PHARMACIA

Pharmacia 69(2): 337–343
DOI 10.3897/pharmacia.69.e82097

Research Article

HPLC-FLD analysis of amino acids content in *Chrysanthemum morifolium*

Alona Savych[1], Olha Polonets[2], Liubov Morozova[3], Kateryna Syrovatko[3], Tetiana Recun[2]

1 *I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine*
2 *National Pirogov Memorial Medical University, Vinnytsia, Ukraine*
3 *Vinnytsia National Agrarian University, Vinnytsia, Ukraine*

Corresponding author: Alona Savych (alonasavych@gmail.com)

Received 10 February 2022 ♦ **Accepted** 14 March 2022 ♦ **Published** 12 April 2022

Citation: Savych A, Polonets O, Morozova L, Syrovatko K, Recun T (2022) HPLC-FLD analysis of amino acids content in *Chrysanthemum morifolium*. Pharmacia 69(2): 337–343. https://doi.org/10.3897/pharmacia.69.e82097

Abstract

Chrysanthemum morifolium (*Asteraceae* family) have long been used as a tonic, antioxidant, antipyretic, analgesic, sedative, antitumor, neuroprotector, hepatoprotector and cardioprotector agent. This species should be reconsidered as possible sources of many biocompounds, especially amino acids. Thus, the aim of this study was to validate the chromatographic method for detection of amino acids and their identification in flowers and leaves of *Ch. Morifolium* of variant *Pectoral*. HPLC-FLD method was evaluated in terms of linearity, precision, repeatability, accuracy, LOD and LOQ. The calibration curves of all analytical standards of amino acids were linear ($R^2 > 0.99$) over the range of 0.015–0.625 μmol/mL, the LODs and the LOQs were in the range of 0.001–0.096 μg/mL and 0.004–0.321 μg/mL, respectively. During the HPLC-FLD assay ten amino acids in free form and fifteen amino acids after hydrolysis in *Ch. Morifolium* flowers were identified. Besides, twelve amino acids were detected in free form and fourteen amino acids after hydrolysis in *Ch. morifolium* leaves. The results of HPLC-FLD analysis showed that the predominant amino acid was *L*-proline in both types of herbal raw materials. Its total content was 31.67±0.02 μg/mg in *Ch. morifolium* flowers and 18.56±0.02 μg/mg in *Ch. morifolium* leaves. This phytochemical study confirms that flowers and leaves of *Ch. Morifolium* (*Pectoral*) are rich sources of amino acids and can exhibit a wide range of pharmacological activities.

Keywords

Chrysanthemum morifolium, amino acids, high-performance liquid chromatography-fluorescence detector, *L*-proline

Introduction

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) – a genus of flowering plants of *Asteraceae* family, which has more than 200 species of annual and perennial herbaceous plants or shrubs that grow in temperate subtropical areas of Southeast China and Japan (Shahrajabian 2019). The diversity and heterogeneity of *Asteraceae* family justifies the great importance of its individual members, which are known and used from ancient times, not only as food sources or as spices, but also for medicinal purposes. Several classes of compounds from *Asteraceae* family and *Chrysanthemum* species were studied and tested for different bio-activities and were reported as having medicinal potential (Marchyshyn et al. 2020; Youssef et al. 2020). Among these compounds, a special attention has been given to amino acids, which provide for these species important uses in the pharmaceutical and food industry, that are due to their important medicinal properties. They play an integral role in protein synthesis, hormone and enzyme production, help stimulate muscle growth and regeneration, are involved in energy production, play a role in fat metabolism and immune function, have

the detoxification activity, help regulate blood sugar levels, stimulate wound healing (Savych 2021; Savych and Mazur 2021; Savych and Milian 2021; Savych and Sinicgenko 2021). Thus, the essential amino acids are at the core of many vital processes. In this context and taking into consideration the fact that in the last decades these compounds have shown a significant importance in the field of medicinal compounds (Savych and Marchyshyn 2021a, b; Savych et al. 2021a, d, f, g, h), the *Asteraceae* family and *Chrysanthemum* species should be reconsidered as possible sources of amino acids.

Many species and varieties of *Chrysanthemum* are valuable ornamental plants that are widely used in landscaping in Europe and Asia. Chrysanthemums are now mainly grown as an ornamental plant but some species are used as medicinal (Marchyshyn et al. 2020; Youssef et al. 2020). In the folk medicine of Japan and China, the raw materials of *Ch. morifolium* have long been used as a tonic, detoxification, antipyretic, analgesic and sedative. Pharmacological studies of *Ch. morifolium* have shown that it influences on lipid and carbohydrate metabolism, has antioxidant, antitumor, neuroprotective, hepatoprotective and cardioprotective activities (Yang et al. 2017; Ryu et al. 2019; Mekapogu et al. 2020).

