# Comparative study of carboxylic acids of plants of the genus Astragalus

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# Comparative research of the carboxylic acids in the plants of the Genus astragalus

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SUMMARY

Considering the important role of carboxylic acids in the metabolic processes occurring in living organisms, an urgent task of modern pharmacy is to search for their sources among poorly studied plants. For this purpose, a comparative study of the carboxylic acids of a number of plants of the numerous genus Astragalus was carried out. The objects of the study were astragalus white stem, astragalus licorice and astragalus sainfoin. For the first time, the qualitative composition and the quantitative content of carboxylic acids in the studied plants were determined by the method of gas-liquid chromatography-mass spectrometry. It has been established that the carboxylic acids of astragalus alba, astragalus licorice and astragalus sainfoin differ in quantitative content, but have a similar qualitative composition. 36 carboxylic acids were identified in the herb astragalus whitestem. The herb astragalus licorice and astragalus esparcet contains 37 compounds classified as carboxylic acids each. The results obtained indicate that the studied raw materials can be further used in medical and pharmaceutical practice as sources for the production of carboxylic acids and drugs based on them.

Key words: white-stemmed astragalus, licorice-leaved astragalus, sainfoin astragalus, carboxylic acids, gas-liquid chromato-mass spectrometry.

RESUME

Considering the important role of carboxylic acids in metabolic processes occurring in living organisms, a very important problem is to find their sources among little-known plants. For this purpose, a comparative study of a number of carboxylic acids in numerous plants of genus Astragalus. The objects of study were Astragalus albicaulis, Astragalus glycyphyllus, Astragalus onobrychis. For the first time the method of gasliquid chromatography-mass spectrometry was used to establish a qualitative composition and quantitative content of carboxylic acid in the studied plants. It was found that the carboxylic acid of Astragalus albicaulis, Astragalus glycyphyllus and Astragalus onobrychis differ from each other in quantitative content, but have a similar composition. 36 carboxylic acids were identified in the herb Astragalus albicaulis. The herbs Astragalus glycyphyllus and Astragalus onobrychis contain 37 compounds classified as carboxylic acids. These results indicate that raw herbs can be further used in pharmacy and medical practice as sources for the production of carboxylic acids and medicines.

keywords:Astragalus albicaulis DC, Astragalus glycyphyllus L, Astragalus onobrychis L, carboxylic acids, gas-liquid chromatography-mass spectrometry.

## INTRODUCTION

Indispensable participants in many metabolic processes occurring in the human body are carboxylic acids (a class of organic compounds whose molecules contain one or more functional carboxyl groups), and each of them has certain functions. Fatty acids protect the brain from aging and prevent the development of Alzheimer's disease; they are indispensable in the treatment of depressive and manic states [2, 4]. Deficiency of fatty acids leads to a weakening of the immune system, an increase in blood pressure, the development of allergic reactions and inflammatory processes [3, 10]. Organic acids (ascorbic, citric) maintain acid-base balance, are directly involved in the processes of digestion, activate intestinal motility, slow down the development of putrefactive diseases.

bacteria and fermentation processes, reduce the risk of developing gastrointestinal diseases [14, 15]. Citric and malonic acids have cardioprotective activity [17], while lilac and vanilla acids have hepatoprotective activity [12]. A number of organic acids (malonic, oxalic, succinic) exhibit a growth-stimulating effect when treated with plant seed solutions [1]. Phenolcarboxylic acids exhibit antibacterial and antioxidant activity [13, 16].

One of the most important tasks of modern pharmacy is to conduct research to find natural sources of biologically active compounds, including carboxylic acids. Among the little-studied plants, some species of the numerous genus of perennial plants Astragalus (Astragalus) of the legume family (Fabaceae) may be of interest. An analysis of literary sources shows that plants of the Astragalus genus contain a whole range of biologically active substances: flavonoids, tannins, triterpene saponins, polysaccharides, nitrogen-containing compounds, vitamins, and essential oils [6, 7, 8].

White-stemmed astragalus (Astragalus albi-caulis DC) (Fig. 1), licorice-leaved astragalus (Astragalus glycyphyllus L) (Fig. 2) and sainfoin astragalus (Astragalus onobrychis L) (Fig. 3) have long been used in folk medicine in different countries as anti-inflammatory , diuretics, lipid-lowering agents, in diseases of the cardiovascular system [9].

Rice. 1. Astragalus white stem (Astragalus albicaulis DC)

Rice. 2. Astragalus licorice (Astragalus glycyphyllus L)

Rice. 3. Astragalus sainfoin (Astragalus onobrychis L.).

