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Biologically Active Substances Elaeagnus Angustifolia

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Abstract:

Currently, close attention in the field of pharmacy and medicine is aimed at finding new sources of biologically active substances of various origins, including plant origin. The woody plant Elaeagnus angustifolia is considered as a promising source. Despite its widespread use in the food industry, folk medicine for diseases of the gastrointestinal tract, as they have an astringent, anti-inflammatory and enveloping effect.

Key words: *Elaeagnus angustifolia L., amino acid, fatty acids, monosaccharides, vitamins.*

Introduction:

Lokh narrow-leaved medicinal - a plant included in most of the world's pharmacopoeias. It is widely used in folk medicine for diseases of the gastrointestinal tract, as it has an astringent, anti-inflammatory and enveloping effect. They are used as an expectorant (for bronchitis), diuretic (for edema), as an anthelmintic and vitamin agent. The infusion of fruits exhibits hypotensive and mild analgesic effects. In Central Asia, baby food is prepared from pericarp powder. In Iranian traditional medicine, the fruits have been used as an analgesic and anti-inflammatory agent in patients with rheumatoid arthritis, as well as to speed up the wound healing process [1].

The main results and findings:

In Armenia, the drug pshatin, a concentrate of polyphenolic compounds, used for colitis and other diseases of the digestive tract, was obtained from the fruits of the narrow-leaved oak tree [2,3].

Fiber of fruits helps to eliminate from the body toxic substances, excess cholesterol, heavy metals, stimulates the processes of bile secretion [4]. On the basis of oak seed oil, compositions of soft dosage forms have been proposed, the regenerative and anti-inflammatory activities of which have been experimentally proven.

Based on the foregoing, the aim of this work is the chemical study of various organs of the narrow-leaved oak tree growing in various climatic conditions of the Republic of Uzbekistan.

The Experimental Part :

Due to the fact that the migration of various chemical compositions from raw materials to extracts is very different and the fact that the elemental, amino acid, polysaccharide, vitamin and fatty acid compositions of plant vegetative organs are also influenced by climatic conditions and the material composition of the soil, various organs were studied these plants, in particular the leaves, flowers, pulp, seeds and peel of the fruit. The object for the study was Elaeagnus angustifolia L. collected from different regions of Uzbekistan: Khodjeyly district (No. 1); Nukus (No. 2); Nukus region (No.3); Samarkand 7 (No. 4); Fergana 6 (No. 5); Samarkand 7.2 (No. 6); Tashkent 16 (No. 7); Sirdarya 8 (No. 8); Tashkent 22 (No. 9); Tashkent 11 (No. 10); Tashkent 2 (No. 11); Tashkent 17 (No. 12).

Polysaccharide content tests:

To study the carbohydrate composition, the mass fraction of sucrose, glucose, fructose, and sorbitol was determined by HELC with a refractometric detector.

An exact sample of about 2,5-3,0 g is extracted with 100 ml of distilled water using a magnetic stirrer at 35-40^{0}C temperature for 3 hours. Then the extract with the flask is placed in an ultrasonic bath for 10 minutes and then filtered through a filter with a pore diameter of 0,20 μm (for water-insoluble substances) or with a pore diameter of 0,45 μm in vials of 1-1,5 cm^3 of this solution. First, quantification of the solutions of standard samples of sucrose, glucose, fructose and maltose is carried out. *Preparation of standard calibration solutions for a mixture of sugars (sucrose, glucose, fructose and maltose).*

Standard calibration solutions of a mixture of sugars (sucrose, glucose, fructose and sorbitol) are prepared for simultaneous calibration dependence according to three points, from a higher mass concentration or mass fraction of sucrose, glucose, fructose and sorbitol to a smaller one based on standard solution No.1 in accordance with Table 1.

Table 1.

