occurs in June–July. The entire plant emits a strong aromatic scent.

The native range of Thymus vulgaris L. is Spain and southern France, where it commonly grows on dry open slopes. It is also regularly found in Portugal, Italy, Algeria, Morocco, and along the Mediterranean coast. It has long been cultivated in North America, Western Europe, South Africa, northern Asia, and Oceania (Kosakowska, 2020; Atowa, 2024).

Amino acids are the most essential compounds of all known modern plants, existing both as bound components in proteins and in their free state (Hamad, 2014). It is known that in plants, amino acids participate in numerous biosynthetic pathways, including the synthesis of polyphenols, flavonoids, tannins, enzymes, hydroxycinnamic acids, alkaloids, steroids, proteins, auxins, and others (Yang, 2020; Mazulin, 2023; Taghouti, 2020). Certain amino acids, such as cysteine, alanine, γ-aminobutyric acid, and proline, significantly increase plant resistance to adverse environmental conditions (salinity, drought, high temperatures, soil erosion) (Ingrisano, 2023).

In the human body, amino acids are used as nutrients for tissue and cell construction, as well as nutritional components. They participate in numerous biochemical reactions in the nervous, vascular, and endocrine systems. Amino acids demonstrate pronounced biological activity in herbal infusions and decoctions and in complex phytopreparations derived from medicinal plants. They exhibit multitarget biological effects, the ability to convert other compounds into safe bioavailable forms, and enhance their action (Iordache, 2023; Afonso, 2018).

Amino acid preparations are widely used in modern medicine for the treatment of gastric ulcers, burns, neuro-psychiatric attacks, epilepsy, regulation of hepatobiliary system function, and for parenteral nutrition. Amino acids are capable of regulating hematopoiesis, supporting the cessation of gastrointestinal and hepatic bleeding, and promoting the healing of various skin injuries, among other effects. Medicinal plants and phytopreparations containing amino acids exhibit more pronounced anti-inflammatory activity. (Afonso, 2020, Мазулін, 2002, Mahdi, 2023).

The purpose of our study was defined as the investigation, by high-performance liquid chromatography, of the qualitative composition and quantitative content of amino acids in the herb and lyophilized extract of *Thymus vulgaris* L.

Materials and methods. For further investigation of bound and free amino acids in the plant raw material and lyophilized extract of *Thymus vulgaris* L., the method of high-performance liquid chromatography (HPLC) was employed using AAA T-339 (Czech Republic) and Agilent Technologies 1100 (Sweden) chromatographs (Moran-Palacio, 2014).

The herb of Thymus vulgaris L., collected in southern Ukraine (June–July 2023) during the flowering period according to the generally accepted methods of the State Pharmacopoeia of Ukraine (SPhU, 2015, Vol. 1), was used for the study. The herb was composed of flowering apical shoots with inflorescences up to 15 cm long, as well as individual leaves and fragments of stems.

The drying process of the raw material was carried out in a «Termolab СНОЛ 24/350» drying oven at a temperature of 35 °C and a layer thickness not exceeding 1 cm for 2 hours, until the moisture content did not exceed 10%.

Lyophilized aqueous extracts (LE) were obtained under laboratory conditions by the method of sublimation drying of aqueous extracts from the plant herb (1:5) under aseptic conditions using a KS–30 unit (Czech Republic).

Previously prepared aqueous extracts, obtained in accordance with SPhU requirements, were dispensed in 200 mL portions into 500 mL vessels and subjected to drying in an alcohol bath for 1 hour (t = −40°C) until complete evaporation of the solvent. The total duration of lyophilized extract production was up to 29 hours. The bottles were removed from the sublimation chamber, quickly closed with special rubber stoppers, sealed with caps, and carefully coated with metalex.

The lyophilized extracts obtained by sublimation drying were characterized by a light-yellow color, a loose air-amorphous consistency, a spicy taste, and a specific aromatic odor. They were well dissolved in water and ethyl alcohol, and were insoluble in chloroform, acetone, and ether.

The yield of the obtained lyophilized extract in powder form from 1 liter of extract amounted to up to 30,44 ± 3,00% for Thymus vulgaris L. (1:5), with a moisture content of 3,22 ± 0,31%.

The advantages of using lyophilized extracts in modern phytotherapy were noted to include their high water solubility, convenience for dosing and standardization, high bioavailability, and the absence of side effects associated with residual solvents (Chevallier, 2016).

During the extraction process of plant raw material, the transfer of biologically active compounds into the corresponding extractant was observed. The solubility of the compounds was determined by such factors as the degree of comminution of the raw material, the completeness and frequency of the extraction process, the nature of the extractant, the ratio between raw material and extractant, hydrodynamic conditions, and temperature.

