DETERMINATION OF THE CONTENT OF AMINO ACIDS IN THE ROOTS OF THE SOPHORA FLAVESCENS

© G. Shumova, I. Nizhenkovska, I. Vladymyrova

Asteraceae family is a perennial plant, distributed in Russia in the Far East, in the Primorsky region, in the south-east of the Amur region, in the southwest of the Khabarovsky region. It is also distributed in China (Japan, Korea, North-East, North and Central China) and in some European countries [1].

Roots of shrubby Sophora are widely used in Chinese and Tibetan medicine when creating various medicines from many diseases. Nanai, in which this plant is valued above other medicinal herbs, it is called "Godyahkin, Godylahin", in China, where the shrub Sophora is very popular too, it is called "Ku shen", which in translation means "bitter root". In Ukraine, this plant can be found in the south of the country in the Crimea, it has a whole range of therapeutic properties, namely: general tonic, antipyretic, hemostatic, diuretic, sedative, antitumor, antispasmodic, expressed anti-inflammatory and antimicrobial properties, but is poorly investigated in phytochemical study [1, 2].

1. Introduction

The Sophora flavesens L. genus of Fabaceae family is a perennial plant, distributed in Russia in the Far East, in the Primorsky region, in the south-east of the Amur region, in the southwest of the Khabarovsky region. It is also distributed in Asia (Japan, Korea, North-East, North and Central China) and in some European countries [1].

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2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

It is known that amino acids are one of the major groups of biologically active substances (BAS) of most plants and are essential for the synthesis of enzymes, alkaloids, vitamins, flavonoids and polyphenolic compounds. They carry out a number of important functions, namely: it is a transport form of nitrogen, precursors of phytohormones, protect against adverse factors, are products of primary metabolism and are found in free form in all plants, and therefore often form part of complex phytopreparations. In this case, amino acids have not only biological activity, but as part of the accompanying substances contribute to improved absorption, prolongation of the therapeutic effect and potentiation of the action of the main plant components [3].

Essential amino acids such as leucine, isoleucine, valine increase human immunity and suppress the growth of malignant neoplasms. Arginine and glutamine have...
antioxidant, hepatoprotective and membrane-stabilizing properties. Alanine and glycine regulate the level of sugar in the blood and are involved in the regeneration of tissues. Serine promotes the accumulation of glycogen in the liver and muscle and affects the exchange of fats. From histidine, a biogenic amine – histamine, which is a local hormone, is formed. Lysine affects the cardiac tone, reduces cholesterol levels in the blood. Methionine prevents deposition of fat in the liver, protects its cells from the effects of toxic substances. Amino acid cysteine is a natural antioxidant [3].

Of particular importance are essential amino acids that are not synthesized in an animal organism. Therefore, the study of the qualitative composition and quantitative content of amino acids in the roots of the shrubby sophora has a scientific and practical significance.

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

Recently, there are many publications devoted to the use of BAS for the correction of various states, metabolic processes, prevention and treatment of various diseases of the human body. Therefore, plants in which the rich composition of the BAS cause more attention from scientists. As noted earlier, the rich chemical composition has Sophora flavescens L. [4, 5]. Scientists D. N. Olennikov and D. V. Sandalov (Institute of General and Experimental Biology, Siberian Branch of the Russian Academy of Sciences) investigated the phenolic compounds of Sophora flavescens and developed a spectrophotometric method for determining the total content of flavonoid compounds in the underground organs of Sophora flavescens [6, 7].

Study of the chemical composition of the roots of the shrubby sophora were also studied at the Pyatigorsk Medical and Pharmaceutical Institute (Ph.D., A.B. Samboryadova), the Institute of Plant Protection and the National University of Mongolia (Ganzul G., Byambasuren M., Sukhdolgor J.) [8, 9]. Panthi Murali Krishna, Rao KNV, Sandhya S., David Banji from Nalanda College of Pharmacy (India) investigated alkaloids in Sophora flavescens Ait. method of high performance liquid chromatography [10]. The study of the amino acid composition of the roots of the shrubby sophora was studied by Zhang Jianhua, Wu Yun, Hou Jianhua, Ma Qingzhi (Department of Bioengineering, Inner Mongolia Institute of Agriculture and Animal Husbandry, Huhhot).

