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An Identification and Study of Amino Acids of *Erigeron annuus* Herb

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Amino acids in the extract of Erigeron annuus herb were determined using an automatic precolumn derivatization with fluorenylmethyl-chloroformate and reversed-phase liquid chromatography with fluorescence and UV Vis detection. This objective is reached with automatic derivatization using o-phthalaldehyde (OPA) for primary amino acids and 9-Fluorenylmethyl chloroformate (FMOC) for secondary amino acids. Then derivatization integrates into high performance liquid chromatography (HPLC). The applied procedure is fast with easily reproduced results. The insufficient knowledge about amino acids composition of herb of Erigeron annuus is the basis for study. This work reports on content of 16 free and bound amino acids (391.408 μg/mg) in the plant raw material and influence's evaluation of different extraction types on the amino acid profile. The total content of free amino acids was 4.66 μg/mg; proline prevailed (2.498 μg/mg). The total content of bound amino acids was 386.748 μg/mg; proline (146.824 μg/mg), arginine (67.861 μg/mg), phenylalanine (25.806 μg/mg), asparagine (24.263 μg/mg), histidine (20.395 μg/mg), alanine (18.204 μg/mg), serine (16.650 μg/mg), valine (15.977 μg/mg) were the dominant amino acids. Nine amino acids were classified as essential.

Keywords: *Erigeron annuus* (L.) Pers., OPA, FMOC, amino acids, HPLC

Ідентифікація та вивчення амінокислот трави Erigeron annuus (Злинка однорічна)

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За допомогою високоефективної рідинної хроматографії (ВЕРХ) вивчали амінокислотний склад трави Erigeron annuus (або Злинка однорічна). У екстракті трави Erigeron annuus було виявлено шістнадцять амінокислот (391.408 мкг/мг). Загальний вміст вільних амінокислот становив 4.66 мкг/мг; переважав пролін (2.498 мкг/мг). Загальний вміст зв'язаних амінокислот становив 386.748 мкг/мг; пролін (146.824 мкг/мг), аргінін (67.861 мкг/мг), фенілаланін (25.806 мкг/мг), аспарагін (24.263 мкг/мг), гістидин (20.395 мкг/мг), аланін (18.204 мкг/мг), домінуючими амінокислотами були серин (16.650 мкг/мг), валін (15.977 мкг/мг). Дев'ять амінокислот, які входять до складу трави Erigeron annuus класифікували як незамінні амінокислоти.

Ключові слова: Злинка однорічна, о-фталальдегід, 9-флуоренілметилхлоро-формат, амінокислоти, високоефективна рідинна хроматографія

There are many synonyms for *Erigeron annuus* (L.) Pers.: *Aster annuus*, *Aster stenactis*, *Cineraria corymbosa*, *Diplopappus annuus, Erigeron annuus, Phalacroloma annuum, Pulicaria annua, Stenactis annua*. Common names include annual fleabane, stenactis, tonkoluchnik, zlynka. *E. annuus* is an invasive species native to North America, spread worldwide. Better known as a weed, this speciesis found mainly in meadows, pastures and well-lit glades. *Erigeron* is the typical plant of the steppe, so it is a well-known fodderfor the cattle. Annual fleabane belongs to *Erigeron* genus (*Asteraceae* family) comprising about400 species. In Ukrainian flora, 8 species are common; *Erigeron acris*, *Erigeron canadensis* and *Erigeron annuus* or annual fleabane are the most spread species [1].

Annual fleabane (*Erigeron annuus* (L.) Pers.) is an annual or perennial, tall and strong plant up to 20-90 centimeters in height; a stem with long spreading hairs; stem leaves abundant; basal leaves medium-hairy, large, coarsely toothed, elliptical, broadly ovate or suborbular, up to 10 centimeters. Typically, leaves directed upwards are shorter and pressed. Flowers: heads 6-10 mm in diameter; inflorescence corymbose;

petals of a variable width and 3-5 mm long, light purple, acuminate or waning, fine-glandular and fine-hairy, with long flattened transparent hairs [2].

In the ethnoscience, an infusion from annual fleabane herb is used as an antidiarrheal remedy and for the treatment of hemorrhoids. *Erigeron canadensis* is an active constituent of antidiarrheal drug "Erican", what gives background for the use of *E. annuus* herb as an additional source of raw materials for the manufacture of this drug. There are literature data on the use of *E. annuus* roots as an insecticidal agent. In Chinese and Korean ethnomedicine, *E. annuus* herb is used for the treatment of hypoglycemia, hematuria, hepatitis, enteritis. The foreign scientists pay much attention to annual fleabane herb; anti-inflammatory, diuretic, antisclerotic activities were established [3-6].