To determine the amino acids bound in the protein composition, a sample of plant material of approximately 0.1 g (accurately weighed) previously ground to a particle size of 2 mm was placed into ampoules (Pyrex glass) under a nitrogen atmosphere and filled with an excess of 6 M hydrochloric acid solution. Acid hydrolysis was carried out at 50–55°C for 24 hours. The ampoules were then cooled, opened, and hydrochloric acid was evaporated at 100°C. The samples were neutralized in a desiccator over sodium hydroxide for 48 hours.

The obtained solution was evaporated under vacuum at 40°C. The dry residue was dissolved in a citrate buffer solution with pH 2.2, and HPLC analysis was performed using columns (length 200 mm, diameter 2.1 mm) packed with LG ANB cation exchange resin and octadecylsilane sorbent («ZORBAX–SB C–18») with a particle size of 3.5 μm. A 100 μL portion of the test solution was used for analysis.

Amino acids were eluted using trisodium citrate buffer solutions. Detection was carried out using a ninhydrin reagent at 100°C in photometer cuvettes at λ = 520 nm, where the corresponding amino acids were recorded as peaks. The components were identified by retention time in comparison with standard samples, and their concentrations were determined based on the area of each peak.

The content of the substances (%) was calculated according to formula 1:

$$X\,\% = \frac{S_{on} \cdot K \cdot M_{в}}{S_{cm}},$$

S_{on} – area of the corresponding amino acid peak;
K – coefficient accounting for the sample weight and dilution;
$M_{в}$ – molecular weight of the corresponding amino acid;
S_{cm} – area of the peak of the corresponding standard amino acid in the mixture.

For the determination of free amino acids, preliminary derivatization of the samples was carried out using o-phthalaldehyde (for primary amino acids) and 9-fluorenylmethyl chloroformate (for secondary amino acids) in the presence of 0.4 M borate buffer solution (pH 10.4). Mobile phases were used as follows: a 20% solution of 100 mM sodium acetate containing 0.018% triethylamine and 0.3% tetrahydrofuran; and a solution consisting of 40% acetonitrile, 40% methanol, and 20% of 100 mM sodium acetate.

Amino acids were identified using the standard addition method. The retention time accuracy, peak area measurement, and linearity were confirmed using five standard samples of 17 amino acids (concentration range 10–1000 pmol/μL). The results were processed using mathematical statistical methods with the licensed software «Statistica 6.0 for Windows» (Stat, Soft. Inc., AXXR712D833214FANS). Statistical significance of the differences in the indicators, according to the requirements of the SPhU, was determined using Student's t-test (p > 95%).

Research results and discussion. The obtained results of the qualitative composition and quantitative content of amino acids in *Thymus vulgaris* L. herb and lyophilized extract are presented in table and fig. 1–2.

When the obtained results (table 1) were analyzed, the presence of up to 17 amino acids in the herb of Thymus vulgaris L. was confirmed, seven of which (methionine, valine, lysine, leucine, threonine, isoleucine, phenylalanine) were identified as essential. In the plant raw material of *Thymus vulgari*s L., the concentrations of bound and free amino acids were determined to be 10.92 ± 0.55% and 2.50 ± 0.13%, respectively.

Table

Amino acid content in the herb and lyophilized extract of *Thymus vulgaris* L. (July 2023, Zaporizhzhia region, Vilnoandriivka village), ($\bar{x} \pm \Delta\bar{x}$), % μ=6

Compound name	Herb of *Thymus vulgaris* L.		Lyophilized extract *Thymus vulgaris* L.	
	Bound	Free	Bound	Free
Aspartic acid	1,07 ± 0,05	0,12 ± 0,01	0,17 ± 0,01	0,02 ± 0,001
Threonine	0,46 ± 0,02	0,09 ± 0,005	0,15 ± 0,01	0,03 ± 0,002
Serin	0,66 ± 0,03	0,09 ± 0,005	0,15 ± 0,001	0,02 ± 0,001
Glutamine acid	1,53 ± 0,08	0,11 ± 0,01	-	-
Proline	0,71 ± 0,04	0,10± 0,01	-	-
Glycine	0,53 ± 0,04	0,03 ± 0,001	0,41 ± 0,02	0,03 ± 0,001
Alanine	0,57 ± 0,03	0,30 ± 0,02		
Cystine	0,07 ± 0,003	0,14 ± 0,01	1,83 ± 0,09	0,06 ± 0,003
Valine	0,61 ± 0,03	0,18 ± 0,01	0,35 ± 0,02	0,03 ± 0,001
Methionine	0,34 ± 0,02	0,12 ± 0,01	0,18 ± 0,01	0,02 ± 0,001
Isoleucine	0,51 ± 0,03	0,22 ± 0,01	0,55± 0,03	0,05± 0,002
Leucine	0,83 ± 0,04	0,30 ± 0,02	0,88 ± 0,04	0,07 ± 0,003
Tyrosine	0,61 ± 0,03	0,03 ± 0,001	0,25 ± 0,01	0,03 ± 0,002
Phenylalanine	0,66 ± 0,03	0,20 ± 0,01	0,40 ± 0,02	0,03 ± 0,002
Histidine	0,61 ± 0,03	0,09 ± 0,005	0,43 ± 0,02	0,03 ± 0,001
Lysine	0,63 ± 0,03	0,19 ± 0,01	1,00 ± 0,05	0,05 ± 0,002
Arginine	0,52 ± 0,03	0,19 ± 0,01	0,86 ± 0,04	0,10 ± 0,01
Sum of amino acids	10,92 ± 0,55	2,50 ± 0,13	8,88 ± 0,44	0,70 ± 0,03