Today, chrysanthemums are grown mainly as ornamental plants, only certain species are used for medicinal purposes, but the phytochemical composition and mechanism of action on the human body is still poorly understood. That is why a detailed study of the genus of *Ch. Morifolium* and its biologically active substances that can have a certain pharmacological effect is an important task in pharmacy, because it will expand the potential sources for the manufacture of herbal medicines.

In addition it is important for medicine and pharmacy to study new promising plant species, as they can be a source of new phytodrugs that can have a numerous of advantages over synthetic agents, namely, they are low-toxic (Savych and Mala 2021), have a mild pharmacological effect and possibility to be used for long periods of time without significant side effects, have a complex activity through a numerous of biologically active compounds (Savych and Basaraba 2021; Savych and Milian 2021; Savych et al. 2021b, c, i, e).

Aim of the research

Thus, the aim of this study was to validate the chromatographic method for detection of amino acids and their identification in the herbal raw materials of *Ch. morifolium* of variant *Pectoral*.

Materials and methods (experimental part)

Plant materials

The herbal raw material, such as flowers and leaves of *Ch. morifolium* (*Pectoral*), cultivated on experimental areas of the National M. M. Hryshko Botanic Gardens of the National Academy of Sciences of Ukraine were used. Aerial parts of *Ch. morifolium* were harvested during a mass flowering period in 2019. The raw materials were dried, crushed and stored according to the general GACP requirements (WHO 2003). Plants were authenticated by prof. Svitlana Marchyshyn, Department of Pharmacognosy with Medical Botany, Ivan Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. A voucher specimen No. 324 was kept in departmental herbarium for future record.

Chemicals and standards

Standard mix of 17 amino acids AAS18 Supelco containing *L*-aspartic acid 2.5 µmol/mL, *L*-serine 2.5 µmol/mL, *L*-glutamic acid 2.5 µmol/mL, *L*-histidine 2.5 µmol/mL, glycine 2.5 µmol/mL, *L*-threonine 2.5 µmol/mL, *L*-arginine 2.5 µmol/mL, *L*-alanine 2.5 µmol/mL, *L*-tyrosine 2.5 µmol/mL, *L*-cystine 1.25 µmol/mL, *L*-valine2.5 µmol/mL, *L*-methionine 2.5 µmol/mL, *L*-phenylalanine 2.5 µmol/mL, *L*-isoleucine 2.5 µmol/mL, *L*-lysine 2.5 µmol/mL, *L*-leucine 2.5 µmol/mL, *L*-proline 2.5 µmol/mL of analytical standard grade was used and was purchased from Sigma-Aldrich Chemical Co. (USA) *o*-Phthalaldehyde (OPA) (≥ 99.0% purity HPLC); 9-fluorenylmethyl chloroformate (FMOC) (≥ 99.0% purity HPLC); acetonitrile (ACN) (≥ 99.9% purity HPLC); methanol (CH_3OH) (≥ 99.9% purity HPLC); hydrochloric acid (HCl) (ACS reagent, 37%); disodium hydrogen phosphate (Na_2HPO_4) (ACS reagent, ≥ 99.0%); sodium hydroxide (NaOH) (ACS reagent, ≥ 97.0%); sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10 H_2O$) (ACS reagent, ≥ 99.5%) were also used. The used in the studies water was produced by MilliQ Gradient water deionizaton system (USA).

Extraction of amino acids

For the extraction of free amino acids the samples of the herbal raw material were grinded into a powder by laboratory mill, then about 0.1 g (accurately weighed) was selected and placed into flask with 2.0 mL of 0.1 N HCl. The extractions were carried out in the ultrasonic water bath at 50 °C for 3 hours.

Extraction of bound amino acids was carried out by adding 2 mL of 6 N HCl to 0.3 g (accurately weighed) of powdered herbal raw materials. Hydrolysis was conducted for 24 hours in a thermostat at 110 °C.

The resulting extracts were centrifuged at 3000 rpm and 0.5 mL of supernatants were evaporated to dryness on a rotary evaporator washing three times with distilled water to remove HCl. Then, resulting product was resuspended in 0.5 mL water and filtered through disposable membrane filters with pores of 0.22 µm (Savych and Nakonechna 2021; Savych et al. 2022).

Pre-column derivatisation

The pre-column derivatization was conducted with a help of an automatic programmable regulations using derivatization reagents, which contained borate buffer 0.4 M

in water (pH 10.2), FMOC 2.5 mg/mL in ACN and OPA 10.0 mg/mL in 0.4 M borate buffer. A fluorescence detector (FLD) was used for identification of the derivatized amino acids.