These plants are widespread in the European part of Russia, Western Siberia, and the Caucasus [7] and have a sufficient source of raw materials. However, their chemical composition is not well understood. All this indicates the relevance of studying these types of astragalus, therefore, the purpose of our study was a comparative study of the qualitative composition and quantitative content of carboxylic acids in the herb of white-stem astragalus, licorice-leaved astragalus and sainfoin astragalus.

## MATERIALS AND METHODS

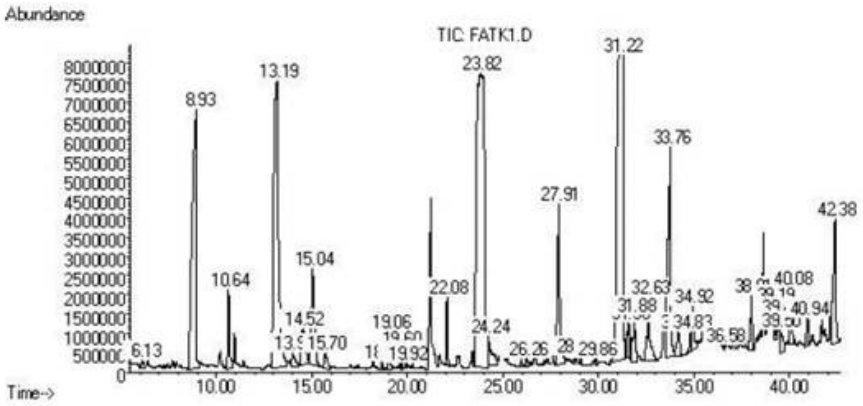
The object of the study was dry air-crushed grass of white-stemmed astragalus, licorice-leaved astragalus and sainfoin astragalus, harvested in 2016 in the Kursk region during the period of mass flowering of plants.

The study of the content of carboxylic acids in the grass of the studied plants was carried out by us by the method of gas-liquid chromato-mass spectrometry [5, 11]. To do this, 50.0 mg of air-dry crushed raw materials of Astragalus whitestem, Astragalus licorice and Astragalus sainfoin were placed in 2.0 ml Agilent vials, 50.0 μg of tridecane in hexane were added as an internal standard and 1.0 ml of methylating agent (14% $BCl_3$in methyl alcohol, Supelco 3-3033). The mixture was kept in hermetically sealed vials for 8 hours at a temperature of 65°C. This time is necessary for the most complete extraction of fatty oil from the plant material, while it is also hydrolyzed into constituent fatty acids and methylated. Simultaneously, free organic and phenolcarboxylic acids are methylated. Next, the reaction mixtures were decanted from plant materials and diluted with 1.0 ml of purified water. The extraction of methyl esters of carboxylic acids was carried out with methylene chloride, after which they were chromatographed on an Agilent Technologies 6890 gas-liquid chromatograph with a 5973N mass spectrometric detector. Analysis conditions: chromatographic column - capillary INNOWAX, 30 m long, inner diameter 0.25 mm; carrier gas - helium, carrier gas velocity - 1.2 ml / min., sample volume - 2 μl; sample injection rate 1.2 ml/ min for 0.2 minutes; thermostat temperature programmable from 50°C to 250°C at a rate of 4°C/min.; sample injection heater temperature 250°C. Identification of carboxylic acids was carried out by comparison with known samples of methyl esters, as well as using the NISTOS5 and WILLEY 2007 mass spectra library with a total number of spectra of more than 470,000 in combination with AMDIS and NIST identification programs. The concentrations of individual carboxylic acids were calculated by the internal standard method [5, 11]. The determination for each type of raw material under study was carried out three times. Identification of carboxylic acids was carried out by comparison with known samples of methyl esters, as well as using the NISTOS5 and WILLEY 2007 mass spectra library with a total number of spectra of more than 470,000 in combination with AMDIS and NIST identification programs. The concentrations of individual carboxylic acids were calculated by the internal standard method [5, 11]. The determination for each type of raw materials under study was carried out three times. Identification of carboxylic acids was carried out by comparison with known samples of methyl esters, as well as using the NISTOS5 and WILLEY 2007 mass spectra library with a total number of spectra of more than 470,000 in combination with AMDIS and NIST identification programs. The concentrations of individual carboxylic acids were calculated by the internal standard method [5, 11]. The determination for each type of raw materials under study was carried out three times.

