PHARMACIA

Pharmacia 70(4): 1231–1238
DOI 10.3897/pharmacia.70.e111279

Research Article

Analysis of carboxylic acids of *Astragalus dasyanthus* Pall. herb

Olha Khvorost[1], Yevheniia Zudova[1], Liliia Budniak[2], Liudmyla Slobodianiuk[2], Hanna Kramar[3], Olha Palamarchuk[3], Anna Ocheretniuk[3]

1 *National University of Pharmacy, Kharkiv, Ukraine*
2 *Ternopil National Medical University, Ternopil, Ukraine*
3 *National Pirogov Memorial Medical University, Vinnytsya, Ukraine*

Corresponding author: Liliia Budniak (stoyko_li@tdmu.edu.ua)

Received 17 August 2023 ♦ **Accepted** 13 October 2023 ♦ **Published** 25 October 2023

Citation: Khvorost O, Zudova Ye, Budniak L, Slobodianiuk L, Kramar H, Palamarchuk O, Ocheretniuk A (2023) Analysis of carboxylic acids of *Astragalus dasyanthus* Pall. herb. Pharmacia 70(4): 1231–1238. https://doi.org/10.3897/pharmacia.70.e111279

Abstract

One of the fundamental issues in modern pharmacy is the expansion of the range of medications based on plant-derived raw materials with specific targeted effects. An example of such a plant is the woolly-flowered milkvetch (*Astragalus dasyanthus* Pall.). This perennial herbaceous medicinal plant belongs to the Fabaceae family and is listed in the Red Book of Europe. *Astragalus dasyanthus* Pall. is cultivated for pharmaceutical purposes. The herb of a plant exhibits sedative, cardiotonic, hypotensive, and diuretic effects, as well as hepatoprotective and antioxidant properties. The herb of *Astragalus dasyanthus* Pall. contains polysaccharides (arabinose, bassorin), flavonoids (quercetin, kaempferol), glycyrrhizin, starch, and triterpenoid saponins, which contribute to the broad spectrum of the plant's pharmacological activity. Continuing the investigation of this plant, it is advisable to study the qualitative and quantitative composition of organic and fatty acids. By the HPLC method, the following organic acids were identified and determined quantitative content: tartaric (8.68 mg/g), citric (7.69 mg/g), oxalic (6.90 mg/g), succinic (5.01 mg/g), isocitric (1.30 mg/g), and malic (0.49 mg/g). The saturated and unsaturated fatty acids were determined by the GC/MS method. The *Astragalus dasyanthus* Pall. herb contained seven fatty acids. High concentrations of fatty acids such as pentadecanoic acid (4.16 mg/g), linoleic acid (1.73 mg/g), and stearic acid (1.14 mg/g) predominate in woolly-flowered milkvetch. The character of many processes in which these organic and fatty acids take part may be associated with the medicinal properties of this plant pursuant to its use in medicine and, therefore, may contribute to the insight into its therapeutic properties.

Keywords

Astragalus dasyanthus Pall., fatty acids, GC/MS, herb, HPLC, organic acids

Introduction

The genus *Astragalus* is the largest in the Fabaceae family, comprising more than 2,500 species (Chaudhary et al. 2008). *Astragalus* plants, including medicinal and poisonous species, are annual or perennial herbs, small shrubs – often spiny, glabrous, or hairy – with hairs basifixed or bifurcate (Xu and Podlech 2010). The center of origin of *Astragalus* plants is Eurasia, especially the mountainous parts of South-Central and South-Western Asia (Podlech 1986; Lock and Schrire 2005).

Species of the *Astragalus* genus are valued in folk medicine throughout the world and utilized as medicinal herbs against chronic bronchitis, cough, stomach

ulcer, hypertension, diabetes, and gynecological disorders (Benchadi et al. 2013). Some plants of the same genus have cardiovascular, antiviral and immunostimulant activities (Verotta and El-Sebakhy 2001b; Yalcin et al. 2012). The plants of the genus *Astragalus* have been intensively analyzed, mainly for flavonoids, polysaccharides, and saponins, which are the main groups of biologically active compounds (Verotta and El-Sebakhy 2001a; Pistelli 2002). Flavonoids represent the largest group of polyphenolic compounds occurring in *Astragalus* species (Bratkov et al. 2016). The plants of this genus also include components such as anthraquinones, alkaloids, amino acids, β-sitosterol, and metallic elements (Li et al. 2014).

An urgent task of modern pharmacy is the search for plants that can supplement the range of official species, as well as the creation of new drugs based on them (Budniak et al. 2021b). Since ancient times, members of the *Astragalus* genus, especially *A. membranaceus*, *A. mongholicus*, *A. dasyanthus*, *A. trigonius*, and *A. gummifera*, have been used as important drugs in traditional Chinese medicine. Antifungal and antibacterial effects of *Astragalus* genus have been established in many researches (Wang et al. 2014; Jaradat et al. 2017). Some species of this genus have shown anti-protozoal effects (Özipek et al. 2005). The protective effect of *Astragalus* L. on the liver (Jia et al. 2012), cardiovascular system (Han et al. 2011; Zhao et al. 2015), and neurodegenerative diseases (Luo et al. 2004) suggest the presence of antioxidant components in their tissues (Chen et al. 2015).

Astragalus dasyanthus Pall. belongs to the family Fabaceae. The plant grows in Moldova, Crimea, along the Black Sea coast, and other areas. *Astragalus dasyanthus* is a rare plant species and is vulnerable in Ukraine; it has also been listed as endangered on the Red List of Europe (Ganea and Gacota 1996). One of the most effective ways to preserve biodiversity is through plant introduction. The introduction of *Astragalus dasyanthus* into plant cultivation in Ukraine also holds significant scientific significance. This is primarily due to their valuable medicinal properties; individual representatives of Astragalus have led to the natural habitats facing intensive anthropogenic pressure (Bondarchuk and Rakhmetov 2018).