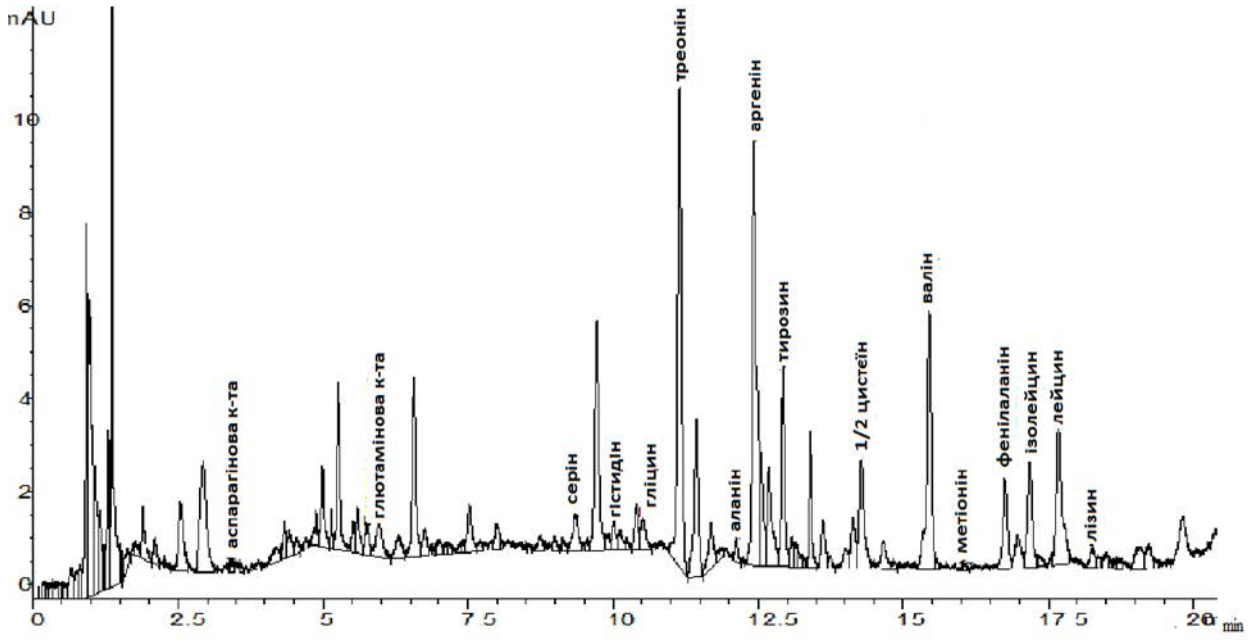

Fig. 1. HPLC chromatogram of amino acids from the herb of *Thymus vulgaris* L. collected in Zaporizhzhia region (July 2023)

Predominant compounds were found to be glutamic acid, aspartic acid, leucine, phenylalanine, alanine, proline, serine, lysine, valine, arginine, and histidine. According to the results, the quantitative content of glutamic acid was determined to be 1.53 ± 0.08%; aspartic acid – 1.07 ± 0.05%; leucine – 0.83 ± 0.04%; phenylalanine – 0.66 ± 0.03%; alanine – 0.57 ± 0.03%; proline – 0.71 ± 0.04%; serine – 0.66 ± 0.03%; lysine – 0.63 ± 0.03%; valine – 0.61 ± 0.03%; arginine – 0.52 ± 0.03%; histidine – 0.61 ± 0.03%.