A phytochemical profile of *E. annuus* herb is characterized by various groups of biologically active substances, namely, phenolic compounds, organic acids, mono- and sesquiterpenoids, polyacetylene compounds, sterols, derivatives of γ-pyrone were reported [7-13].

Currently, this raw material is poorly studied, and this fact highlights the preconditions for a comprehensive pharmacognostic study of *E. annuus* herb in order to obtain new substances from this plant. This work aims at the identification and quantification of amino acids in *E. annuus* herb.

It is known that the qualitative composition and quantitative content of amino acids significantly vary between different plants. The analysis of aqueous and hydroalcoholic extracts (70 % ethanol extracts) from raw materials showed that concentrationsof various amino acids in aqueous extracts were 2-5-fold higher andsometimes even more higher than those in hydroalcoholic extracts. For example, in aqueous extracts from yarrow 11 amino acids (350.98 μmol per 100 g of dry biomass) were detected; in St. John's wort, 13 amino acids (13075.0 μmol) were found, in wormwood, 12 amino acids (982.09 μmol) were detected; in white mistletoe, 13 amino acids (1316.82 μmol) were reported; in the leaves and stems of Jerusalem artichoke, 9 amino acids (19855.71 and 914.4 μmol) were found [14].

One of the promising research directions of Pharmacognosy Department of the National University of Pharmacy is a development of themodified drugs based on extracts from raw herbal drugs with amino acid additives. Twelve amino acids were used to obtain modified dry extracts from motherwort tincture. Based on the pharmacological studies, authors concluded that extracts modified with lysine, glycine, alanine, valine, phenylalanine and arginine are the promising substances. After the modification, these substances showed the anxiolytic activity. Moreover, the extract modified with phenylalanine showed the pronounced diuretic activity, which is a new type of pharmacological activity for preparations from motherwort herb [15].

An aim of the study was to establish the content of free and protein-bound amino acids in *E. annuus* herb.

Experimental Part

The *E. annuus* herb collected in the flowering stage in the Kharkiv and Sumy regions (Ukraine) during summer 2019.

Reagents: ACN (acetonitrile, CHROMASOLV® for HPLC, >=99.9%, *Sigma-Aldrich);* MeOH (methanol CHROMASOLV® for HPLC, >=99.9%, *Sigma-Aldrich);* Na_2HPO_4, anhydrous 99.99 % Suprapur® for trace analysis, Supelco®; H_2O Deionized Milli-Q; Agilent FMOC reagent, 2.5 mg/mL in acetonitrile, 9-fluorenylmethylchloroformate, 10x1mL; Agilent OPA reagent, 10 mg/mL each of *o*-phthalaldehyde and 3-mercaptopropionic acid in 0.4 M borate buffer mixture of amino acid standards (Agilent 5061-3334); HCl 37%, Sigma-Aldrich.

All amino acids were purchased from Sigma (Sigma-Aldrich, St. Louis, Mo., USA).

Determination of amino acids. A presence of amino acids was confirmed by studies of aqueous extracts from *E. annuus* herb using chromatographic paper Filtrak FN-7, *n*-BuOH-HOAc-H_2O (4:1:2) and three-fold chromatography. Amino acids were detected by spraying with a solution of ninhydrin (0.1 %) followed by heating in an oven at 96°C until spots of amino acids appear.

In raw materials, free protein-forming amino acids were quantified after the extraction of free amino acids; bound amino acids were determined after an acid hydrolysis of the preparations with subsequent HPLC analysis of hydrolysates using a pre-column derivatization by 9-fluorenylmethoxycarbonyl chloride and a fluorescence detector [16]. Each assay was performed in quintuplicate. The samples of raw materials were prepared and analyzed as follows:

a) free amino acids: 100 mg of the powdered preparation were placed in a vial, treated with 2 ml of 1N aqueous HCl solution and kept at 50 °C for 3 hours in an ultrasonic bath;

b) total content of amino acids: 100 mg of the preparation were placed into the vial, treated with 2 ml of 6 N aqueous HCl solution, and placed into a thermostatic chamber at 110°C for 24 h. Then, 0.5 ml of the centrifuged extract/hydrolysate was evaporated in a rotary vacuum evaporator, rinsed three times with distilled H_2O to remove HCl, re-suspended in 0.5 ml of distilled H_2O, and filtered through a 0.2 μm regenerated cellulose membrane. Amino acids identification was performed by comparison of their retention times with a mixture of amino acid standards (Agilent 5061-3334). The content of bound amino acids was determined by subtracting the content of free amino acids from their total content.