In this paper, the content of free amino acids in the roots of Sophora flavescens L. was analyzed. The total content of sixteen kinds of free amino acids was 194.52 μg / 100 mg; glutamic acid content was highest (47.68 μg / 100 mg), and the methionine content was the lowest (0.20 μg / 100 mg) [11].

4. The field of research considering the general problem, which is described in the article

Scientists Zhang Jianhua, Wu Yun, Hou Jianhua, Ma Qingzhi studied the content of free amino acids in the roots of Sophora flavescens L. An actual question for us was the analysis of the content of amino acids in both the free and bound state in the studied sample of medicinal plant material. It should be noted that the proteins are complex polymers containing amino acids that come from the digestive tract in the case of digestion of protein nutrients. A special place in metabolic transformations belongs to amino acids. It is the amino acids in the bound state that are the main elements of the construction of proteins and biologically active substances (hormones, vitamins, enzymes) and act as regulators of immune processes.

Sophora flavescens L. is a promising medicinal plant material for use in official medicine and makes it possible to create new drugs of combined action on the basis of the specified type of medicinal plant material and requires a comprehensive phytochemical study. Information on the study of the chemical composition of the Sophora flavescens roots available in Ukraine, in particular studies on the study of the amino acid composition, is very limited.

5. Formulation of goals (tasks) of article

The aim of the work was to study the content of amino acids in the shrubby sophora roots (roots of Sophora flavescens L.).

6. Presentation of the main research material (methods and objects) with the justification of the results

For the study, we used shrubby sophora roots (supplier – PP "Naidionova", Lviv, the producer – China). Quantitative determination of the amino acids of the sophores of the yellowing roots was carried out by the HPLC method. The method is based on the extraction of free amino acids from vegetable raw materials and acid hydrolysis of herbal preparations with the subsequent analysis of hydrolyzates by the HPLC method with percoloceral derivatization of 9-fluorenylmethoxycarbonyl chloride (FMOC) and o-phthalic aldehyde (OPA) followed by detection with a fluorescence detector.

Chromatographic separation was carried out on an Agilent 1200 (Agilent technologies, USA) liquid chromatograph. Porabax AAA column length 150 mm, internal diameter 4.6 mm, sorbent grain diameter 3 μm. Mobile phase A – 40 mM Na2HPO4 pH 7.8; B – ACN: MeOH: water (45:45:10, v/v/v). Gradient separation mode with a constant flow rate of 1.5 ml/min. Temperature thermostat column 40 °C. Precolumn derivatization was performed in an automatic programmed mode using FMOCl reagent (Agilent 5061–3337) and an OPA reagent (Agilent 5061–3335). Detection of derivatized amino acids was carried out using a fluorescence detector.

Sample preparation and analysis of plant raw materials:

a. Free amino acids. The weight of the powdered drug was placed in the vial, 4 ml of aqueous solution of 0.1 N hydrochloric acid was added and held in an ultrasonic bath at 80 °C for 3 hours.

b. Common amino acids. The weight of the drug was placed in vial, 1 ml of an aqueous solution of 6N hydrochloric acid was added and placed in a thermostat at 110 °C. Hydrolysis was carried out for 24 hours.
An aliquot of centrifuged extract / hydrolyzate is evaporated on a rotary evaporator, rinsed three times with distilled water to remove the hydrochloric acid. Resuspended in a suitable volume of distilled water and filter through membrane filters from regenerated cellulose with pores of 0.2 μm. The fluorescence derivatives were obtained in an automatic programmable mode before entering the sample into a chromatographic column.

Identification of the amino acids was carried out by comparing the 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 [12–14].

Chromatograms obtained as a result of the study of the amino acid composition of the investigated raw material are shown in Fig. 1. Results of determining the quantitative content of amino acids are presented in Table 1.

As a result of a free and bound chromatographic study, the content of 15 amino acids, of which 6 are irreplaceable (threonine, valine, methionine, leucine, isoleucine, phenylalanine) are detected and determined in the free and bound state. Among the identified amino acids, most (11 compounds) belong to the aliphatic group; there is also the presence of 2 aromatic and 2 heterocyclic amino acids, which is consistent with the literature data on the content of amino acids in plants [3, 15].