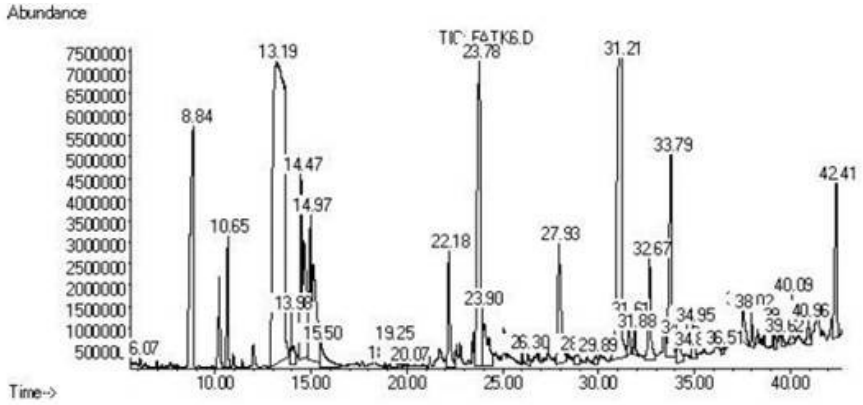
## RESULTS AND ITS DISCUSSION

The results of a comparative study of the qualitative composition and quantitative content of carboxylic acids of the herb astragalus white stem, astragalus licorice and astragalus sainfoin are presented in Table. 1 and in fig. 4–6.

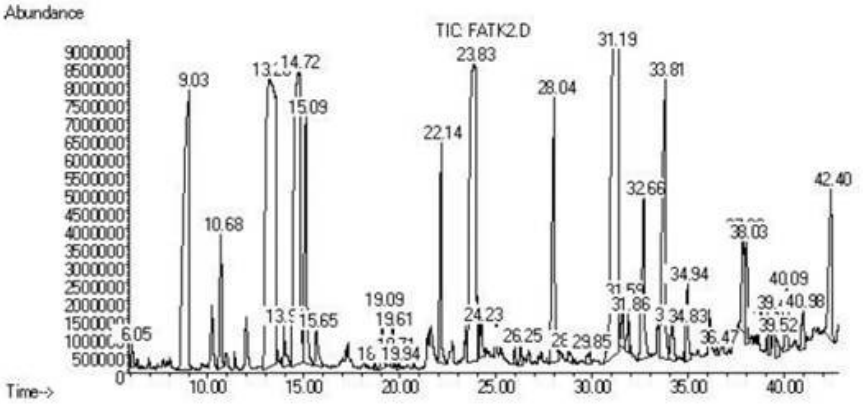
Our studies have made it possible to establish that astragalus white stem, astragalus licorice and astragalus sainfoin contain a wide range of carboxylic acids represented by aromatic, aliphatic and heterocyclic compounds. Among the carboxylic acids of the studied astragalus species, representatives of dicarboxylic, phenolcarboxylic, hydroxycarboxylic and fatty acids were identified. Fatty acids, in turn, include both saturated and mono- and polyunsaturated compounds, and some of them (linoleic and linolenic acids) are indispensable for the human body. At the same time, the astragalus species studied by us have a similar qualitative composition, differing only in the quantitative content of the identified compounds.




Rice. 4. Scheme of the chromatogram of the chromato-mass spectral analysis of carboxylic acids of astragalus white stem.




Rice. 5. Scheme of the chromatogram of the chromato-mass spectral analysis of carboxylic acids of astragalus licorice.




Rice. 6. Scheme of the chromatogram of the chromato-mass spectral analysis of carboxylic acids of astragalus sainfoin.

36 carboxylic acids were identified in the herb Astragalus whitestem, of which 17 compounds belong to fatty acids and 7 to phenolcarboxylic acids. Among the saturated fatty acids, palmitic acid is predominant, among monounsaturated fatty acids, oleic acid occupies the first place in terms of quantitative content, among polyunsaturated fatty acids in the herb Astragalus white stem, the highest content of linoleic and linolenic acids is noted. Among phenolcarboxylic acids, ferulic and gentisic acids are dominant. In addition, white stem astragalus contains significant amounts of citric and malic acids (Table 1).

The presence of 37 compounds classified as carboxylic acids was found in the herb Astragalus licorice, including 17 fatty and 8 phenolcarboxylic acids. Within each group of Astragalus licorice carboxylic acids identified by this method, the greatest amount is contained: among saturated acids, palmitic acid prevails, among monounsaturated acids, oleic acid, and among polyunsaturated fatty acids, linoleic and linolenic acids. Of the phenolcarboxylic acids of astragalus licorice, ferulic and p-coumaric acids are predominant. This species also contains significant amounts of malic and malonic acids (Table 1).