Preparation of standard calibration solutions for a mixture of sugars (sucrose, glucose, fructose and maltose)

No. of p/p	No. of standard solution	Capacity of volumetric flask, cm	Method of preparation	Mass concentration, g/dm^3, mass fraction, ‰
1	1 (basic)	100	2.0 g of sucrose, glucose, fructose and sorbitol of each are weighed in a 50 cm volumetric flask with the result written down to the fourth decimal place after comma, dissolved in 40 cm of water by 4.18, transferred to a 100 cm volumetric flask, adjusted with water by 4.18 to the mark and thoroughly mix solution No.1.	2,0
4	2	50	Solution No.2: 25 cm^3 is selected from solution No.1, transfer to a volumetric flask, bring to the mark with water and mix thoroughly.	1,0
3	3	50	25 cm^3 is selected from solution No.2, transfer to a volumetric flask, make up to the mark with water and mix thoroughly.	0,5

The standard calibration solutions of the sugar mixture are prepared immediately before the measurements. To prepare the calibration solution No.1 (basic), weigh accurately 2,0 g of sucrose, glucose, fructose and sorbitol of each in a volumetric flask with the result written down to the fourth decimal place after comma, dissolve in 50 cm^3 of water, quantitatively transfer to a volumetric flask with a capacity of 100 cm^3, make up to the mark with water and mix. To prepare calibration solutions No.2 and 3, the corresponding aliquots of the calibration solution No.1 in

accordance with Table 3 are pipetted, placed in volumetric flasks and adjusted to the mark with water, mixing thoroughly. The elution order of sugars and sorbitol is as follows: sucrose, glucose, fructose and sorbitol.

Chromatographic analysis conditions:

- Chromatograph Agilent 1260 Infinity (USA)
- Analytical column: *Exlipse XDB. 8 mkm 4,6x250 mm*
- Eluent: solution Ca-EDTA 0,03-0,1 mmol/dm^3
- Column temperature: 70°C - 90°C
- Detection: refractometric
- Flow rate of the eluent: 1,0 cm^3/min
- The volume of the injected sample 10 µl

Samples Analysis:

Each sample is analyzed three times under repeatability conditions in accordance with the requirements of GOST ISO 5725-1 and GOST ISO 5725-2. The peak areas of sugars are recorded. If the area of the corresponding peak is outside the range of the chromatograph's calibration range, a new less or more diluted sample is prepared and the analysis is repeated.

Tests for the content of vitamins:

To study water-soluble vitamins, the vegetative organs of *Elaeagnus angustifolia* were used. The analyses were conducted by HELC method with a detector on a diode matrix (Diode Array Detector-DAD).

Chromatography conditions:

Chromatograph - Agilent 1200 Infinity with autosampler (USA)

Mobile phase (gradient mode) - acetonitrile - buffer solution pH = 2,92 (4%: 96%) 0-6 min., (10%: 90%) 6-9 min., (20%: 80%) 9-15 min., (4%: 96%) 15-20 min.

The injection volume - 20 µl.

The speed of the mobile phase is 1,000 ml/min.

Column - Eclipse XDB - C18.

The detector is a diode-matrix detector, wavelengths of 272 nm, 292 nm, 254 nm, 297 nm and 360 nm.

Recommended concentrations of vitamins in standard and test solutions:

Vitamin B_1 - from 5 to 15 µg/ml;
B_2 - from 3 to 8 µg/ml;
B_3 - from 2 to 5 µg/ml;
B_c - from 3 to 8 µg/ml;
B_6 - from 5 to 10 µg/ml;
C - from 150 to 300 µg/ml;
Rutin - from 100 to 200 µg/ml;

Preparation of the mobile phase.

Solution A. About 0,240 g (accurately weighed) of sodium pentanesulfonate and 5 ml of glacial acetic acid are dissolved in methanol-water mixture (25:75), transferred to a volumetric flask with a capacity of 250 ml, the solution volume is adjusted to the mark with the same solvent and stirred.

Solution B. About 0,275 g (accurately weighed) of sodium heptanesulfonate and 5 ml of glacial acetic acid are dissolved in methanol-water mixture (25:75), transferred to a volumetric flask with a capacity of 250 ml, the solution volume is adjusted to the mark with the same solvent and stirred.
To obtain the mobile phase, solutions A and B are mixed in a ratio of 5:3.
Preparation of standard solution. Accurate weights of 0,1 g of standard samples of vitamins B_1, B_2, B_6, nicotinamide, B_C (folic acid), C are placed in a 100 ml volumetric flask, 50 ml of the mobile phase are added, heated for 20 minutes in a water bath at 60°C, cooled to room temperature, adjusted the volume of the solution with the mobile phase to the mark, and stirred (SSS). The concentration of the standard solution sample (SSS) is 1 mg/ml.
Carrying out of the analysis. Sequentially chromatography is carried out by preliminarily filtered through a filter with a pore size of 0,5 µm, first a solution of (SSS) and solutions of test samples 3 times each. For calculations of betur, the average value of three injections [5].