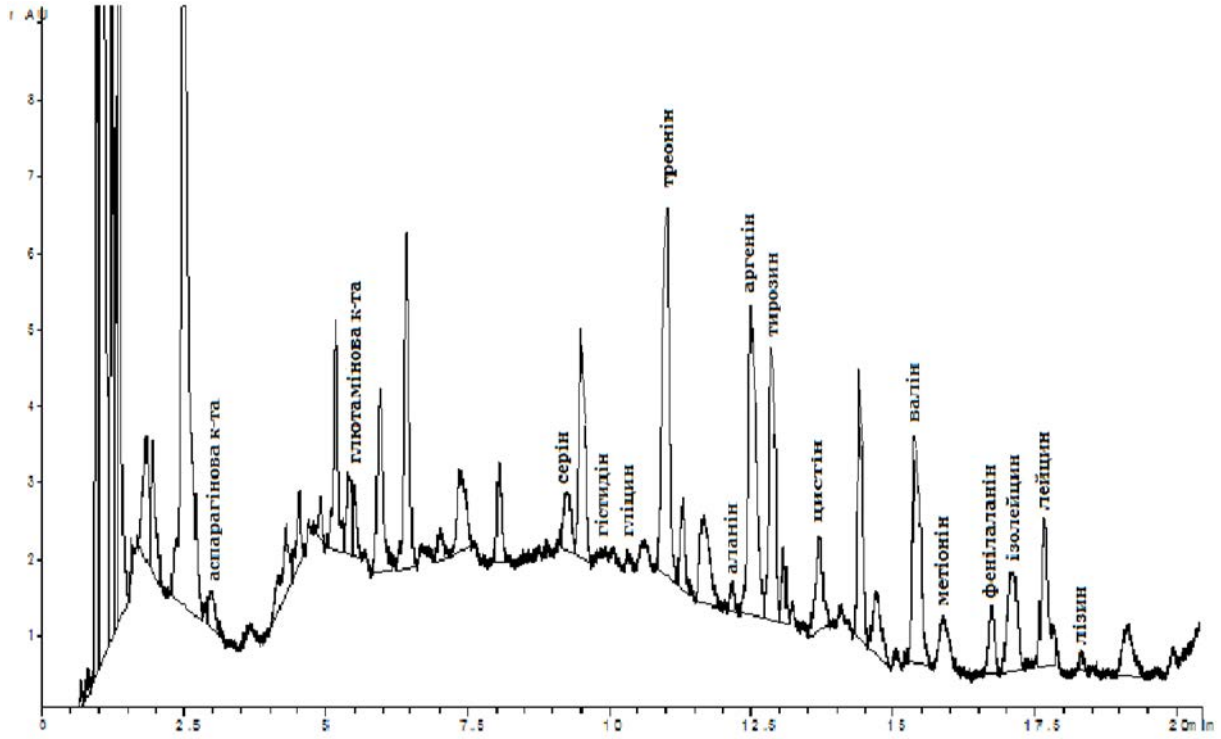

Fig. 2. HPLC chromatogram of amino acids obtained during quantitative analysis of the lyophilized extract from *Thymus vulgaris* L.

Higher concentrations were found for alanine (0.30 ± 0.02%), leucine (0.30 ± 0.02%), isoleucine (0.22 ± 0.01%), phenylalanine (0.20 ± 0.01%), lysine (0.19 ± 0.01%), and arginine (0.19 ± 0.01%).

It was established that up to 15 amino acids were present in the lyophilized extract obtained from the herb of Thymus vulgaris L., seven of which were essential (methionine, valine, leucine, lysine, isoleucine, phenylalanine, threonine). The total content of these compounds was determined to be up to 9.58 ± 0.48%. The highest concentrations were recorded for cysteine (up to 1.83 ± 0.09%), lysine (up to 1.00 ± 0.05%), leucine (up to 0.88 ± 0.04%), and arginine (up to 0.86 ± 0.04%). The content of free amino acids was found to be significantly lower and reached only up to 0.70 ± 0.03%.

Conclusions

1. The obtained results indicated that 17 amino acids were identified in the herb of Thymus vulgaris L. and 15 amino acids were determined in the lyophilized extract, seven of which (methionine, valine, lysine, leucine, threonine, isoleucine, phenylalanine) were found to be essential.

2. The highest accumulation of bound amino acids was observed in the lyophilized extract of Thymus vulgaris L., including cysteine (1.83 ± 0.09%), lysine (1.00 ± 0.05%), leucine (0.88 ± 0.04%), and arginine (0.86 ± 0.04%). Their concentrations in the herb differed significantly and were determined as follows: 0.07 ± 0.006%, 0.63 ± 0.09%, 0.83 ± 0.04%, and 0.52 ± 0.03%, respectively. The absence of proline, alanine, and glutamic acid was established in the lyophilized extract.

3. The established total composition and content of bound and free amino acids in the herb of Thymus vulgaris L. demonstrated its suitability for obtaining complex water-soluble phytopharmaceutical preparations.

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Prospects for further research. The plant raw material of Thymus vulgaris L. contains biologically active amino acid compounds with pronounced anti-inflammatory, antioxidant, and wound-healing activity, and represents a promising source for the development of complex water-soluble phytomedicines

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Contribution of the authors:
Fukleva L.A. – ідея, дизайн дослідження, аналіз та інтерпретація результатів, затвердження статті;
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