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ANALYSIS OF FREE AMINO ACIDS, VITAMINS, AND CARBOHYDRATES IN THE STEMS, LEAVES AND FRUITS OF NITRARIA SCHOBERI

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Abstract
In recent years, many results have been achieved in determining the potential of biological resources of the Aral Sea, the rational use of plant resources of the Aral Sea, the separation of biologically active compounds from them. The Nitraria schoberi plant, which is distributed in the region, contains biologically active substances: alkaloids, flavonols, tannins, catechins, anthocyanin, pectin, polysaccharides and many other chemical antioxidant compounds. No special scientific research has been conducted on the chemical composition, biological properties and biologically active substances of N. schoberi plant. The purpose of this study was to determine the amount of biologically active substances in the plant N.schoberi belonging to the family Nitrariaceae, selected as the object of study. The results of the study were the first to determine the amount of free amino acids, vitamins, carbohydrates in the plant N. schoberi.
At the end of the research, N.schoberi plant, which is distributed on the dried bottom of the Aral Sea, is recommended for the production of new generation drugs as a reserve, given its richness in biologically active substances and high activity.
Keywords: Nitraria schoberi, Aral Sea, biologically active substances, protein, free amino acids, vitamins, carbohydrates.

1. Introduction.

Particular attention is paid to the use of plants in poorly studied areas of Uzbekistan as phytomeliorant and medicinal raw materials. In recent years, research has been conducted to determine the potential of biological resources in the Aral Sea, to use them in various fields of medicine, pharmaceuticals and chemical production, to use biologically active compounds from the Aral Sea plant resources.

2. The degradation of the marineandcoastalecosystems

In recent years, interest in herbal medicines has been growing significantly due to their superiority, toxicity, and broad spectrum of action compared to synthetic drugs. In particular, the Nitraria schoberi plant is one of these [1,2,3].Scientific sources state that most species of the family Nitrariaceae contain peptides, proteins, alkaloids, aminocysts, vitamins and many other biologically active substances. N.schoberi L. and N.sibirica Pall, first grown in the Siberian region by Russian scientists. The content of biologically active substances in the leaves and fruits of plant species: flavonols, tannin, catechins, anthocyanin, pectin, glucose has been determined and their antioxidant properties have been proven [4,5].Iranian scientists have conducted experiments to study the drought tolerance of the N.schoberi plant. Studies have determined the amount of total carbohydrates in the dry leaves of the N. schoberi plant: the irrigation interval increased due to the increase in the amount of proline and sugar [6].Biological preparations based on biologically active substances contained in N.schoberi plant have a high activity and are of great importance in the treatment of many diseases. Also, for many years, biologically active additives made from the fruits of the plant have been widely used in folk medicine, scientific medicine and the food industry. The N.schoberi plant is widespread in desert areas in Central Asia, Europe, North Africa, and southeastern Australia. The N.schoberi plant grows naturally in the dry southern regions of the Aral Sea and occupies large areas [7,8,9].Freshly cut and dried fruits of the N.schoberi plant can be consumed. The fruit of the plant contains sugars, proteins, amino acids, vitamins, pectin, mineral elements, from which juices, jams and food

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colors are made in the food industry [10,11].Extracts from the fruits of N. schoberi are not only antioxidants, but also effective against microorganisms, fungal and inflammatory diseases [12].

3.Methodology

For laboratory analysis, samples were collected from the above-ground vegetative and generative organs of the N.schoberi plant growing in the South Aral Sea region in different seasons of 2015-2018.

Scientific research to determine the amount of biologically active substances in the plant N.schoberi was carried out in the laboratory "Chemistry of proteins and peptides" of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan.

Determination of the amount of free amino acids. Derivation (combination) of free amino acids with phenylthiocarbamail (FAP) was carried out on the basis of analysis of high-performance liquid chromatography (HPLC). The proteins and peptides contained in the aqueous extraction of the samples were precipitated. Take 1 ml of the supernatant and add 1 ml of 20% trichloroacetic acid (STA). After 10 min, the rotation was centrifuged for 15 min at 8000 rpm. 0.1 ml of the residual liquid was dried in a lyophilic dryer. Phenylthiocarbamail (FAP) synthesis of free amino acids was performed by Steven, Cohen Daviel method. FAA amino acid identification was performed on a 75x4.6 mm Discovery HS C18 column on an Agilent Technologies 1200 chromatograph. The following mixtures were used: 0.14M CN3SOONa + 0.05% TEA pH 6.4 and CH3CN. Flow rate 1.2 ml per minute, absorption 269 nm. Gradient% B / min: 1-6% /0-2.5 min; 6-30% / 2.51-40 minutes; 30-60% / 40.1-45 minutes; 60-60% / 45.1-50 min; 60-0% / 50.1-55min.