Astragalus dasyanthus Pall. is a valuable herb. In medicine, the overhead parts are used and gathered during the blooming period. The herb of this plant contains flavonoids and triterpenoid glucosides (Ganbarov and Ibrahimov 2015). Experimental studies have shown that preparations of *Astragalus dasyanthus* exhibit hypotensive, diuretic, and sedative properties, and also expand coronary vessels (Khoron'ko and Glyzin 1973). In folk medicine, a decoction of the woolly herb of *Astragalus dasyanthus* is used as an expectorant and diuretic, as well as for asthenia, kidney disease, burns, rheumatism, rinsing the mouth and pharynx in cases of angina, and stomatitis (Kodirova 2021).

The genus *Astragalus* is well-known for being a rich source of bioactive secondary metabolites. However, no phytochemical investigations of primary metabolites in *Astragalus dasyanthus* have ever been reported. This deficiency in experimental data motivated us to determine the presence of organic and fatty acids in the herb of the *Astragalus dasyanthus*, cultivated in Ukraine.

Materials and methods

Plant materials

Astragalus dasyanthus herb was selected as the object of study. The raw materials were provided by the Department of Cultural Flora of M. Gryshko National Botanic Garden of the National Academy of Sciences of Ukraine (Marchyshyn et al. 2021b, c). The herb was collected in 2021. The plant was identified by Prof. Olha Khvorost, National University of Pharmacy (Kharkiv, Ukraine), where a voucher specimen, with the identification number (763/ACCE), was preserved. The herb was dried using the conventional method and then stored in paper bags in a dry, protected from direct sunlight place (Budniak et al. 2021d; Feshchenko et al. 2021; Slobodianiuk et al. 2021a).

Chemicals and standards

Standards of organic acids, including pyruvic acid, tartaric acid, citric acid, isocitric acid, malic acid, and succinic acid, obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) were of analytical grade (≥95% purity).

Fatty acids were identified by the reference standard mixture FAME (Supelco, Belle fonte, PA, USA). The internal standard undecanoic acid (≥98% purity) used for metabolite quantification was purchased from Sigma-Aldrich (St. Louis, MO).

HPLC method determination of organic acids

Instrumentation and conditions

HPLC analysis of organic acids was performed using Agilent 1200 (Agilent Technologies, USA) (Agius et al. 2018; Marchyshyn et al. 2021a). Mobile phase: A – acetonitrile (CH3CN); mobile phase B – 0.1% solution of phosphoric acid in water (1:99, v/v). Elution was performed in isocratic mode. Separation was performed on a Zorbax SB-Aq chromatographic column (4.6 mm ± 150 mm, 3.5 μm) (Agilent Technologies, USA), column flow rate 0.5 ml/min, the temperature of the thermostat column is 30 °C, volume injection 3 μl (Budniak et al. 2021a; Slobodianiuk et al. 2021b, c). Detection was performed using a diode-matrix detector with signal registration at 210 nm and fixation of absorption spectra in the range of 210–700 nm.

Standard solutions

Standard solutions (1000 ppm) of pyruvic, tartaric, citric, isocitric, succinic, and malic acids were prepared in the mobile phase consisting of 0.1% phosphoric acid solution. Stock solutions of every organic acid were made in the mobile phase by respective dilutions.

Extraction of organic acids

700 mg of powdered raw material was placed in a vial and extracted in 10 ml of 0.1% solution of phosphoric acid. Extraction was performed in the ultrasonic bath at 80 °C for 4 h. The obtained extract was centrifuged at 3000 rpm/min for 30 minutes and filtered through the Supelco Discovery DSC-18 filter.

Identification and quantification content of organic acids was performed using standard solutions of dicarboxylic compounds (pyruvic, tartaric, citric, isocitric, malic and succinic acids).

GC/MS determination of fatty acids

Chromatographic condition

GC/MS analysis of fatty acids was performed using gas chromatograph Agilent 6890N with mass detector 5973 inert (Agilent Technologies, USA). Samples were analyzed on a silica capillary column HP-5MS (30 m × 0.25 mm × 0.25 mkm, Agilent Technologies, USA). The interface was operated at 250 and 380 °C respectively. The initially set up oven temperature at 60 °C for 4 min, then at the rate of 4 °C/min raised to 250 °C and kept at this point for 6 min and maintained at a final temperature for 7 min. The carrier gas was used helium at a constant flow rate of 1.0 ml/min. The sample with a volume of 1 µl was injected in a splitless mode using a 7683 series Agilent Technologies injector. Detection was performed in scan mode in the range (38–400 m/z) (Slobodianiuk et al. 2022).

Sample preparation with pre-column derivatization

Samples of herbal raw materials were ground into a powder by laboratory mill and about 0.5 g (accurately mass) were selected and placed into a glass vial. Then 3.3 ml of reacting mixture (methanol: toluene: sulfuric acid (44:20:2 v/v)) with 1.7 ml of internal standard solution (undecanoic acid in heptane solution) was added. The obtained samples were stood at 80 °C for 2 hours, refrigerated and centrifuged for 10 minutes at 5000 rpm. It was taken 0.5 ml of the upper heptane phase, which contains methyl esters of fatty acids (Atolani et al. 2015; Budniak et al. 2021c).

The compositions of the product obtained were identified by comparison of their mass-spectrums with data obtained from the National Institute Standard and Technology (NIST 2008) database. The quantitative content of fatty acids was done using the internal standard of undecanoic acid in heptane solution added to the sample.