In the herb Astragalus sainfoin, the presence of 37 carboxylic acids was also established, of which 17 are fatty and 8 are phenol carboxylic acids. Among the saturated acids of this type, palmitic acid is predominant, among monounsaturated acids - oleic, among polyunsaturated acids - linoleic and linolenic. Of the phenolcarboxylic acids, the highest content of ferulic and p-coumaric acids was noted (Table 1).

Thus, white stem astragalus, licorice astragalus and sainfoin astragalus can be used as sources for obtaining a number of carboxylic acids (citric, malonic, malic, succinic), including essential linoleic and linolenic acids.

Table 1

The composition of carboxylic acids of the herb astragalus white stem, astragalus licorice and astragalus sainfoin

| № п/п | Наименование карбоновых кислот | Содержание карбоновых кислот, мг/кг | | |
|---|---|---|---|---|
| | | Астрагал белостебельный | Астрагал солодколистный | Астрагал эспарцетный |
| 1 | 3-окси-2-метилглютаровая кислота | 456,63 | 1166,50 | 748,31 |
| 2 | 2-окси-2-метилянтарная кислота | 109,04 | 43,35 | 113,76 |
| 3 | 2-оксипальмитиновая кислота | 94,85 | 35,09 | 48,73 |
| 4 | п-кумаровая кислота | – | 447,92 | 620,82 |
| 5 | п-оксибензойная кислота | 274,24 | 240,48 | 93,58 |
| 6 | Азелаиновая кислота | 75,21 | 120,67 | 84,78 |
| 7 | Арахиновая кислота | 450,03 | 317,95 | 324,89 |
| 8 | Бегеновая кислота | 478,87 | 259,55 | 315,63 |
| 9 | Бензойная кислота | 246,16 | 653,60 | 213,10 |
| 10 | Ванилиновая кислота | 378,47 | 434,49 | 229,69 |
| 11 | Гексадикарбоновая кислота | 38,44 | 10,44 | 22,74 |
| 12 | Гентизиновая кислота | 491,28 | 434,87 | 247,04 |
| 13 | Гептадекановая кислота | 51,69 | 129,06 | 71,29 |
| 14 | Капроновая кислота | 84,29 | 62,27 | 36,18 |
| 15 | Лауриновая кислота | 44,58 | 33,88 | 28,31 |
| 16 | Левулиновая кислота | 732,16 | 3235,42 | 3549,93 |
| 17 | Лимонная кислота | 10186,29 | 7605,34 | 4242,88 |
| 18 | Линолевая кислота | 888,75 | 1069,97 | 847,69 |
| 19 | Линоленовая кислота | 3036,87 | 2713,76 | 1961,61 |
| 20 | Малоновая кислота | 6945,90 | 15272,08 | 5269,49 |
| 21 | Миристиновая кислота | 438,82 | 1264,84 | 163,26 |
| 22 | Олеиновая кислота | 640,13 | 218,56 | 135,99 |
| 23 | Пентадекановая кислота | 57,64 | 71,73 | 52,67 |
| 24 | Пальмитиновая кислота | 1816,38 | 1845,86 | 1453,87 |
| 25 | Пальминтолеиновая кислота | 71,61 | 180,01 | 78,63 |
| 26 | Салициловая кислота | 271,24 | 262,80 | 266,98 |
| 27 | Сиреневая кислота | 297,64 | 125,15 | 108,77 |
| 28 | Стеариновая кислота | 356,84 | 343,31 | 236,86 |
| 29 | Тетракозановая кислота | 256,92 | 283,79 | 214,20 |
| 30 | Трикозановая кислота | 56,77 | 51,63 | 18,63 |
| 31 | Фумаровая кислота | 247,80 | 416,09 | 172,74 |
| 32 | Фенилуксусная кислота | 44,18 | 46,19 | 23,06 |
| 33 | Феруловая кислота | 1497,60 | 1741,69 | 796,38 |
| 34 | Хенейкозановая кислота | 28,44 | 84,05 | 24,61 |
| 35 | Щавелевая кислота | 574,69 | 924,01 | 499,97 |
| 36 | Яблочная кислота | 10950,76 | 4741,23 | 3763,47 |
| 37 | Янтарная кислота | 1491,41 | 3457,60 | 1091,36 |

CONCLUSIONS

1. For the first time, the study of

qualitative composition and quantitative content of carboxylic acids of white-stemmed astragalus, licorice-leaved astragalus and sainfoin astragalus.