Determination of the amount of vitamins. Laboratory analyzes to determine the amount of water-soluble vitamins in the vegetative and generative organs of the plant N. schoberi were carried out using the method of high-efficiency liquid chromatography (HELC). 5-10 g of each of the crushed samples was weighed on an electronic scale. The samples were placed in flat 300 ml volumetric flasks and 50 ml of 40% ethanol solution was placed on top. The mixtures were boiled with intensive stirring for 1 h, equipped with a magnetic stirrer, reverse coolant, and then stirred for 2 h at room temperature for 2 h. The mixtures were precipitated and filtered. The remainder was re-extracted twice by adding 25 ml of 40% ethanol. The filtrates were combined and transferred to a 100 ml volumetric flask to the line and filled with 40% ethanol (5-10%). The resulting solutions were centrifuged at 7000 rpm for 10 min. The resulting solutions were taken from the top for analysis.

Working solutions of water-soluble vitamins at a concentration of 1mg / ml were prepared. To do this, 50.0 ml of clear weighing was weighed on an analytical balance from each vitamin standard and dissolved in 40% ethanol in a 50 ml volumetric flask, filled to the line. In the literature, phosphorous, acetate buffer solutions and acetonitrile were used as eluent in the determination of water-soluble vitamins by high-efficiency liquid chromatography (HELC). We decided to use acetonitrile with an acetate buffer system.

Chromatography conditions:Chromatograph Agilent-1200 (equipped with Avtodozator), Column Exlipse XDB C 18 (round-phase), 5mkm, 4.6x1500mm,Diode-matrix detector (DAD), identified 204 nm, 245 nm, 254 nm, 290 nm, flow rate 1ml / min. Eluent acetate buffer: acetonitrile: 0-5 min 96: 4, 6-8 min 90-10, 9-15 min 80-20, 15-17 min 96: 4, Thermostat temperature 25 C, Introduced amount of 5 µl (vcol).

The chromatograph was first introduced into the working standard solutions and then into the prepared working solutions.

Determining the amount of carbohydrates. Carbohydrate quantification analyzes were performed using the High Efficiency Liquid Chromatography (HELC) method. Agilent 1100 liquid chromatograph, equipped with a Degasser G1379A degasser, QuatPump G1311A pump, ALS G1313A auto sampler, Colcom G1316A column thermostat, RID G1362A refractometer detector and Agilent ChemStation Rev. Data processing system B.01.03. Column SupelcosilLC-NH2 5micron 4.6x250 mm, “Supelco”, USA. 100 and 1000 µl micropipettes, “VWR”, Poland. 5 ml pipette, “Biohit”, Finland. Analytical balance AnD GR-202 (accuracy 0.00001 g), “AnD”, Japan. Water deionizers Millipore Simplicity, “Millipore”, France. Ultrasonic bath S 30 H Elmasonic, “Elma”, Germany. Filter Nylon 0.45 micron 13 mm. Fructose is standard, imp. Glucose standard, imp. Standard sucrose, imp. Maltose standard monohydrate, imp. Acetonitrile for HELC “Sigma-Aldrich”, USA.

When developing the methodology, a standard solution was prepared according to an exact weight. To ensure long-term storage, the standard solution was mixed with acetonitrile in a ratio of 1/1. Working solutions of the standards were prepared by diluting the standard solution with a mixture of water-acetonitrile 1/1. In the analysis of honey samples, the solutions were prepared as follows: 1 g of the studied honey (accurately weighed) was dissolved in 50 ml of deionized water. The solution was stirred until honey was completely dissolved in the ultrasonic bath at room temperature in the "mixing" mode. Then the solution was filtered through a membrane filter with a pore diameter of