Method validation

The performance of the proposed method of determining fatty acids was tested using a number of qualitative and quantitative parameters. The identification criteria for working range determination (calibration curve range) were LOQ, linearity, and calibration model fits (correlation) (Slobodianiuk et al. 2022). Table 1 shows the values obtained for the linearity ranges, the determination coefficients (R2), and the limits of detection (LOD) and quantification (LOQ). The working range was set as the range of concentrations from the LOQ to the maximum of the calibration curve, maintaining the correlation coefficient (R^2) above 0.995. The calibration curves were constructed over the range of 0.4–0.004 mg/ml by replicate injections (n = 3) of standard mixtures. The calibration curves, determined by the least-squares regression method were linear over the range, with R^2 above 0.995 (Table 1). It was found that the linear fit was an appropriate calibration model for all fatty acids in the analyzed samples.

Table 1. Performance parameters of the fatty acid determination method.

Fatty acid	Correlation coefficient R^2	Limit of detection LOD, µmol/ml	Limit of quantification LOQ, µmol/ml
undecanoic acid	0.999	0.0086	0.0143
pentadecanoic acid	0.996	0.0159	0.0265
benzenepropanoic acid	0.995	0.0204	0.034
linoleic acid	0.998	0.0128	0.0256
stearic acid	0.999	0.0265	0.0318
arachidic acid	0.997	0.0157	0.0262
behenic acid	0.995	0.0206	0.0343
lignoceric acid	0.999	0.0229	0.0383

Quality parameters of the proposed HPLC method were established with pure organic acid standards and selected from the raw materials samples. Results have been summarized in Table 2. Linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ)were investigated as method validation parameters. The compound peaks were identified by their retention times and compared with the standards. Quantification was performed with five-point external calibration curves. Precision was determined as repeatability (six successive injections) and intermediate precision (three injections on five different days in one week). Accuracy was determined as a recovery for two different concentration levels. The LOD was determined as LOD = 3 × SD/slope and the LOQ as LOQ = 10 × SD/slope at the low concentration calibration level.

Table 2. Performance parameters of the organic acid determination method.

Organic acid	Correlation coefficient R^2	Limit of detection LOD, µg/ml	Limit of quantification LOQ, µg/ml
tartaric acid	0.9974	0.81	2.7
oxalic acid	0.9948	0.22	0.75
citric acid	0.9999	0.26	0.87
isocitric acid	0.9930	0.10	0.33
malic acid	1.0000	0.16	0.55
succinic acid	0.9982	0.13	0.43

Results and discussion

The quantitative content of fatty acids is present in Table 3.

As shown in Fig. 1, Table 3, *Astragalus dasyanthus* herb contains a mixture of fatty acids saturated (7.06 mg/g; 80.32%) and polyunsaturated (1.73 mg/g; 19.68%).

Unsaturated fatty acids play an essential role in the body's vital functions. These acids exhibit F-vitamin activity, which improves blood circulation in all bodily organs and tissues, consequently facilitating the renovation of the entire human body (Magnusdottir et al. 2014; Molfino et al. 2014; Makhatova et al. 2016).

Fatty acids play various roles in biological systems, such as being components of plasma membranes and functioning as signaling molecules that regulate cell growth, differentiation, and gene expression (Das 2006; Calder 2015; Czumaj and Śledziński 2020).

Saturated fatty acids serve a significant biological function as they act as an energy source for the human body. Additionally, they play a significant role in constructing cell membranes, synthesizing hormones, and facilitating the transfer and absorption of vitamins and trace elements (Marchyshyn et al. 2021d). As a result, fatty acids possess a vast range of opportunities to regulate the immune functions of the cell by impacting its structure, metabolism, and function (Brown and Marnett 2011; Radzikowska et al. 2019). These acids in humans are ingested with foods and they are synthesized by cells (Radzikowska et al. 2019). Polyunsaturated fatty acids are important cellular components that significantly impact the proper development and functioning of various organisms (Ganbarov and Ibrahimov 2015; Czumaj and Śledziński 2020).

The results of the study showed that the major components of *Astragalus dasyanthus* herb were pentadecanoic acid (4.16 mg/g; 47.33%), linoleic acid (1.73 mg/g; 19.68%), and stearic acid (1.14 mg/g; 12.97%). The content of other fatty acids is lower. Mounting experimental evidence supports the nutritionally beneficial role of

Table 3. The results of the determination of fatty acids in *Astragalus dasyanthus* Pall. herb.

No.	Retention time	Common name of fatty acid (IUPAC)	Quantitative content of methyl esters of fatty acids	
			mg/g	% of the total number of identified
Saturated acids				
1.	14.29	(Pentadecanoic)	4.16	47.33
2.	14.65	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	0.16	1.82
3.	18.89	Stearic (octadecanoic)	1.14	12.97
4.	23.16	Arachidic (eicosanoic)	0.58	6.60
5.	27.12	Behenic (docosanoic)	0.82	9.33
6.	30.81	Lignoceric (tetracosanoic)	0.20	2.27
Polyunsaturated acids				
7.	18.13	Linoleic (octadecadienoic)	1.73	19.68
The amount of saturated fatty acids			**7.06**	**80.32**
The amount of unsaturated fatty acids			**1.73**	**19.68**
Total			**8.79**	**100**