Instrumentation and conditions of liquid chromatography-mass spectrometry

The amino acids composition in the herbal raw materials was studied by high-performance liquid chromatography with fluorescence detector (HPLC-FLD) using the liquid chromatograph Agilent 1200 (Agilent Technologies, USA) equipped with a G1313A autosampler, a G1311A quaternary pump, a G1316A thermostatted column and a G1315A fluorescence detector. The separation was performed on a Zorbax Eclipse-AAA chromatographic column (4.6 mm ± 150 mm, 3.5 µm) (Agilent Technologies, USA).

Table 1. Chromatographic conditions.

Mobile phase A	40 mM Na_2HPO_4, pH 7.8 [5.5 g NaH_2PO_4, monohydrate + 1 L of water, adjust to pH 7.8 with 10 N NaOH]
Mobile phase B	ACN : CH_3OH : H_2O (45:45:10, v/v/v)
Flow rate	2 mL/min
Column temperature	40 °C
Injection volume	2.5 mL
Stoptime	26 min

Table 2. Gradient mode.

Chromatography time, min	Mobile phase A, %	Mobile phase B, %
0:00	100	0
2:00	100	0
18:00	43	57
19:00	0	100
23:00	0	100
26:00	100	0

Identification and calculation by HPLC-FLD

Amino acid identification in the herbal raw materials was performed by comparing the retention times (t_R) of amino acid standards. The content of bound amino acids was determined by subtracting the content of free amino acids from their total content (Savych and Nakonechna 2021; Savych et al. 2022).

Method validation

HPLC-FLD method to quantify of amino acids was validated for linearity, limit of detection (LOD), limit of quantitation (LOQ), precision and repeatability according to the International Conference on Harmonization (ICH) guidelines. The standard mix of amino acids was used as a stock solution. Amino acids in this standard was 2.5 µmol/mL in 0.1 N HCl, except *L*-cystine at 1.25 µmol/mL. To obtained standard calibration solutions, the stock solution was dissolved in 0.1 N HCl to give concentrations in range of 0.015–0.625 µmol/mL. Linearity was performed by injecting a series of standard solutions with a threefold derivatization procedure and a single injection for standard of amino acids. The mean value and standard deviation, as well as regression analysis were calculated using Microsoft Excel software package 2016 (USA). The values for LOD and LOQ were calculated based on the data obtained during linearity testing in the low concentration range of the working in the test solution, using the following formulas: LOD = 3.3 * s / Slope; LOQ = 10 * s / Slope. Linearity testing was repeated with the same samples after a complete restart of the system with removement and re-installation of the column. Repeatability precision was determined by six-fold injection of the same sample in a row. For the resulting relative peak area of the quantifier ions the relative standard deviation (RSD) was calculated. To determine intra-day precision, six injection of amino acids reaction mixtures with the same concentration were single injected and the resulting relative peak areas were used to calculate the RSD. Inter-day precision for the day of sample preparation and the two following days was specified by injecting six standard sample of amino acids reaction mixtures once each on all three days. The RSD of the samples on that day together with the previous samples were calculated as above (Wang et. al 2020).

Results and discussion

The analytical procedure has been validated to confirm its reliability. All the peaks of analytical standard of amino acids showed good linearity ($R^2 > 0.99$) in a wide concentration range (0.015–0.625 µmol/mL). The results showed that the LODs and the LOQs of amino acids were in the range of 0.001–0.096 µg/mL and 0.004–0.321 µg/mL, respectively, indicating that the sensitivity of the method was satisfactory (Table 2). The repeatability of the subsequent derivatization and HPLC-FLD measurement of six standard samples with the same concentration resulted in precision values for the derivatization procedure. For intra- and inter-day precision, the RSD was in a range of 1.17% to 5.11%, which is acceptable.

According to the results of the HPLC-FLD assay, it was identified ten amino acids in free form in flowers (Fig. 1) and twelve amino acids in leaves of *Ch. morifolium* (*Pectoral*) (Fig. 3). HPLC analysis of amino acids after hydrolysis showed that *Ch. morifolium* flowers contained fifteen species of these compounds (Fig. 2) and *Ch. morifolium* leaves – fourteen (Fig. 4).

The results of quantitative study showed that the predominant amino acid in free form was *L*-proline in flowers and leaves of *Ch. morifolium* (*Pectoral*), its content was 2.55±0.01 µg/mg and 3.79±0.01 µg/mg, respectively. As for amino acids after hydrolysis, the predominant compound was *L*-proline in both types of herbal raw materials too. Its content was 29.12±0.02 µg/mg in *Ch. morifolium* flowers and 14.76±0.02 µg/mg in *Ch. morifolium* leaves (Table 4). In plants, *L*-proline accumulation is a common physiological response to various stresses but is also part of the developmental

Table 3. Results of linearity data obtained for analytical standards of amino acids after HPLC-FLD analysis.