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0.45 microns. The filtrate was mixed with acetonitrile in a ratio of 1/1 and analyzed by HELC.In the process of testing the methodology, the analysis conditions were determined: the elution mode is isocratic; the composition of the mobile phase is acetonitrile / water in a volume ratio of 82/18 without mixing from two separate containers. The composition of the mobile phase can vary to achieve complete separation of the peaks of glucose and fructose. The volumetric rate of elution of 1.0 ml / min .; injection volume 10 µl; column thermostat temperature 35 ° C; retention times of standards: -fructose –6.7 ± 0.2 min, glucose –7.6 ± 0.2 min, sucrose –11.1 ± 0.2 min, maltose –12.8 ± 0.2 min. Calibration dependence the detector signal from the content of each carbohydrate was determined in the concentration range of 0.1–10.0 mg / ml (corresponding to 10–1000 g / kg of honey) using 6 points; 3 measurements were performed for each point.

4. Results

Some free amino acids in the cytoplasm of a plant cell, such as phenylalanine, proline, play a major role in response to various adverse environmental factors. The effect of free phenylalanine is accelerated, especially under the influence of stress factors.The amount of free amino acids in various organs of the N.schoberi plant was studied. As a result of the analysis, it was found that the stems, leaves and fruits of the N.schoberi plant contain 20 amino acids, as well as a large amount of free amino acids such as alanine, proline and phenylalanine. (Table 1).

Name of amino acids	Stem	Leaf	Fruit
	Concentration mg/ml		
Asparagine acid	0,39	0,25	0,74
Glutamic acid	0,4	0,57	0,48
Serine	0,29	0,46	0,38
Glycine	0,28	0,44	0,45
Asparagine	0,28	0,44	0,45
Glutamine	1,86	5,52	0,69
Cysteine	1,73	1,07	0,92
Threonine	0,15	0,35	0,23
Arginine	2,26	1,45	1,18
Alanine	1,62	1,08	3,85
Proline	3,89	5,63	3,66
Tyrosine	0,19	0,81	0,63
Valin	0,31	1,33	0,46
Methionine	0,1	0,46	0,54
Isoleucine	0,16	1,19	0,22
Lucien	0,3	1,09	0,75
Histidine	0,07	0,70	0,79
Tryptophan	2,42	2,36	2,53
Phenylalanine	12,99	13,67	5,96
Lysine HCl	0,18	0,48	0,06

TABLE 1. The amount of free amino acids in the N. schoberi plant distributed in the Southern Aral Sea region

The main sources of vitamins are plants. Vitamins enhance chemical reactions in the body, affect the body's absorption of nutrients, promote normal cell growth and development of the whole organism, enter the enzymes in the body and ensure their normal activity and activity studied. Studies have shown that the plant contains vitamins B, PP, C, and the analysis is rich in vitamins B9 and PP (Table 2).

Name of Vitamins	Stem	Leaf	Fruit
	Concentration mg/ml		
B1	0,01	0,02	0,03
B2	0,23	0,98	0,72
B6	0	0	0
B9	4,26	5,43	4,38
C	0,52	1,03	0,07
PP	17,86	27,95	1,34

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TABLE2. The amount of water-soluble vitamins in the plant Nitraria schoberi

Carbohydrates are one of the main components of plant cell nutrition and structure. They make up 85-90 percent of the plant organism.

The amount of carbohydrates in the N. schoberi plant was studied in a research study. As a result, the amount of fructose, glucose, sucrose and maltose in the plant N. schoberi, which was first distributed in the South Aral Sea region, was determined and chromatographed. (Table 3). The results of the analysis show that the plant contains a large amount of fructose.

Carbohydrates	stalk	leaf	fruits
	Concentration mg/ml		
Fructose	1,19	2,63	11,98
Glucose	0,1	2,66	0
Sucrose	0,01	0	0
Maltose	0,14	0	0
	1,44	5,29	11,98

TABLE3 . The amount of carbohydrates in the N. schoberi plant

4. Conclusion

Based on the results of laboratory analysis of the amount of biologically active substances in the plant Nitraria schoberi L., first distributed in the dry southern regions of the Aral Sea, the plant contains 20 amino acids, as well as abundance of free amino acids such as alanine, proline and phenylalanine, vitamins B9, PP group in the stems and leaves, and fructose content in the plant was found to be higher than glucose.

According to the results of scientific research, N.schoberi plant is recommended for the production of new generation drugs as a reserve, given its richness in biologically active substances and high activity.

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