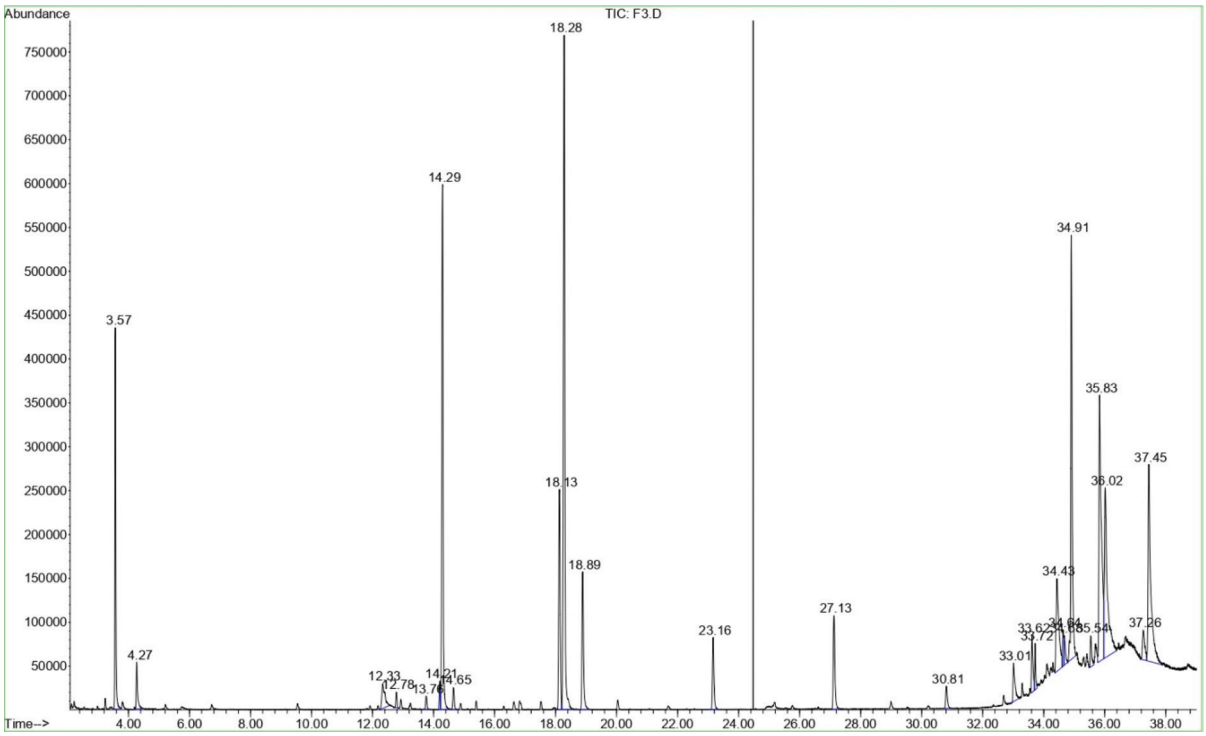

Figure 1. GC/MS chromatogram of fatty acids in the *Astragalus dasyanthus* Pall. herb.

odd-chain saturated fatty acids for human health. Notably, pentadecanoic acid exhibited specific cytotoxic effects in MCF-7/SC cells compared to the parental cells. This suggests that pentadecanoic acid could act as a novel inhibitor of the JAK2/STAT3 signaling pathway in breast cancer cells, indicating the potential benefits of consuming pentadecanoic acid-rich foods during breast cancer treatments (To et al. 2019). This herb is also a source of the fatty acid omega-6 (linoleic acid), also known as linoleate. This fatty acid must be obtained through the diet because the body requires them but cannot synthesize them. Therefore, this acid is considered essential and must be supplied by the diet to be the starting point for the synthesis of a variety of other unsaturated fatty acid (De Lorgeril et al. 2001; Anez-Bustillos et al. 2018; Radzikowska et al. 2019).

Linoleic acid serves a multitude of crucial functions within the body, including the synthesis of bile acids in the liver, the regulation of metabolic processes, the production of prostaglandins, the maintenance of hormonal balance, and the enhancement of digestive enzymes. Within the body, linoleic acid is converted into γ-linolenic acid, which exhibits high activity and further transforms into prostaglandin E1, contributing to an improved immune response. Prostaglandins also play a role in suppressing inflammatory processes, regulating brain function, reducing the risk of vascular and cardiac diseases, normalizing the nervous system, and controlling metabolism and insulin levels. Additionally, linolenic acid helps in regulating blood pressure and blood cholesterol levels (Stoyko et al. 2015; Marchyshyn et al. 2021d).

Stearic acid is a saturated fatty acid found in fats derived from both animal and plant sources. After palmitic acid, it takes the lead as the most extensively consumed fatty acid in both the United States and the Western population at large (Ervin et al. 2004). Stearic acid amounts to 26% of the total intake of saturated fatty acids (Valenzuela et al. 2011). Also, stearic acid, another example of an even-chain saturated fatty acid, was found to inhibit breast cancer cell proliferation in vitro (Wickramasinghe et al. 1996; Evans et al. 2009) and breast tumorigenesis in vivo (Habib et al. 1987). This underscores the dual impact of saturated fatty acids on cancer cells (To et al. 2020). Certain saturated fatty acids are known to raise low-density lipoprotein (bad) cholesterol levels, but stearic acid, in contrast, does not have this effect (FAO 2010). Currently, stearic acid is the only suitable choice for producing solid fats that are considered safe (Crupkin and Zambelli 2008; Valenzuela et al. 2011; Anushree et al. 2017).

The absorption of fatty acids in the human digestive system primarily relies on the position they occupy in dietary triglycerides (Mu and Høy 2004), which constitute approximately 90–95% of our fat intake (Valenzuela et al. 2011).

In total, six organic acids were determined in the *Astragalus dasyanthus* herb, including tartaric, oxalic, citric, isocitric, malic and succinic acids by means of the HPLC method (Fig. 2, Table 4)..

As shown in Table 4, the highest tartaric (8.68 mg/g), citric (7.69 mg/g), oxalic (6.90 mg/g) and succinic (5.01 mg/g) acids content were found in *Astragalus dasyanthus* herb. Tartaric acid possesses anti-inflammatory and antioxidant properties, which help in keeping the immune system healthy. It finds pharmaceutical applications in formulating effervescent tablets, powders, and antibiotic tablets, as well as drugs for heart diseases. Moreover, in cosmetic industry extensively utilizes tartaric acid, making it a common ingredient in various cosmetic products such as creams, moisturizers, and serums. It acts as an anti-aging acid that helps maintain the skin's pH level (Uses of tartaric acid 2023).

