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STUDY OF THE PRIMARY METABOLITS OF SEEDS OF THE LOCAL PLANT AMARANTH TAILED (*AMARANTHUS CAUDATUS L.)*

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Seeds of Amaranthus caudatus L. were collected in October in the Andijan region of the Republic of Uzbekistan. The collected raw materials were dried in air directly under the sun. Extraction was performed with hexane in a soxhlet extractor. In this studyon the native plant Amaranthus caudatus L., the chemical composition of seeds, in particular lipids, proteins and carbohydrates, was studied.

Keywords: Amaranthus caudatus L., lipids, phospholipids, neutral lipids, glycolipids, carbohydrate, protein, amino acid, amaranth oil

The oils of most wild plants and cultivars have long been valued for their use in medical practice. These oils are used as independent medicinal preparations for the treatment of burns and wounds and as auxiliary substances for the preparation of injectable solutions, ointments and suppository bases. In addition, vegetable oils and their production wastes can serve as a source for obtaining new drugs with a unique set of pharmacological properties. The therapeutic effect of fatty vegetable oils is due to the presence of a whole complex of biologically active compounds, such as tocopherols, essential phospholipids, polyunsaturated fatty acids, and phytosterols. In recent years, there has been a tendency to expand the raw material base for the production of vegetable oils through the introduction of new nontraditional plants and food industry waste. Currently, pilot production of amaranth seed oil has been organized in Uzbekistan and is actively promoted to the pharmaceutical market as a biologically active additive.

Amaranth seeds are characterized by high contents of protein, lysine and other amino acids. The value of the proteins in the seeds exceeds the biological value of the proteins in milk. Amaranth is more similar to animal protein. The nutritional value of proteins can be assessed by the number of coincidences according to a 10ϑ point system: soy protein is equal to 68 conventional units, barley is equal to 63, wheat is equal to 57, corn is equal to 44, and amaranth is equal to 75. Moreover, cow's milk protein has a coefficient of 72 points. [1]

One of the main components of the lipid fraction of the seeds is phospholipids, which have hepatoprotective, immunomodulating, antioxidant and regenerative effects. The content of phospholipids in vegetable oils follows the requirements of regulatory documents and is provided in summary form. However, not all phospholipids have the same pharmacotherapeutic effect. Therefore, the study of the phospholipid composition of plants seeds used in pharmaceuticals, as well as the development of modern methods for the separation and analysis of this group of substances, is very relevant.

MATERIALS AND METHODS

We studied the amino acids in the seeds of Amaranth plants grown in the Andijan, Uzbekistan. Amaranth L. belongs to the Amaranthaceae family and is a local plant of Uzbekistan.

The quantitative contents of amino acids of seeds Amaranth caudate was determined by HPLC withUV-detector . Identification of the FTC amino acids was carried out with an Agilent Technologies 1200 chromatograph and a 75×4.6 mm Discovery HS C18 column.

Solutions A and B. Mobile phase A - 0.14 M CH_3COONa + 0.05% TEA (triethylamine), pH 6.4. Mobile phaseB - HPLC-grade acetonitrile.

Chromatographic conditions: flow rate - 1.2 ml/min; column temperature - 25°C; wavelength - 269 nm; injection volume -5 μl;

The test sample and the standard were dissolved in ethanol and filtered through a Millipore filter with a pore size of 0.2 μm before HPLC analysis.

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Preparation of a working standard sample (WSS) solution. Standard samples of the following amino acids were used. In addition, distilled water, acetonitrile, isopropyl alcohol, sodium acetate, phenyl isothiocyanate, hydrochloric acid and sodium hydroxide were used. To construct the calibration curve, an initial concentrated solution of amino acids in 1 M hydrochloric acid was used. For this, 150, 100, 50, 25, and 15 μL of the WSS solution was placed into five test tubes and dried at 65°C under a stream of air that entered through a capillary under vacuum generated by a water jet pump. Then, 0.10 ml of 0.15 M NaOH was added to the dried aliquots followed by thorough mixing. Next, 0.35 ml of a solution of phenyl isothiocyanate in isopropyl alcohol was added, the mixture was stirred and 0.05 ml of distilled water was added. In the case of turbidity in the solution, the test tube was heated for 10-15 s in a water bath at 60 °C until the solution became clear. The tubes were then maintained at room temperature for 20 min and immediately dried in a bath at 60 °C for 10-15 min. The dry residue was dissolved in 1 ml of distilled water and filtered through a membrane filter with a pore diameter of 0.45 μm [1]. The resulting solutions were sequentially injected onto the chromatographic column.