The chromatographic separation was performed on an Agilent 1200 liquid chromatograph (Agilent Technologies, USA) using Zorbax AAA column (150 mm × 4.6 mm × 3 μm), mobile phases A

(Na_2HPO_4, 40 mm, pH 7.8) and B (ACN– MeOH–H_2O, 45:45:10, v/v/v) in a gradient mode at a constant flow rate of 1.5 ml/min. The column was thermostated at 40 °C. The pre-column derivatization was carried out in automated programmed mode using FMOC and OPA. The derivatized amino acids were detected using a fluorescence detector [17-21].

Results and Discussion

The amino acid composition of *E. annuus* herb is given in Table 1.

As the result of HPLC analysis, 16 amino acids (391.4 μg/mg) were detected in *E. annuus* herb. The content of free amino acids was (4.66 μg/mg), the content of bound amino acids was (386.7 μg/mg); Figs.1, 2.

Nine amino acids were classified as essential amino acids (158.0 μg/mg), the content of non-essential amino acids was (233.2 μg/mg); Table 1. Proline (2.498 ± 0.035μg/mg), valine (0.302 ± 0.086 μg/mg), glutamic acid (0.30 ± 0.18 μg/mg), aspartic acid (0.24 ± 0.14 μg/mg), arginine (0.233 ± 0.078 μg/mg) were quantitatively dominating free amino acids; among the bound amino acids, proline (146.82 ± 0.03 μg/mg), arginine (67.861 ± 0.096 μg/mg), aspartic acid (24.263 ± 0.061 μg/mg), phenylalanine (25.806 ± 0.061 μg/mg) and histidine (20.40 ± 0.04 μg/mg) prevailed. Comparing the data obtained with the literature data on the content of amino acids in some plants, it should be noted that they differ depending both on the studied raw materials (leaves, flowers, herb, roots) and the plant species. For example, the HPLC analysis of amino acid composition of herb and roots of *Smallanthus sonchifolius* revealed the presence of 14 free and 15 protein-bound amino acids in herb, and 3 free and 14 protein-bound amino acids in roots. Among the free amino acids, proline (0.44±0.05 μg/mg), aspartic acid (0.12±0.05 μg/mg) were dominant in the herb; proline (0.28±0.05 μg/mg) dominated in roots. Aspartic acid (18.58±0.94 μg/mg), glutamic acid (16.33±0.58 μg/mg), proline (14.5±1.9 μg/mg) prevailed among the protein-bound amino acids in the herb; proline (3.14±0.16 μg/mg) dominated in roots. *E. annuus* contains a significant amount of amino acids, is widely used in the folk medicine; also, *E. annuus* is characterized by a sufficient raw material base, but in the chemical aspect is poorly studied, and these facts justify the prospect of further studies of *E. annuus.*

Table 1. The content of amino acids in *E. annuus* herb (μg/mg).

№	Aminoacid	RT, min	Free	Bound	Total
1.	Asp	1.715	0.24±0.14	24.263±0.061	24.502±0.001
2.	Glu	3.122	0.30±0.18	11.69±0.07	11.99±0.06
3.	Ser	6.256	0.20±0.25	16.650±0.056	16.849±0.021
4.	His*	7.325	0.09±0.08	20.40±0.04	20.481±0.092
5.	Gly	7.706	0.039±0.075	4.28±0.15	4.32±0.04
6.	Thr*	7.935	0.098±0.071	5.722±0.073	5.820±0.012
7.	Arg*	8.812	0.233±0.078	67.861±0.096	68.09±0.09
8.	Ala	9.366	0.182±0.096	18.204±0.005	18.38±0.37
9.	Tyr	10.775	0.045±0.013	7.70±0.02	7.841±0.003
10.	Val*	12.953	0.302±0.086	15.98±0.05	16.28±0.94
11.	Met*	13.253	-	1.131±0.042	1.13±0.52
12.	Phe*	14.648	0.220±0.064	25.806±0.061	26.03±0.53
13.	Iso*	14.875	0.125±0.043	5.480±0.080	5.61±0.49
14.	Leu*	15.629	0.094±0.031	5.387±0.065	5.48±0.51
15.	Lys*	16.046	-	9.29±0.06	9.29±0.07
16.	Pro	20.082	2.498±0.035	146.82±0.03	149.32±0.73
Total essential amino acids			1.158	157.045	158.203
Total non-essential amino acids			3.502	229.703	233.205
Total amino acids			4.66	386.748	391.408

Note: * essential amino acids.