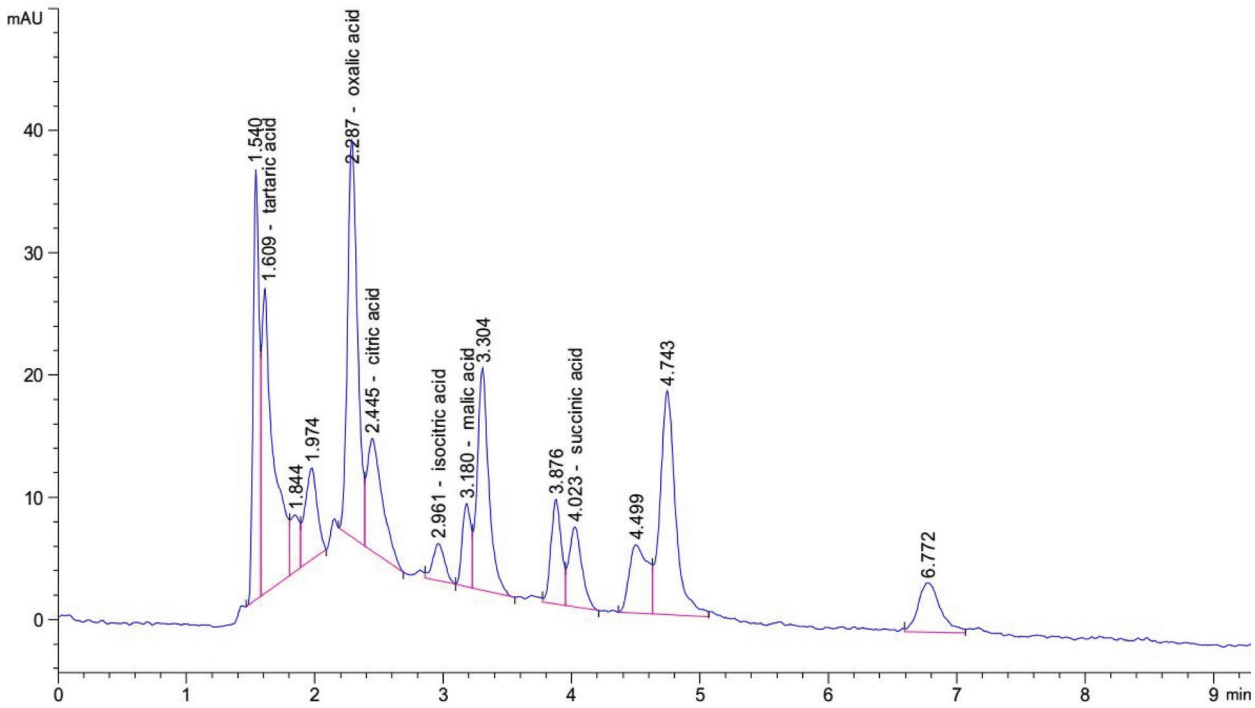

Figure 2. HPLC chromatogram of organic acids in the *Astragalus dasyanthus* Pall. herb.

Table 4. The results of the organic acids determination in *Astragalus dasyanthus* Pall. herb.

No.	Retention time	Common name of organic acid (IUPAC)	Molecular formula	Quantitative content (mg/g) $\bar{x}\pm\Delta\bar{x}$
1.	1.609	Tartaric (2,3-dihydroxybutanedioic) acid	$C_4H_6O_6$	8.68 ± 0.17
2.	2.287	Oxalic (ethanedioic) acid	C2H2O4	6.90 ± 0.09
3.	2.445	Citric (2-hydroxypropane-1,2,3-tricarboxylic) acid	$C_6H_8O_7$	7.69 ± 0.11
4.	2.961	Isocitric (1-hydroxypropane-1,2,3-tricarboxylic) acid	$C_6H_8O_7$	1.30 ± 0.05
5.	3.180	Malic ((2*S*)-2-hydroxybutanedioic) acid	$C_4H_6O_5$	0.49 ± 0.02
6.	4.023	Succinic (butanedioic) acid	$C_4H_6O_4$	5.01 ± 0.13

Oxalic acid is characterized by metabolic stability and prolonged action. It also selectively acts on malignant cells without affecting healthy cells in the body, leading to apoptosis (death) of cancer cells. Oxalic acid is used in the production of a therapeutic anticancer drug (WO2011/119126 A1 2011).

Citric acid is a naturally occurring component and a common metabolite found in plants. It is the most widely used organic acid in both pharmaceuticals and foods. Citric acid and its salts are used in different industries as pH adjustment, chelating, derivatization and buffering (Crolla and Kennedy 2001; Celik et al. 2014).

Succinic acid is the precursor to various biological compounds and plays a crucial role in the accumulation of the mitochondrial metabolite succinate (in the citric acid cycle) (Rousová et al. 2014; Ozpinar et al. 2015). Additionally, it regulates reperfusion injury during ischemia by controlling mitochondrial reactive oxygen production (Jarukas et al. 2018). In the living organism, succinic acid exists in the form of an anion called succinate, which serves various biological functions as a metabolic intermediate. It is converted into fumarate, involved in ATP production, and acts as a signaling molecule, reflecting the cellular metabolic state (Tretter et al. 2016).

The research findings indicate that *Astragalus dasyanthus* is a promising plant due to the important role of organic and fatty acids in various biological processes.

Conclusion

The results of the research indicate that *Astragalus dasyanthus* Pall. herb contained fatty and organic acids. Using the GC/MS method, the composition of saturated and unsaturated fatty acids. Among seven identified fatty acids dominant acids of *Astragalus dasyanthus* Pall. herb were pentadecanoic, linoleic, and stearic, the content of which was 4.16 mg/g, 1.73 mg/g, and 1.14 mg/g respectively. The qualitative composition and quantitative content of organic acids were studied by the HPLC method. Six organic acids in the herb of *Astragalus dasyanthus* Pall. was determined. Among them, tartaric (8.68 mg/g), citric (7.69 mg/g), oxalic (6.90 mg/g), and succinic acid (5.01 mg/g) acids were dominant. Thus, *Astragalus dasyanthus* Pall. displayed a particular composition of fatty and organic acids which could be of interest for pharmaceutical manufacturing, and this plant's raw material can be used as a source for new medicines in the future.