Preparation of the test sample by the precipitation of proteins and peptides from the aqueous extract into centrifuge tubes. For this procedure, 1 ml (exact volume) of 20% TCA was added to 1 ml of the test sample. After 10 minutes, the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. Then, 0.1 ml of the sedimentary liquid was freeze-dried.

The objects of the study were samples of Amaranth seed oil obtained in 2020 by an extraction method. Chloroform solutions of phospholipids from ICN BIOMEDICAL were used as standards: phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PEA), phosphatidylserine (PS), phosphatidic acid (PC), and lysophosphatidylcholine (LPH).

Preparation of the Phospholipid Complex (PLC). The extraction of phospholipids from amaranth seed oil was carried out according to the Bligh and Dyer method, which is a simplified version of the Folch method. The deposition of the PLC from the chloroform extract was carried out with cold acetone. The separation of the PLC into individual phospholipids was carried out by three methods: column chromatography on sorbents AV-17-2P, KRS-2P; TLC on Sorbfil plates (Sorbpolymer, Krasnodar) in a chloroform-methanol-water solvent system (65: 25: 4); high-voltage electrophoresis on an LFGB device (Hungary) with a horizontal chamber at 600 V.

The determination of the fatty acid composition of the lipid fraction of amaranth seed oil was carried out by GLC according to state standards (GOST R 51483 99) on a Crom-5 chromatograph.

The analysis of monosaccharides in the free state was carried out by direct phase HPLC. In their quantitative determination, 100 mg of plant material was poured into 1 ml of water in a test tube with a screw cap, heated at 90°C until it swelled, and then carbohydrates were extracted for an hour at a temperature of 250°C with shaking. The resulting extract was centrifuged for 10 minutes at 1400r pm, activated charcoal was added, shaken and centrifuged again for 10 minutes at 1400 rpm. A 20 μl aliquot of the supernatant was analyzed on a Liena NH 24.6x250 mm or similar column with a mobile phase: acetonitrile-water (70:30) at a flow rate of 1 ml/min at room temperature with refractometric detection. In this case, a Gilson isocratic chromatograph was used, which included a pump with a 5 SSC analytical head, an injector with a 20 micron loop, a column thermostat, and a refractive index detector. The collection and processing of chromatograms was carried out using the Ecochrome program, the assignment of peaks and the calculation of carbohydrate concentrations were carried out according to an external standard containing a mixture of fructose, glucose, and glycerol at a concentration of 1 g/l.

RESULTS AND DISCUSSION

The resulting solutions were filtered through a Millipore filter with a pore size of 0.2 μm and chromatographed under the above conditions. The chromatography results showed s**um of**amino a cid in amaranth seeds 7.56%: **aspartic acid** 0.411, **glutamic acid** 0.602, **serine** 0.128, **glycine** 0.12, a**sparagine** 0.12, **glutamine** 0.431, **cysteine** 3.22, **threonine** 0.25, **arginine** 0.39, **alanine** 0.28, **proline** 0.175, **ty-**

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rosine 0.192, **valine** 0.117, **methionine** 0.083, **isoleucine** 0.103, **leucine** 0.102573, **histidine** 0.147, **tryptophan** 0.26, **phenylalanine** 0.186, l**ysine** 0.25%.

Intermediate precision. To determine the intermediate precision, the content of the active pharmaceutical ingredient was measured in six samples prepared by different analysts on different days. By evaluating intermediate precision with the quantitative determination of amino acids, this HPLC technique gave good results.

Neutral lipids (NLs, oil) were isolated from air-dried crushed seeds subjected to a Soxhlet extractor using gasoline extraction (bp 72-80°C) [1].