Table 1

| No. | Retention time, min. | The name of the amino acid | Amino acid content, μg / mg |
|-----|---------------------|---------------------------|----------------------------|
| 1.  | 1.61                | L-Aspartic                | 0.961 / 0.732 / 0.229      |
| 2.  | 2.79                | L-Glutamic                | 0.952 / 0.145 / 0.806      |
| 3.  | 5.98                | L-Serine                  | 0.986 / 0.139 / 0.846      |
| 4.  | 7.12                | L-Histidine               | 0.708 / 0.066 / 0.642      |
| 5.  | 7.41                | Glycine                   | 1.292 / 0.040 / 1.252      |
| 6.  | 7.63                | L-Threonine               | 0.472 / 0.046 / 0.426      |
| 7.  | 8.70                | L-Arginine                | 1.034 / 0.163 / 0.871      |
| 8.  | 9.049               | L-Alanine                 | 0.583 / 0.132 / 0.451      |
| 9.  | 10.49               | L-Tyrosine                | 0.506 / 0.098 / 0.409      |
| 10. | 12.60               | L-Valine                  | 0.575 / 0.149 / 0.426      |
| 11. | 12.81               | L-Methionine              | 0.255 / 0.024 / 0.231      |
| 12. | 14.23               | L-Phenylalanin            | 0.687 / 0.062 / 0.625      |
| 13. | 14.44               | L-Isoleucine              | 0.407 / 0.093 / 0.314      |
| 14. | 15.18               | L-Leucine                 | 0.807 / 0.049 / 0.758      |
| 15. | 19.65               | L-Proline                 | 3.689 / 3.611 / 0.079      |
Aliphatic acids are represented by 8 monoamino-carboxylic acids (glycine, alanine, valine, isoleucine, leucine), including containing threonine, serine and sulfur-containing (methionine) compounds. Monoamino-carboxylic acids are represented by aspartic and glutamic acid, diaminomono-carboxylic acid by arginine. Of the aromatic amino acids in the shrubby sophora roots, tyrosine and phenylalanine have been detected. As representatives of heterocyclic acids, there are proline and histidine.

During the study, it was found that in the free state in large quantities, proline (3.61 μg/mg) and aspartic acid (0.73 μg/mg) accumulate; in the bound state – glycine (1.25 μg/mg), arginine (0.87 μg/mg), serine (0.84 μg/mg) and glutamic acid (0.80 μg/mg).

In the free state, methionine (0.024 μg / mg), glycine (0.040 μg / mg) and threonine (0.046 μg / mg) accumulate in the smallest amount; in the bound state – proline (0.079 μg / mg), aspartic acid (0.229 μg / mg) and methionine (0.231 μg / mg).

Such an amino acid as lysine was not defined in the shrubby sophora roots.

7. Conclusions from the conducted research and prospects for further development of this field

1. Thus, by the HPLC method, the content of 15 amino acids, of which 6 are irreplaceable (threonine, valine, methionine, leucine, isoleucine, phenylalanine) are detected and determined in the shrubby sophora roots in a free and bound state. In the series of analyzed acids, monoamionmono-carboxonic, monoamino-dicarboxonic, dianamono-carboxylic, aromatic and heterocyclic amino acids were detected in the sophora gravelecens roots, proline (3.61 %) is accumulated in the free state of the identified amino acids and in the bound state it is glycine (1.25 %).

2. In the free state, methionine (0.024 %) and glycine (0.040 %) accumulate in the smallest amount; in the bound state – proline (0.079 %), aspartic acid (0.229 %) and methionine (0.231 %). The amino acid of lysine was not defined in the sophora gravelecens roots. The obtained data contribute to the expansion of information on the chemical composition of medicinal plant material and will be used in future for specified substance-markers.

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Ganna Shumova, PhD, Assistant, Department of Pharmaceutical, Biological and Toxicological Chemistry, Bogomolets National Medical University, T. Shevchenka blvd., 13, Kyiv, Ukraine, 01601
E-mail: shumova_ganna@i.ua
ANTIPYRETIC ACTIVITY OF THE NEW 2-(((3-MERCAPTO-5-METHYL-4H-1,2,4-TRIAZOL-4-YL)IMINO)METHYL)-5-R-BENZOATES

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Thermoregulation of the body relies on the balance of physiological processes of thermogenesis and thermolysis, which are controlled by neural and hormonal mechanisms. Antipyretic activity, which involves the increase of thermolysis through angiectasis...