References

Agius C, Tucher S, Poppenberger B, Rozhon W (2018) Quantification of sugars and organic acids in tomato fruits. MethodsX 5: 537–550. http://doi.org/10.1016/j.mex.2018.05.014

Anez-Bustillos L, Dao DT, Fell GL, Baker MA, Gura KM, Bistrian BR, Puder M (2018) Redefining essential fatty acids in the era of novel intravenous lipid emulsions. Clinical nutrition (Edinburgh, Scotland) 37(3): 784–789. https://doi.org/10.1016/j.clnu.2017.07.004

Anushree S, André M, Guillaume D, Frédéric F (2017) Stearic sunflower oil as a sustainable and healthy alternative to palm oil. A review. Agronomy for Sustainable Development 37: 18. https://doi.org/10.1007/s13593-017-0426-x

Atolani O, Adeniyi O, Kayode OO, Adeosun CB (2015) Direct preparation of fatty acid methyl esters and determination of in vitro antioxidant potential of lipid from fresh *Sebal causarium* Seed. Journal of Applied Pharmaceutical Science 5: 24–28. https://doi.org/10.7324/JAPS.2015.50305

Benchadi W, Haba H, Lavaud C, Harakat D, Benkhaled M (2013) Secondary metabolites of *Astragalus cruciatus* Link. and their chemotaxonomic significance. Records of Natural Products 7(2): 105–113.

Bondarchuk OP, Rakhmetov DB (2018) Evaluation of the introduction effectiveness of plants of *Astragalus* spp. in conditions of right-bank of forest-steppe of Ukraine. Introduction of plants 4: 23–29.

Bratkov VM, Shkondrov AM, Zdraveva PK, Krasteva IN (2016) Flavonoids from the genus *Astragalus*: Phytochemistry and biological activity. Pharmacognosy Reviews 10: 11–32. https://doi.org/10.4103/0973-7847.176550

Brown HA, Marnett LJ (2011) Introduction to lipid biochemistry, metabolism, and signaling. Chemical Reviews 111(10): 5817–5820. https://doi.org/10.1021/cr200363s

Budniak L, Slobodianiuk L, Marchyshyn S, Demydiak O, Dakhym I (2021a) Determination of amino acids of some plants from Gentianaceae family. Pharmacia 68(2): 441–448. https://doi.org/10.3897/pharmacia.68.e67052

Budniak L, Slobodianiuk L, Marchyshyn S, Ilashchuk P (2021b) Determination of polysaccharides in *Gentiana cruciata* L. herb. Pharmacologyonline 2: 1473–1479.

Budniak L, Slobodianiuk L, Marchyshyn S, Kostyshyn L, Horoshko O (2021c) Determination of composition of fatty acids in *Saponaria officinalis* L. ScienceRise: Pharmaceutical Science 1(29): 25–30. https://doi.org/10.15587/2519-4852.2021.224671

Budniak L, Slobodianiuk L, Marchyshyn S, Parashchuk E (2021d) Determination of carbohydrates in burnet saxifrage (*Pimpinella saxifraga* L.) Pharmacologyonline 2: 1374–1382.

Calder PC (2015) Functional roles of fatty acids and their effects on human health. Journal of Parenteral and Enteral Nutrition 39(1): 18S–32S. https://doi.org/10.1177/0148607115595980

Celik G, Ucar FB, Akpınar O, Çorbacı C (2014) Production of citric and isocitric acid by *Yarrowia lipolytica* strains grown on different carbon sources. Turkish Journal of Biochemistry 39(3): 285–290. https://doi.org/10.5505/tjb.2014.92005

Chaudhary LB, Rana TS, Anand KK (2008) Current status of the systematics of *Astragalus* L. (Fabaceae) with special reference to the Himalayan Species in India. Taiwania 53: 338–355.

Chen RZ, Tan L, Jin CG, Lu J, Tian L, Chang QQ, Wang K (2015) Extraction, isolation, characterization and antioxidant activity of polysaccharides from *Astragalus membranaceus*. Industrial Crops and Products 77: 434–443.

Crolla A, Kennedy KJ (2001) Optimization of citric acid production from *Candida lipolytica* Y-1095 using *n*-paraffin. Journal of Biotechnology 89(1): 27–40. https://doi.org/10.1016/S0168-1656(01)00278-4

Crupkin M, Zambelli A (2008) Detrimental impact of trans fats on human health: stearic acid-rich fats as possible substitutes. Comprehensive Reviews in Food Science and Food Safety 7(3): 271–279. https://doi.org/10.1111/j.1541-4337.2008.00045.x

Czumaj A, Śledziński T (2020) Biological role of unsaturated fatty acid desaturases in health and disease. Nutrients 12(2): 356. https://doi.org/10.3390/nu12020356

Das UN (2006) Biological significance of essential fatty acids. The Journal of the Association of Physicians of India 54: 309–319.

De Lorgeril M, Salen P, Laporte F, De Leiris J (2001) Alpha-linolenic acid in the prevention and treatment of coronary heart disease. European Heart Journal Supplements 3: D26–D32. https://doi.org/10.1016/S1520-765X(01)90115-4

Ervin RB, Wright JD, Wang C Y, Kennedy-Stephenson J (2004) Dietary intake of fats and fatty acids for the United States population: 1999–2000. Advance Data 348: 1–6.

Evans LM, Cowey SL, Siegal GP, Hardy RW (2009) Stearate preferentially induces apoptosis in human breast cancer cells. Nutrition and Cancer 61(5): 746–753. https://doi.org/10.1080/01635580902825597

FAO (2010) Fats and fatty acids in human nutrition. Report of an expert consultation. FAO Food and Nutrition Paper 91: 1–166.