Amino Acid Test:
There is data on the determination of amino acids content using a sequencer according to the Edman method and chromatography in a thin layer of a sorbent, as well as using an amino acid analyzer AAA 339 M (Czech Republic) [6-9].
For analysis of amino acids we used 1 g of the fruits of the oleagus and 5 ml of 5,7 N HCl hydrolyzed at 110°C for 24 hours without access to air. The hydrolyzate is evaporated, the dry residue is dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1) and dried. This operation is repeated twice to neutralize the acids. The reaction with phenylthioisocyanate gives phenylthiocarbamyl-derivatives (PTC) of amino acids according to the method [10].
The identification of amino acid derivatives was carried out by HELC (Highly Effective Liquid Chromatography) method. The conditions of carrying out the chromatography:
Agilent technologies 1200 C chromatograph with DAD detector,
Column 75x4,6 mm Discovery HS C_{18}, 3 µm.
Solution A: 0,14 M CH_3COONa+0,05% TEA (tetraethyl acetate) pH-6,4; B: acetonitrile.
Flow rate 1,2 ml/min,
Detection - 269 nm (nanometer).
Qualitative analysis and quantitative calculation of the concentration of the studied amino acids were carried out by comparing the retention times and peaks areas of standard and test samples from the PTC- derivatives of amino acids.

Tests for the content of fatty acids.
The study of lipid compounds (fatty acids) was carried out by gas-liquid chromatography [11]. To study the fat-acid composition, the crushed seeds of the plant *Elaeagnus angustifolia L.* were extracted with chloroform 1:50. Chloroform was distilled off on a rotary evaporator. The resulting oils were methylated and methyl esters were obtained according to the method [12]. Next, chromate mass-spectrometric studies of the obtained methylated derivatives of fatty acids were carried out.
For the qualitative determination of the fat-acid composition, the method of gas chromatography of mass spectrometry was used.
Device of the company Thermo Fisher Scientific, USA with Triple quadrupole.
The chromate mass-spectrometry conditions:
Column - capillary column (0,2 µm x 0,25 mm x 30 m), 5% biphenyl-dimethylsiloxane.
The carrier gas is helium with a constant flow of 1 ml/min.

The initial temperature of the column thermostat is 40°C with a delay of 1 min. Then the thermostat was heated to 280°C at a rate of 20°C/min with a delay of 3 minutes at 280°C, followed by a decrease in temperature to its original state for 6 minutes at a speed of 40°C.
The temperature of the injector and mass-detector is 250ºC.
The extract was injected in a volume of 1 µl in the mode of dividing the stream (split) 1/5.
The ionization method is electron ionization at 70 eV.
The chromatographic profile was recorded immediately after the start of the chromatographic analysis. The chromatography process was controlled with the help of the XCalibur program in the range of limits of m/z 50-1500 values. The components were identified with application of the library of reference mass-spectra of natural compounds "NIST".
Further, a quantitative analysis of the fat-acid composition was carried out by gas chromatography using FID (Flame Ionization Detector) detector.
The analysis was carried out on a Clarus-400 Perkin-Elmer (USA) chromatograph.
- Column Restek, Stabilwax
- Column length - 60m
- Diameter - 0,32 mm ID
- Detector - FID
- Carrier gas - nitrogen
- Gradient temperature: 1-8 min – 80^0C
  8-18 min - 10^0C
  18-22 - 180^0C
- Division stream (split) 1/10

Tests for the content of macro- and microelements:
The quantitative determination of macro and microelements was carried out by the method of Optics of emission spectrometry with inductively coupled argon plasma (ICP OES) on an Optima-2100 DV Perkin-Elmer instrument (USA).
Sample preparation: Accurately weighed about 0.5000-0.1000 g of the test sample is weighed on an analytical balance and transferred to Teflon autoclaves. Then 4 ml of concentrated nitric acid and 3 ml of hydrogen peroxide are poured onto the autoclaves. Close the autoclaves and place them on a BERGHOF microwave digester with SpeebwaveTM MWS-3 + software. The decomposition time of the samples is from 25 to 40 minutes relative to the selected program. After decomposition, the weight of the contents in the autoclaves is quantitatively transferred into a 100 ml volumetric flask and the volume is adjusted to the mark with a blank solution (2% nitric acid). After receiving data from the device, the final Win-Lab processing (offline) is carried out. The device automatically calculates the noise, the form of the solution in the designated areas of the investigated elements. I use a multi-element standard solution as standards. The analysis is repeated 5 times and the arithmetic mean is calculated. The RSD for each element should be between 0.01 and 1.0%.
Used autosampler S-200 Perkin-Elmer,
generator power 1500W,
the flow rate of the peristaltic pump is 1.2 ml / min,
argon flow 10-15 l / min,
axial overview,
nebulizer 0.8 l / min.