Lipides polaires (NS) were extracted from the oil by hydrolysis with a 10% KOH solution in methanol [2]. After extraction of NLs, the meal was dried in air and then with a mixture of chloroform and methanol (2: 1) according to the Folch method [3]. A concentrate of polar lipids (PLs) was the extracted, consisting of residues of NLs, glycolipids (GLs), and phospholipids (PLs). The crude PL extract was treated with a 0.04% aqueous solution of $CaCl_2$ to remove nonlipid components. Then, the PLs were fractionated by column chromatography (CC) on silica gel into separate groups of lipids, while the NLs were eluted with chloroform, GLs with acetone, and PLs with methanol. The yield of the lipid groups was established gravimetrically.

The qualitative composition of NLs, unsaponifiable substances, GLs and PLs was found by TLC on silica gel and silufol plates.

In the NLs of the seeds, according to TLC data in solvent systems (hexane: ether: acetic acid at 7:3:0.1 and heptane: benzene at 9:1), paraffinic hydrocarbons, isoprenoid hydrocarbon squalene, fatty acid esters with phytosterols and triterpenols, triacylglycerides (main component), free fatty acids, phytosterols and triterpenols were identified. (tab.1)

Table 1.

Characteristics of lipids (oil) from amaranth seeds

Index	Content
Moisture and volatile substances, % by weight of seeds	10, 41
The yield of neutral lipids (oil content) at the actual moisture content, % of the mass of seeds	8,37
The yield of neutral lipids per absolutely dry matter, % of the mass of seeds	7,11
The content of unsaponifiable substances, % of the mass of neutral lipids	7,34
Polar lipids (PL), % of the seed mass, including: glycolipids phospholipids	1,08 0,42 10,66

Among the unsaponifiables in the solvent systems (hexane: ether at 6:4, and heptane: benzene at 9:1), paraffinic hydrocarbons, squalene, triterpenols and phytosterols were found. In the composition of GLs (TLC, chloroform: acetone: methanol: acetic acid-water at 65:20:10:10:3), esters of steryl glycosides with FA, monogalactosyldiacylglycerides, steryl glycosides (main component), and digalactosyldiacylglycerides were found. Phospholipids (TLC, chloroform: methanol: concentrated ammonia at 65:35:5) were represented by phosphatidylcholines, phosphatidylethanolamines, phosphatidic acid, and phosphatidylinositols. [4]

To identify the composition of fatty acids of the neutral lipid, glycolipid and phospholipid were hydrolysed with an alcoholic alkali solution, and the isolated fatty acids were methylated with freshly prepared diazomethane. FAs in the form of methyl esters were analysed by GC using an Agilent 6890 N device with a flame ionization detector and a capillary column (30 m x 0.32 mm) with a stationary phase of SP-5, carrier gas of helium, and programming temperature of 150-270°C.

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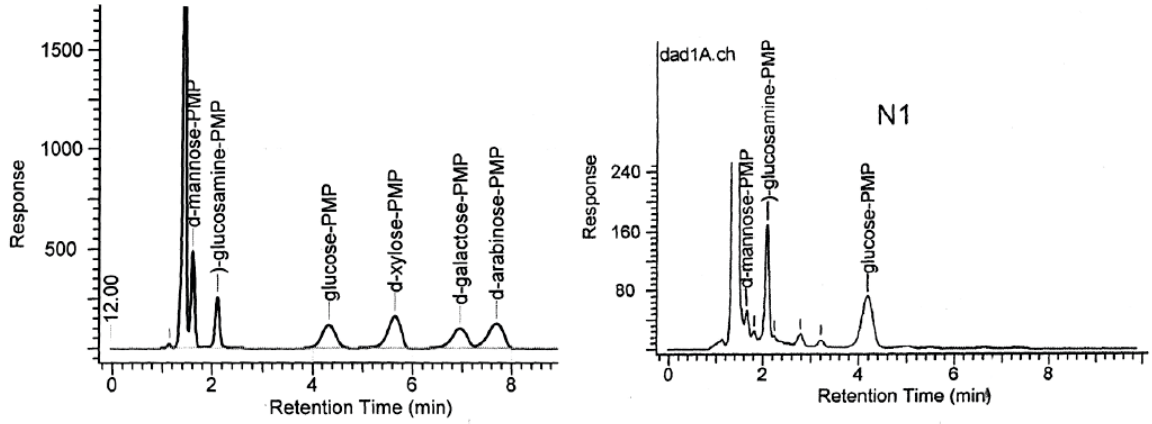
Composition of fatty acids in the neutral lipids, glycolipids and phospholipids of Amaranth seeds, as determined by GC.: there are analised Lauric, Myristic, Pentadecane, Palmitic, Palmitoleic, Margarine, Stearic, Oleinic (omega – 9), Linolenic (omega – 3), Arachinic, Linoleic (omega -6), Behenic, Lignoceric acids. Therefor sum of saturated fatty acids are 15,23% in NL, 49,59% in GL, 19,67% in FL and sum of unsaturated fatty acids are 84,77% in NL, 50,41% in GL, 80,33% in FL.