Feshchenko H, Marchyshyn S, Budniak L, Slobodianiuk L, Basaraba R (2021) Study of antibacterial and antifungal properties of the lyophilized extract of fireweed (*Chamaenerion angustifolium* L.) herb. Pharmacologyonline 2: 1464–1472.

Ganbarov DSh, Ibrahimov Ash (2015) *Astragalus dasyanthus* L. (Fabaseae) a New Species to the Flora of Azerbaijan. International Journal of Multidisciplinary Research and Development 2(2): 426–427.

Ganea A, Gacota A (1996) Moldova: Country Report to the FAO International Technical Conference on Plant Genetic Resources. Leipzig, 30 pp.

Habib N, Wood C, Apostolov K, Barker W, Hershman M, Aslam M, Heinemann D, Fermor B, Williamson R, Jenkins W (1987) Stearic acid and carcinogenesis. British Journal of Cancer 56: 455–458. https://doi.org/10.1038/bjc.1987.223

Han XH, Liu P, Zhang YY, Zhang N, Chen FR, Cai JF (2011) Astragaloside IV regulates expression of ATP-sensitive potassium channel subunits after ischemiareperfusion in rat ventricular cardiomyocytes. Journal of Traditional Chinese Medicine 31: 321–326. https://doi.org/10.1016/S0254-6272(12)60012-0

Jaradat NA, Zaid AN, Abuzant A, Khalaf S, AbuHassan N (2017) Phytochemical and biological properties of four *Astragalus* species commonly used in traditional Palestinian medicine. The European Journal of Integrative Medicine 9: 1–8. https://doi.org/10.1016/j.eujim.2017.01.008

Jarukas L, Kamarauskaitė J, Marksa M, Trumbeckaitė S, Banienė R, Ivanauskas L (2018) Bio-based succinic acid sample preparation and derivatization procedure optimisation for Gas chromatography-mass spectrometry analysis. ScienceRise – Pharmaceutical Science 4(14): 9–13. https://doi.org/10.15587/2519-4852.2018.135132

Jia R, Cao L, Xu P, Jeney G, Yin G (2012) In-vitro and in-vivo hepatoprotective and antioxidant effects of *Astragalus* polysaccharides against carbon tetrachloride-induced hepatocyte damage in common carp (*Cyprinus carpio*). Fish Physiology and Biochemistry 38: 871–881. https://doi.org/10.1007/s10695-011-9575-z

Khoron'ko AT, Glyzin VI (1973) Flavonoids of *Astragalus dasyanthus*. Chemistry of Natural Compounds 9: 403. https://doi.org/10.1007/BF00565707

Kodirova MS (2021) *Astragalus dasyanthus* Pall. Central Asian Journal of Medical and Natural Sciences 2(2): 72–74. https://doi.org/10.47494/cajmns.v2i2.126

Li X, Qu L, Dong Y, Han L, Liu E, Fang S, Zhang Y, Wang T (2014) A review of recent research progress on the *Astragalus* genus. Molecules 19(11): 18850–18880. https://doi.org/10.3390/molecules191118850

Lock M, Schrire BD (2005) Galegeae. In: Lewis GP, Schrire BD, Mackinder BA, Lock M (Eds) Legumes of the world, Royal Botanic Gardens, Kew, India.

Luo Y, Qin Z, Hong Z, Zhang X, Ding D, Fu JH, Zhang WD, Chen J (2004) Astragaloside IV protects against ischemic brain injury in a murine model of transient focal ischemia. Neuroscience Letters 363: 218–223. https://doi.org/10.1016/j.neulet.2004.03.036

Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Åkesson B, Rosqvist F, Schwab U, Herzig KH, Hukkanen J, Savolainen MJ, Brader L, Hermansen K, Kolehmainen M, Poutanen K, Uusitupa M, Risérus U, Thorsdottir I (2014) Whole grain rye intake, reflected by a biomarker, is associated with favorable blood lipid outcomes in subjects with the metabolic syndrome – a randomized study. PLoS ONE 9(10): e110827. https://doi.org/10.1371/journal.pone.0110827

Makhatova BG, Datkhayev UM, Burda NY, Kyslychenko VS (2016) Fatty acids from *Verbascum songaricum* herb. Research Journal of Pharmaceutical, Biological and Chemical Sciences 7(2): 277–279.

Marchyshyn S, Budniak L, Slobodianiuk L, Ivasiuk I (2021a) Determination of carbohydrates and fructans content in *Cyperus esculentus* L. Pharmacia 68(1): 211–216. https://doi.org/10.3897/pharmacia.68.e54762

Marchyshyn S, Slobodianiuk L, Budniak L, Ivasiuk I (2021b) Hypoglycemic effect of *Cyperus esculentus* L. tubers extract. Pharmacologyonline 2: 1383–1392.

Marchyshyn S, Slobodianiuk L, Budniak L, Ivasiuk I (2021c) Study the anti-inflammatory and wound-healing activity of the dry extract of the herb *Cyperus esculentus* L. Pharmacologyonline 3: 282–290.

Marchyshyn S, Slobodianiuk L, Budniak L, Skrynchuk O (2021d) Analysis of carboxylic acids of *Crambe cordifolia* Steven. Pharmacia 68(1): 15–21. https://doi.org/10.3897/pharmacia.68.e56715

Molfino A, Gioia G, Fanelli F, Muscaritoli M (2014) The role for dietary omega-3 fatty acids supplementation in older adults. Nutrients 6(10): 4058–4072. http://doi.org/10.3390/nu6104058

Mu H, Høy CE (2004) The digestion of dietary triacylglycerols. Progress in lipid research 43(2): 105–133. https://doi.org/10.1016/S0163-7827(03)00050-X

NIST (2008) National Institute Standard and Technology.