Analytical standard of amino acids	t_R, min (SD±0.01)	R^2	LOD, µmol/mL	LOQ, µmol/mL
L-aspartic acid	2.62	0.999	0.005	0.017
L-serine	4.92	0.999	0.004	0.014
L-glutamic acid	7.43	0.999	0.001	0.004
L-histidine	8.28	0.998	0.001	0.004
glycine	8.67	0.999	0.002	0.005
L-threonine	8.85	0.999	0.018	0.060
L-arginine	9.38	0.999	0.010	0.035
L-alanine	10.07	0.998	0.003	0.011
L-tyrosine	11.15	0.999	0.004	0.013
L-cystine	12.43	0.999	0.002	0.006
L-valine	13.12	0.999	0.002	0.007
L-methionine	14.06	0.999	0.017	0.065
L-phenylalanine	14.52	0.999	0.004	0.013
L-isoleucine	14.72	0.999	0.012	0.044
L-lysine	15.33	0.999	0.096	0.321
L-leucine	15.58	0.998	0.002	0.007
L-proline	19.06	0.999	0.003	0.010

program in generative tissues (Meena et al. 2019). Proline – nonessential amino acid that have vital role in the structure of proteins and, also, exhibits significant hypoglycemic activity, which is due to a decrease in hepatic glucose production owing to inhibition of glycogenolysis, gluconeogenesis and glucose-6-phosphatase activity (Alqudah et al. 2021; Patriarca et al. 2021; Savych and Polonets 2021).

In addition, a high content of *L*-serine in bound form was detected in flowers and leaves of *Ch. morifolium* (*Pectoral*), its content was 11.81±0.02 µg/mg and 6.36±0.02 µg/mg, respectively (Table 4). Serine, as essential amino acid, is necessary for the full construction of DNA and RNA, as well as to produce muscle tissue and, accordingly, muscle growth (Šponer et al. 2018). It is also used by the body to produce the hemoglobin molecule. This amino acid is important for the full functioning of our immunity, promotes the production of immunoglobulins and antibodies. It is the starting product for the formation of other essential amino

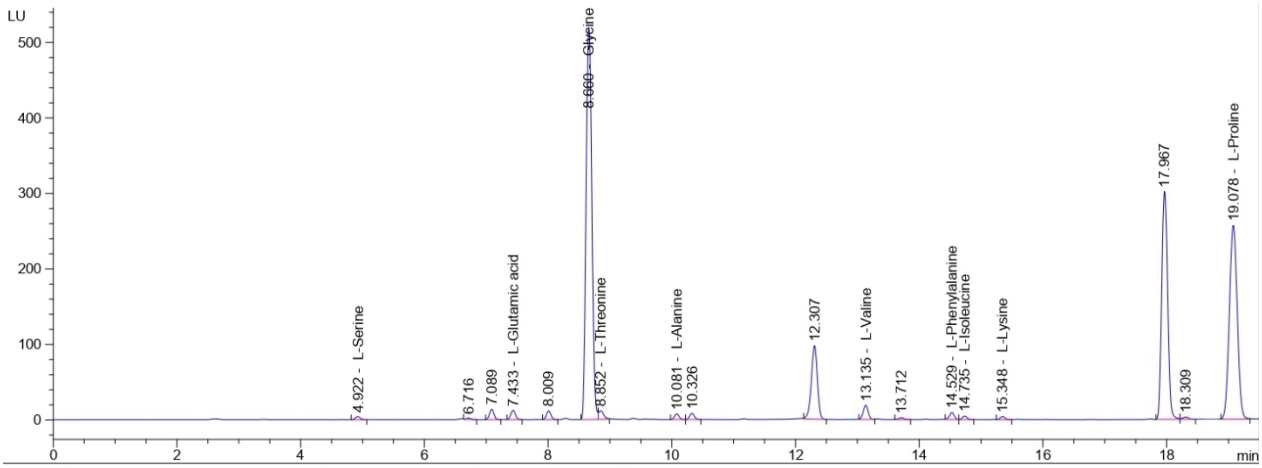

Figure 1. HPLC-FLD chromatogram of derivatives of free amino acids in flowers of *Ch. morifolium* (*Pectoral*).