The results of studies on the detection of free and bound sugars in caudate amaranth seeds. From the results of studies of the carbohydrate composition of amaranth seeds of the Fergana Valley variety, it follows those monosaccharides in the free state were not detected in them, and a fairly significant glucose content was determined from bound sugars (fig.1).



а *б*

Fig. 1. Electrophoregram of a mixture of sugars of standard samples (a) and related monosaccharides of aqueous extraction of amaranth seeds of the Fergana Valley (b)

Summary of the results of research, the tailed amaranth, grown on the territory of Uzbekistan, was selected and the composition of its nutrients was studied. The results of the studies are presnted in Table 2.

Table 2.

Composition of nutrients in amaranth seeds

Index	Seeds of the amaranth
Proteins	18,0%
Lipids	6,8%
Starch	64%
Mono and disaccharides	5%

CONCLUSION

A method for the qualitative detection and quantitative determination of amino acids in the seed meal of the local raw plant material Amaranth caudatus by the modern HPLC technique was developed. This method was validated and can be recommended for use for the determination of amino acids. The main component of the phospholipid group was phosphatidylcholines; in addition, phosphatidylethanolamines, phosphatidylinositols and phosphatidic acids were found. By the method of capillary electrophoresis, a fairly high glucose content was found in the seeds of the Ferghana Valley variety of Amaranth grown in the Andijan region.

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ИЗУЧЕНИЕ ВЕЩЕСТВ ПЕРВОГО МЕТАБОЛИЗМА СЕМЯН МЕСТНОГО РАСТЕНИЯ АМАРАНТА ХВОСТАТОГО

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Семена Amaranthus caudatus L. были собраны в октябре в Андижанской области Республики Узбекистан. Собранное сырье сушили на воздухе непосредственно под солнечными лучами. Экстракцию проводили гексаном в экстракторе сокслета. В этом исследовании на местном растении Amaranthus caudatus L. был изучен химический состав семян, в частности липидов, белков и углеводов.

Ключевые слова: Amaranthus caudatus L., липиды, фосфолипиды, нейтралные липиды, гликолипиды, углевод, белок, аминокислота, амарантовое масло

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МАҲАЛЛИЙ ДУМЛИ АМАРАНТ УРУҒИНИНГ БИРЛАМЧИ МОДДАЛАР АЛМАШИНУВИ МОДДАЛАРНИ ЎРГАНИШ

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Amaranthus caudatus L. уруғлари Ўзбекистон Республикасининг Андижон вилоятида октябр ойида йиғиб олинган. Йиғилган хом ашё тўғридан-тўғри қуёш нурларидан ҳаво билан қуритилган. Экстракция Сокслет экстракторида гексан билан амалга оширилган. Ушбу ишда маҳаллий ўсимлик Amaranthus caudatus L. уруғларининг кимёвий таркиби, хусусан липидлар, оқсил ва углеводлар ўрганилган.

Калит сўзлар: Amaranthus caudatus L., липидлар, ГХ (газ хроматографияси), фосфолипидлар, нейтрал липидлар, гликолипидлар, amaranth мойи