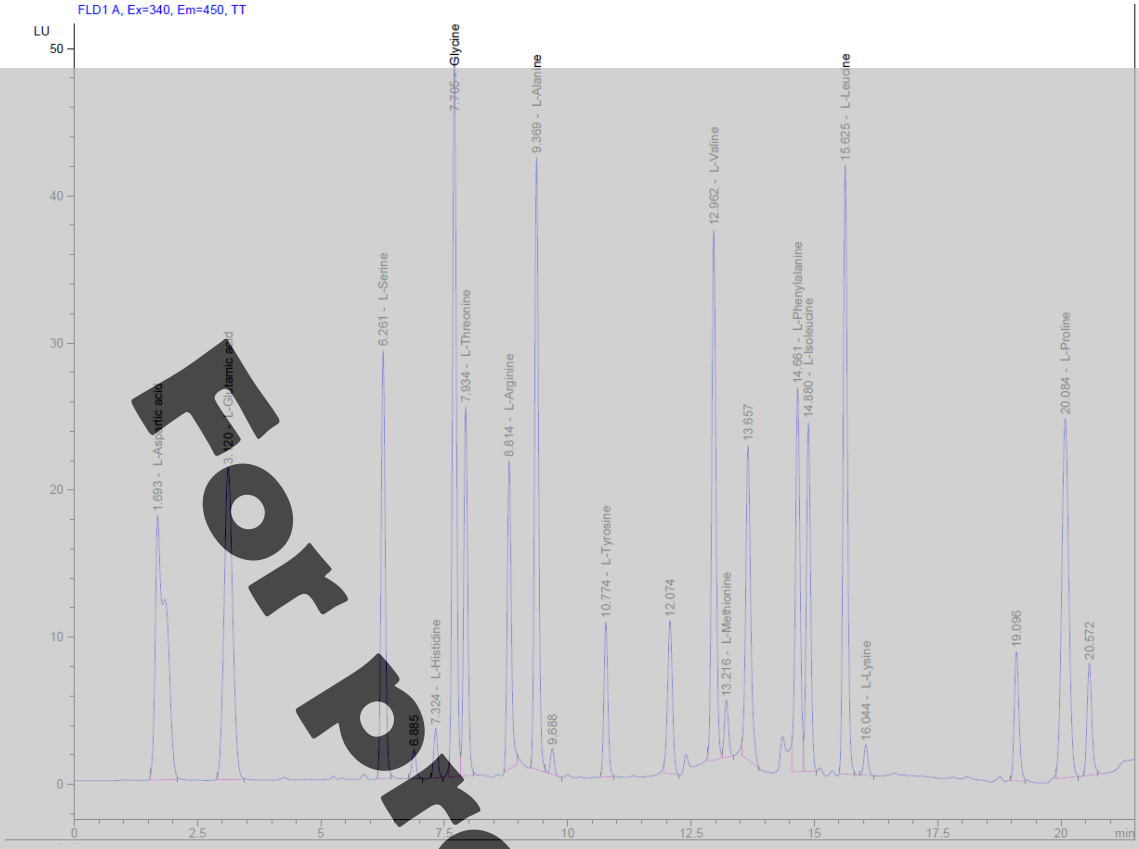

Fig. 1. HPLC chromatogram of bound amino acids of *E. annuus* herb.

Fig. 2. HPLC chromatogram of free amino acids of *E. annuus* herb.

Conclusions

The results obtained in this study emphasize the extreme importance of the amino acid composition of *Erigeron annuus*. The amino acids from the plant are involved in the important metabolic processes in human body. In light of the potential role of free and bound amino acids in pharmacological or physiological conditions, further studies of other parts of the plant are carried out to assess the possibility of their inclusion in dietary supplements and pharmaceuticals products.

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