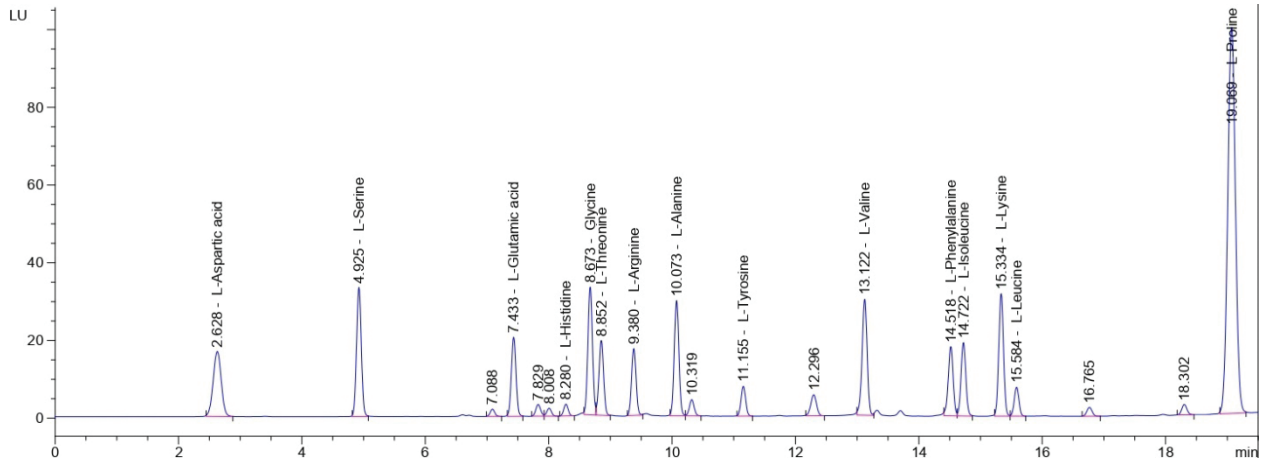

Figure 2. HPLC-FLD chromatogram of derivatives of amino acids after hydrolysis in flowers of *Ch. morifolium* (*Pectoral*).

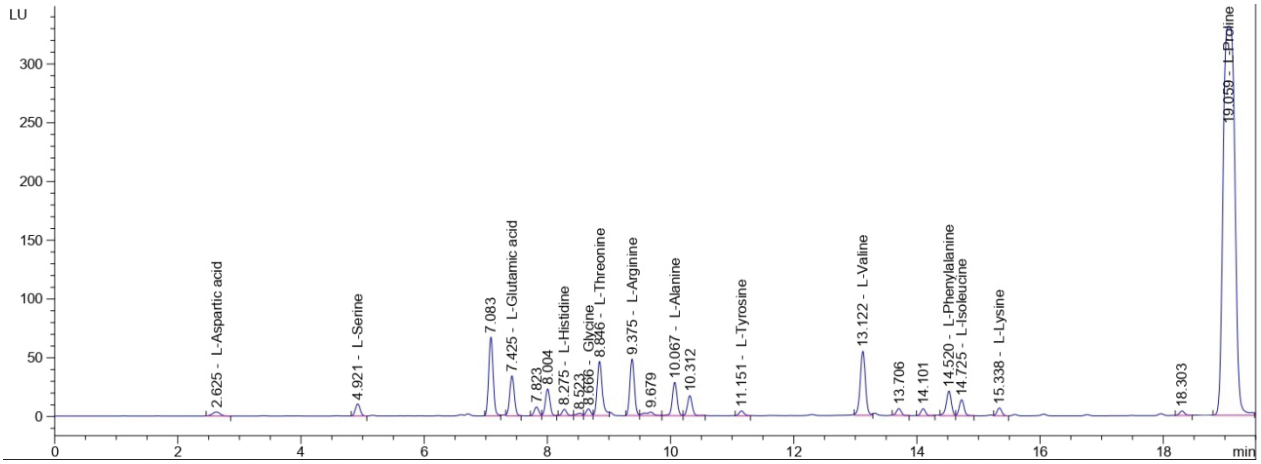

Figure 3. HPLC-FLD chromatogram of derivatives of free amino acids in leaves of *Ch. morifolium* (*Pectoral*).