Özipek M, Dönmez AA, Çalış İ, Brun R, Rüedi P, Tasdemir D (2005) Leishmanicidal cycloartane-type triterpene glycosides from *Astragalus oleifolius*. Phytochemistry 66: 1168–1173. https://doi.org/10.1016/j.phytochem.2005.04.019

Ozpinar A, Weiner GM, Ducruet AF (2015) Succinate: A promising therapeutic target for reperfusion injury. Neurosur-gery 77(6): 13–14. https://doi.org/10.1227/01.neu.0000473807.30361.29

Pistelli L (2002) Secondary metabolites of genus *Astragalus*: Structure and biological activity. In: Atta-ur-Rahman (Ed.) Studies in Natural Products Chemistry (Bioactive Natural Products, Part H). Amsterdam: Elsevier Science, 443–545. https://doi.org/10.1016/S1572-5995(02)80043-6

Podlech D (1986) Taxonomic and phytogeographical problems in Astragalus of old and south west Asia. Proceedings of the Royal Society of Edinburgh 89: 37–43. https://doi.org/10.1017/S0269727000008885

Radzikowska U, Rinaldi AO, Çelebi Sözener Z, Karaguzel D, Wojcik M, Cypryk K, Akdis M, Akdis, Sokolowska M (2019) The Influence of Dietary Fatty Acids on Immune Responses. Nutrients 11(12): 2990. https://doi.org/10.3390/nu11122990

Rousová J, Ondrušová K, Karlová P, Kubátová A (2014) Determination of impurities in bioproduced succinic acid. Journal of Chromatography and Separation Techniques 6(2): 1–8. http://doi.org/10.4172/2157-7064.1000264

Slobodianiuk L, Budniak L, Feshchenko H, Sverstiuk A, Palaniza Y (2022) Quantitative analysis of fatty acids and monosaccharides composition in *Chamerion angustifolium* L. by GC/MS method. Pharmacia 69(1): 167–174. https://doi.org/10.3897/pharmacia.69.e76687

Slobodianiuk L, Budniak L, Marchyshyn S, Demydiak O (2021a) Investigation of the anti-inflammatory effect of the dry extract from the herb of *Stachys sieboldii* MIQ. Pharmacologyonline 2: 590–597.

Slobodianiuk L, Budniak L, Marchyshyn S, Kostyshyn L, Ezhned M (2021b) Determination of amino acids content of the *Tagetes lucida* Cav. by GC/MS. Pharmacia 68(4): 859–867. https://doi.org/10.3897/pharmacia.68.e73325

Slobodianiuk L, Budniak L, Marchyshyn S, Skrynchuk O, Kudria V (2021c) Amino acids content of *Crambe cordifolia* and *Crambe koktebelica* leaves. International Journal of Applied Pharmaceutics 13(4): 111–116. https://doi.org/10.22159/ijap.2021v13i4.41265

Stoyko LI, Gusak LV, Marchishin SM, Demidyak OL (2015) Research of fatty acid composition of common centaury herbs and crosne herbs. Journal of Siberian Medical Sciences 6: 1–9.

To NB, Nguyen YT, Moon JY, Ediriweera MK, Cho SK (2020) Pentadecanoic acid, an odd-chain fatty acid, suppresses the stemness of MCF-7/SC human breast cancer stem-like cells through JAK2/STAT3 signaling. Nutrients 12(6): 1663. https://doi.org/10.3390/nu12061663

Tretter L, Patocs A, Chinopoulos C (2016) Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis. Biochimica et Biophysica Acta (BBA) – Bioenergetics 1857(8): 1086–1101. https://doi.org/10.1016/j.bbabio.2016.03.012

Uses of tartaric acid (2023) Uses of Tartaric Acid. https://www.vedantu.com/evs/uses-of-tartaric-acid

Valenzuela A, Delplanque B, Tavella M (2011) Stearic acid: A possible substitute for trans fatty acids from industrial origin. Grasas y Aceites 62(2): 131–138. https://doi.org/10.3989/gya.033910

Verotta L, El-Sebakhy NA (2001a) Cycloartane and oleanane saponins from *Astragalus* sp. In: Atta-ur-Rahman (Ed.) Studies in Natural Products Chemistry (Bioactive Natural Products, Part F). Elsevier Science, Karachi, 179–234. https://doi.org/10.1016/S1572-5995(01)80008-9

Verotta L, El-Sebakhy NA (2001b) Studies in natural products chemistry: Bioactive natural products, Vol. 25, (Part F), Elsevier, Amesterdam.

Wang QH, Han NRCKT, Dai NYT, Wang XL, Ao WLJ (2014) The structural elucidation and antimicrobial activities of two isoflavane glycosides from *Astragalus membranaceus* (Fisch) Bge. var. *mongholicus* (Bge) Hsiao. The Journal of Molecular Structure 1076: 535–538. https://doi.org/10.1016/j.molstruc.2014.08.025

Wickramasinghe NS, Jo H, McDonald JM, Hardy RW (1996) Stearate inhibition of breast cancer cell proliferation. A mechanism involving epidermal growth factor receptor and G-proteins. The American journal of pathology 148(3): 987–995.

WO2011/119126 A1 (2011) WO2011/119126 A1. https://patentimages.storage.googleapis.com/83/ce/96/5ee46cc9b8d69f/WO2011119126A1.pdf

Xu L, Podlech D (2010) Astragalus. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China, Vol. 10 (Fabaceae). Science Press: Beijing, and Missouri Botanical Garden Press, St. Louis.

Yalcin FN, Piacente S, Perrone A, Capasso A, Duman H, Calis I (2012) Cycloartane glycosides from *Astragalus stereocalyx* Bornm. Phytochemistry 73: 119–126. https://doi.org/10.1016/j.phytochem.2011.09.011

Zhao M, Shao D, Yu L, Sun X, Wang Y, Hu H, Feng R, Gao Q, Guo F, Hao L (2015) Electrophysiological effect and the gating mechanism of astragaloside IV on l-type Ca^{2+} channels of guinea-pig ventricular myocytes. European Journal of Pharmacology 760: 27–35.