2. It has been established that the studied species of astragalus contain a wide range of carboxylic acids, have a similar qualitative composition, differing in the quantitative content of individual compounds.

3. The results obtained indicate that the studied species of astragalus in the future can be used in medical and pharmaceutical practice as sources for obtaining citric, malonic, malic, succinic acids, as well as essential linoleic and linolenic acids.

LITERATURE

1. Vereshchagin A.L., Kropotkina V.V., Khmeleva A.N. About the mechanism of growth-stimulating action ultra-low doses of natural organic acids // Bulletin of the Altai State Agrarian University. - 2010. - No. 1 (63). – P. 46–48.

2. Govorin A.V., Filev A.P. Omega-3 polyunsaturated fatty acids in the treatment of patients with cardiovascular diseases // Rational pharmacotherapy in cardiology. - 2012. - No. 8. - P. 95–102.

3. Zakharova I.N., Surkova E.N. The role of polyunsaturated fatty acids in the formation health in children // Pediatrics. - 2009. - T. 88; 6. – P. 84–91.

4. Osipenko A.N. Fatty acids and their aldehydes as participants in the atherosclerotic process // Siberian medical journal. - 2012. - V.27, No. 2. - S. 122-126.

5. Pozdnyakova T.A., Bubenchikov R.A. Geranium Siberian: content of fatty and organic acids // Pharmacy. - 2014. - No. 8. – P. 13–15.

6. Pozdnyakova T.A., Bubenchikov R.A. The study of tannins of astragalus white stem (Astragalus albicaulis DC) // Traditional medicine. - 2016. - No. 3 (46). – P. 42–43.

7. Plant resources of Russia: Wild flowering plants, their composition and biological activity. T.3. Fabaceae-Apiaceae families. (under the editorship of A. L. Budantsev). - St. Petersburg. M.: Association of scientific publications KMK, 2010. - 601 p.

8. Sergalieva M.U., Mazhitova M.V., Samotrueva M.A. Biological activity of extracts plants of the genus Astragalus // Modern problems of science and education. - 2015. - No. 5. - P. 648.

9. Sergalieva M.U., Mazhitova M.V., Samotrueva M.A. Plants of the genus Astragalus: perspectives applications in pharmacy // Astrakhan Medical Journal. – 2015. V. 10, No. 2. – S. 17–31.

10. Tereshina E. V. The role of fatty acids in the development of age-related oxidative stress. Hypothesis // Successes of gerontology, 2007. - T. 20; 1. - S. 59-65.

11. Carrapiso AI, Carcia C. Development in lipid analisis: some new extraction techniques and in situ transesterification // Lipids, 2000. - Vol. 35. - R. 1167-1177.

12. Hepatoprotective effect of syringic acid and vanillic acid on CCl4-induced liver injury / A. Itoh, K. Isoda, M. Kondoh et al. // Biol Pharm Bull. 2010. - Vol. 33(6). - P. 983-987.

13. Inhibitory effect of oxalic acid on bacterial spoilage of raw chilled chicken / DM Anang, G. Rusul, S. Radu et al. // J. Food Prot. 2006. Vol. 69, No. 8. - R. 1913-1919.

14. Michalska M., Wasek M. Antioxidant capacities of natural supplements with high doses of vitamin C // Actual problems of creation of new medicinal preparations of natural origin PHYTOPHARM 2003: proceedings of the 7th Intern. Kong. (July 3-5; 2003; St. - Peterburg - Pushkin, Russia). - Spb, 2003. - P. 491-494.

15. Podmore ID, Griffiths HR, Herbert KE Vitamin C exhibits pro-oxidant properties // Nature. - 1998. Vol. 392. – P. 559.

16. Roman Merkl, Iveta Hrádková, Vladimír Filip and Jan Šmidrkal. Antimicrobial and Antioxidant Properties of Phenolic Acids Alkyl Esters// Czech J. Food Sci. 2010. - Vol. 28, No. 4. - P. 275-279.

17. The Cardioprotective Effects of Citric Acid and L-Malic Acid on Myocardial Ischemia / Reperfusion Injury / Xilan Tang, Jianxun Liu, Wei Dong et al. // Evidence-Based Complementary and Alternative Medicine. 2013. - Article ID 820695. - R. 1-11.

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Pozdnyakova, T.A. Comparative study of carboxylic acids of plants of the genus Astragalus / T.A. Pozdnyakova, R.A. Bubenchikov // Traditional medicine. - 2017. - No. 1 (48). - P.39-43.