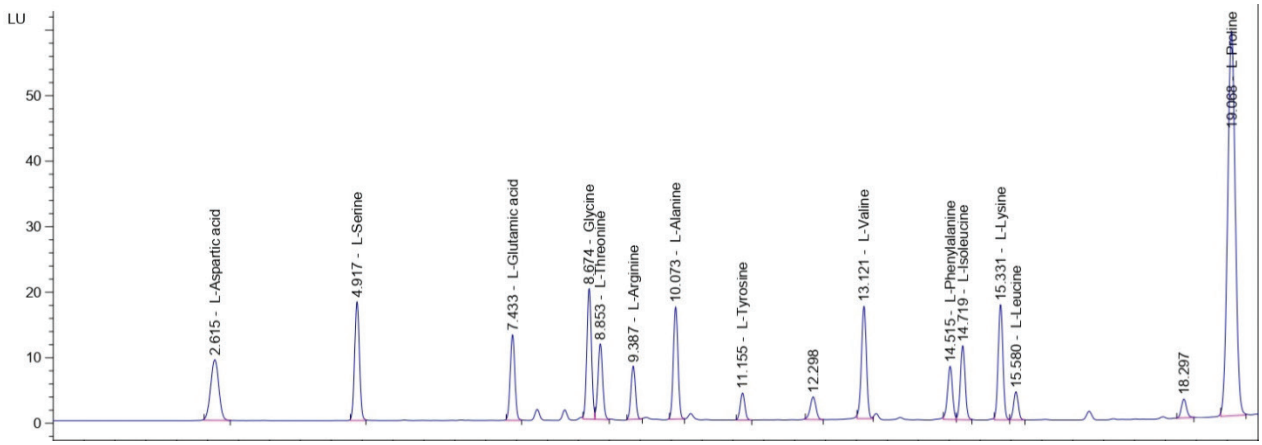

Figure 4. HPLC-FLD chromatogram of derivatives of amino acids after hydrolysis in leaves of *Ch. morifolium* Bailey (*Pectoral*).

Table 4. The results of the HPLC-FLD analysis of amino acids in the herbal raw materials of *Ch. morifolium*.

Identified substance	Content in *Ch. morifolium* (*Pectoral*), µg/mg					
	Flowers			Leaves		
	Free	Bound	Total	Free	Bound	Total
L-aspartic acid	n/d	10.35±0.02	10.35±0.02	n/d	5.75±0.02	5.75±0.02
L-serine	0.04±0.01	11.81±0.02	11.86±0.01	0.12±0.01	6.36±0.02	6.48±0.02
L-glutamic acid	0.14±0.01	7.14±0.02	7.27±0.02	0.16±0.01	4.47±0.02	4.63±0.01
L-histidine*	n/d	3.32±0.01	3.32±0.01	0.24±0.01	n/d	0.24±0.01
glycine	1.44±0.02	4.21±0.02	5.65±0.02	0.03±0.01	3.36±0.02	3.39±0.02
L-threonine*	0.11±0.01	5.44±0.01	5.55±0.01	0.15±0.01	3.22±0.01	3.37±0.01
L-arginine	n/d	6.40±0.02	6.40±0.02	0.04±0.01	2.93±0.01	2.97±0.01
L-alanine	0.05±0.01	6.17±0.02	6.21±0.01	0.17±0.01	3.37±0.01	3.55±0.01
L-tyrosine	n/d	3.30±0.01	3.30±0.01	n/d	1.75±0.01	1.75±0.01
L-cystine	n/d	n/d	n/d	n/d	n/d	n/d
L-valine*	0.10±0.01	5.34±0.01	5.45±0.01	0.28±0.01	2.79±0.01	3.07±0.01
L-methionine*	n/d	n/d	n/d	n/d	n/d	n/d
L-phenylalanine*	0.11±0.01	6.62±0.02	6.73±0.02	0.22±0.01	2.83±0.01	3.05±0.01
L-isoleucine*	0.04±0.01	5.07±0.01	5.10±0.01	0.13±0.01	2.91±0.01	3.04±0.01
L-lysine*	0.05±0.01	12.05±0.02	12.10±0.02	0.10±0.01	6.62±0.02	6.72±0.02
L-leucine*	n/d	5.64±0.02	5.64±0.02	n/d	3.22±0.01	3.22±0.01
L-proline	2.55±0.01	29.12±0.02	31.67±0.02	3.79±0.01	14.76±0.02	18.56±0.02

Note: 1. * – essential amino acid; 2. n/d – not detected; 3. Values are expressed as mean ± SD (n=6).

acids, in particular tryptophan, glycine, cysteine, and methionine. The amino acid – serine is a constituent of choline, ethanolamine, sarcosine, and phospholipids. When serine breaks down, pyruvate is formed, which is necessary for the conversion of glycogen into glucose (Akashi et al. 2018; Okamura et al. 2021).

Besides, another essential amino acid was identified in large quantities – *L*-lysine with content 12.05±0.02 μg/mg in *Ch. morifolium* flowers and 6.62±0.02 μg/mg in *Ch. morifolium* leaves (Table 4). Lysine has a wide range of biological effects, and above all, lysine is vital as a component of body proteins. This amino acid was found in large quantities in collagen, which provides strength to muscles, cartilage, ligaments and tendons. Indirectly, lysine strengthens bones, as it promotes the absorption of calcium from the intestines, with its deficiency, osteoporosis (increased bone fragility) can develop. Lysine plays an important role in the immune system, as it is needed in large quantities to produce antibodies (immunoglobulin). Lysine is part of the hormones and enzymes that regulate the body's metabolic processes (Min et al. 2018; Green and Lamming 2019; Severyanova et al. 2019).

This phytochemical study confirms that flowers and leaves of *Ch. morifolium* (*Pectoral*) are a rich sources of amino acids and these herbal raw materials can exhibit a wide range of pharmacological activities.

The authors have no funding to report.

The authors have declared that no competing interests exist.

The authors have no support to report.

Conclusion

The method was validated in terms of linearity, precision, repeatability, LOD and LOQ. HPLC-FLD assay of amino acids revealed that flowers and leaves of *Ch. morifolium* (*Pectoral*) represent important sources of bioactive compounds with a wide range of pharmacological activities. Ten amino acids in free form and fifteen amino acids after hydrolysis in *Ch. morifolium* flowers were identified. Twelve amino acids in free form and fourteen amino acids after hydrolysis were detected in *Ch. morifolium* leaves. The results of HPLC-FLD analysis showed that the predominant amino acid in free and bound form was *L*-proline in both types of herbal raw materials. Its content was 2.55±0.01 μg/mg and 29.12±0.02 μg/mg in *Ch. morifolium* flowers and in *Ch. morifolium* leaves it was 29.12±0.02 μg/mg and 14.76±0.02 μg/mg, respectively.

References

Akashi H, Okamura E, Nishihama R, Kohchi T, Hirai MY (2018) Identification and biochemical characterization of the serine biosynthetic enzyme 3-phosphoglycerate dehydrogenase in *Marchantia polymorpha*. Frontiers in Plant Science 9: e956. https://doi.org/10.3389/fpls.2018.00956

Alqudah A, Wedyan M, Qnais E, Jawarneh H, McClements L (2021) Plasma amino acids metabolomics' important in glucose management in type 2 diabetes. Frontiers in Pharmacology 12: e695418. https://doi.org/10.3389/fphar.2021.695418

Green CL, Lamming DW (2019) Regulation of metabolic health by essential dietary amino acids. Mechanisms of ageing and development 177: 186–200. https://doi.org/10.1016/j.mad.2018.07.004

Marchyshyn S, Polonets O, Savych A, Nakonechna S (2020) Determination of carbohydrates of *Chrysanthemum morifolium* L. leaves and flowers by GC-MS. Pharmakeftiki Journal 32(4): 202–212.

Meena M, Divyanshu K, Kumar S, Swapnil P, Zehra A, Shukla V, Yadav M, Upadhyay RS (2019) Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. Heliyon 5(12): e02952. https://doi.org/10.1016/j.heliyon.2019.e02952

Mekapogu M, Vasamsetti B, Kwon OK, Ahn MS, Lim SH, Jung JA (2020) Anthocyanins in floral colors: biosynthesis and regulation in *Chrysanthemum* flowers. International journal of molecular sciences 21(18): e6537. https://doi.org/10.3390/ijms21186537

Min K, Yoon HJ, Matsuura A, Kim YH, Lee HH (2018) Structural basis for recognition of L-lysine, L-ornithine, and L-2,4-diamino butyric acid by lysine cyclodeaminase. Molecules and cells 41(4): 331–341. https://doi.org/10.2210/pdb5yu4/pdb

Okamura E, Ohtaka K, Nishihama R, Uchida K, Kuwahara A, Mochida K, Hirai MY (2021) Diversified amino acid-mediated allosteric regulation of phosphoglycerate dehydrogenase for serine biosynthesis in land plants. The Biochemical Journal 478(12): 2217–2232. https://doi.org/10.1042/BCJ20210191

Patriarca EJ, Cermola F, D'Aniello C, Fico A, Guardiola O, De Cesare D, Minchiotti G (2021) The multifaceted roles of proline in cell behavior. Frontiers in cell and developmental biology 9: e728576. https://doi.org/10.3389/fcell.2021.728576

Ryu J, Nam B, Kim BR, Kim SH, Jo YD, Ahn JW, Kim JB, Jin CH, Han AR (2019) Comparative analysis of phytochemical composition of gamma-irradiated mutant cultivars of *Chrysanthemum morifolium*. Molecules 24(16): e3003. https://doi.org/10.3390/molecules24163003

Savych A (2021) Anti-inflammatory effect of antidiabetic mixture on a model of carrageenan edema. PharmacologyOnLine 3: 38–44.

Savych A, Basaraba R (2021) Ascorbic acid content in the herbal mixture with antidiabetic activity. PharmacologyOnLine 2: 76–83.

Savych A, Mala O (2021) Acute toxicity studies of aqueous extracts of plant antidiabetic mixtures. PharmacologyOnLine 3: 716–723.

Savych A, Marchyshyn S (2021a) Inhibition of pancreatic lipase by water extracts of some herbal mixtures. PharmacologyOnLine 2: 1457–1463.

Savych A, Marchyshyn S (2021b) Inhibition of pancreatic α- glucosidase by water extracts of some herbal mixtures. PharmacologyOnLine 2: 1450–1456.

Savych A, Mazur O (2021) Antioxidant activity *in vitro* of antidiabetic herbal mixtures. PharmacologyOnLine 2: 17–24.

Savych A, Milian I (2021) Total flavonoid content in the herbal mixture with antidiabetic activity. PharmacologyOnLine 2: 68–75.

Savych A, Nakonechna S (2021) Determination of amino acids content in two herbal mixtures with antidiabetic activity by GC-MS. Pharmakeftiki 33(2): 116–123.

Savych A, Polonets O (2021) Study of hypoglycemic activity of antidiabetic herbal mixture on streptozotocin-nicotinamide-induced rat model of type 2 diabetes. PharmacologyOnLine 2: 62–67.

Savych A, Sinichenko A (2021) Screening study of hypoglycemic activity of the herbal mixtures used in folk medicine (message 4). PharmacologyOnLine 2: 1254–1262.

Savych A, Basaraba R, Gerush O (2021a) Comparative analysis of hypoglycemic activity of herbal mixtures by glucose tolerance tests (message 2). PharmacologyOnLine 2: 1118–1127.

Savych A, Bilyk O, Vaschuk V, Humeniuk I (2021b) Analysis of inulin and fructans in *Taraxacum officinale* L. roots as the main inulin-containing component of antidiabetic herbal mixture. Pharmacia 68(3): 527–532. https://doi.org/10.3897/pharmacia.68.e66266

Savych A, Duchenko M, Shepeta Y, Davidenko A, Polonets O (2021c) Analysis of carbohydrates content in the plant components of antidiabetic herbal mixture by GC-MS. Pharmacia 68(4): 721–730. https://doi.org/10.3897/pharmacia.68.e69107

Savych A, Gerush O, Basaraba R (2021d) Determination of hypoglycemic activity of the herbal mixtures by means of glucose loading tests (message 3). PharmacologyOnLine 2: 1128–1137.

Savych A, Marchyshyn S, Kyryliv M, Bekus I (2021e) Cinnamic acid and its derivatives in the herbal mixtures and their antidiabetic activity. Farmacia 69(3): 595–601. https://doi.org/10.31925/farmacia.2021.3.23

Savych A, Marchyshyn S, Milian I (2021f) Inhibition of pancreatic α-amylase by water extracts of some herbal mixtures. PharmacologyOnLine 2: 1443–1449.

Savych A, Marchyshyn S, Mosula L, Kravchyk L (2021g) HPLC analysis of flavonoids contained in the plant components of antidiabetic mixture. PharmacologyOnLine 3: 129–139.

Savych A, Marchyshyn S, Mosula L, Kryskiw L (2021h) Spectrophotometric determination of *L*-ascorbic acid in the herbal antidiabetic mixtures. PharmacologyOnLine 3: 118–128.

Savych A, Vorontsova T, Marchyshyn S (2021i) Study of polysaccharide fractions content in plant antidiabetic mixtures. PharmacologyOnLine 3: 975–982.

Savych A, Marchyshyn S, Mosula L, Bilyk O, Humeniuk I, Davidenko A (2022) Analysis of amino acids content in the plant components of the antidiabetic herbal mixture by GC-MS. Pharmacia 69(1): 69–76. https://doi.org/10.3897/pharmacia.69.e77251

Severyanova LA, Lazarenko VA, Plotnikov DV, Dolgintsev ME, Kriukov AA (2019) L-lysine as the molecule influencing selective brain activity in pain-induced behavior of rats. International Journal of Molecular Sciences 20(8): e1899. https://doi.org/10.3390/ijms20081899

Shahrajabian MH (2019) A review of *Chrysanthemum*, the eastern queen in traditional Chinese medicine with healing power in modern pharmaceutical sciences. Applied Ecology and Environmental Research 17: 13355–13369. https://doi.org/10.15666/aeer/1706_1335513369

Šponer J, Bussi G, Krepl M, Banáš P, Bottaro S, Cunha RA, Gil-Ley A, Pinamonti G, Poblete S, Jurečka P, Walter NG, Otyepka M (2018) RNA structural dynamics as captured by molecular simulations: a comprehensive overview. Chemical Reviews 118(8): 4177–4338. https://doi.org/10.1021/acs.chemrev.7b00427

WHO (2003) WHO guidelines on good agricultural and mixture practices (GACP) for medicinal plants. World Health Organization, Geneva, 72 pp. https://apps.who.int/iris/handle/10665/42783

Yang L, Nuerbiye A, Cheng P, Wang JH, Li H (2017) Analysis of floral volatile components and antioxidant activity of different varieties of Chrysanthemum morifolium. Molecules 22(10): e1790. https://doi.org/10.3390/molecules22101790

Youssef FS, Eid SY, Alshammari E, Ashour ML, Wink M, El-Readi MZ (2020) *Chrysanthemum indicum* and *Chrysanthemum morifolium*: chemical composition of their essential oils and their potential use as natural preservatives with antimicrobial and antioxidant activities. Foods 9(10): e1460. https://doi.org/10.3390/foods9101460