Results and its discussion.

In the composition of the polysaccharide complex, glucose, mannose, galactose, fructose, xylose and rhamnose were identified, the R_f of which were, respectively, 0,18; 0,20; 0,21; 0,23; 0,25; 0,41 in the butanol-acetic acid-water system (4:1:5). Chromatographically, in solvent systems ethyl acetate-formic acid-acetic acid-water (18:1:4:3) and ethyl acetate-pyridine-acetic acid-water (5:5:1:3), galacturonic acid (R_f=0,271) was identified, the content of reducing sugars in the fruits of narrow-leaved oleagus is 50,67-55,75% and total sugar (sucrose) -60,0±5,0%. The total content of pectins (water-soluble and insoluble in water) in the fruits of the narrow-leaved oleagus was determined gravimetrically, which was 3,58±0,3%. [13].

Statistically reliable results of determining the technological yield and physical properties of carbohydrate fractions are presented in Table 2. The data obtained indicate a high content of carbohydrates. The water solubility of this fraction, like water-soluble carbohydrates, indirectly implies their high bioavailability in the human body. Polysaccharides of narrow-leaved oleagus may be of interest by analogy with polysaccharides of marshmallow, flax, licorice, plantain, which cause the expectorant and anti-inflammatory effect of the corresponding drugs [15].

Table 2.

The quantitative determination of the content of monosaccharides in the fruit of narrow-leaved oleagus by HELC with a refractometric detector.

№	Name of the sample	Fructose %	Glucose %	Sucrose %	Maltose %	Sum %	№	Name of the sample	Fructose %	Glucose %	Sucrose %	Maltose %	Sum %
1	№1, pulp	26,26	33,1	0,078	0,011	59,45	**13**	№7, pulp	27,89	24,48	1,26	-	53,64
2	№1, peel	20,7	24,48	0,02	0,032	45,23	**14**	№7, peel	27,95	24,69	1,33	0,17	54,14
3	№2 pulp	27,37	34,62	0,065	0,092	62,15	**15**	№8, pulp	24,74	30,18	0,14	0,09	55,15
4	№2 peel	23,01	28,79	0,023	0,064	51,89	**16**	№8, peel	19,28	22,43	0,11	0,15	41,97
5	№3, pulp	27,68	35,44	0,045	0,077	63,24	**17**	№9, pulp	21,7	26,3	0,47	0,09	48,56
6	№3, peel	26,32	32,93	0,016	0,088	59,35	**18**	№9, peel	20,36	25,72	0,20	0,10	46,39
7	№4 pulp	30,43	27,59	0,19	0,11	58,32	**19**	№10, pulp	28,49	26,85	1,02	0,11	56,46
8	№4peel	28,30	25,20	0,15	0,50	54,16	**20**	№10, peel	28,67	26,73	1,32	0,21	56,92
9	№5, pulp	23,66	26,62	0,04	0,04	50,35	**21**	№11, pulp	27,81	23,31	1,74	-	52,86
10	№5, peel	21,57	24,94	0,08	0,16	46,74	**22**	№11, peel	27,63	26,88	0,30	0,04	54,84
11	№6 pulp	29,71	28,46	0,08	0,19	58,44	**23**	№12, pulp	29,02	26,58	2,39	0,21	58,19
12	№6 peel	26,72	24,08	0,04	0,35	51,19	**24**	№12, peel	27,76	24,45	4,30	0,03	56,54

Vitamins in raw materials are represented by ascorbic acid (vitamin C), as well as vitamins B_6, B_1, B_C and B_2. The study of water-soluble vitamins in 16 types of oleagus revealed that folic acid is found in a significant high content. Its content reaches up to 2,323 mg/g. The quantitative content of water-soluble vitamins was determined relatively to the peak areas of the standard samples. The chromatogram of standard samples of vitamins is shown in Fig.1.

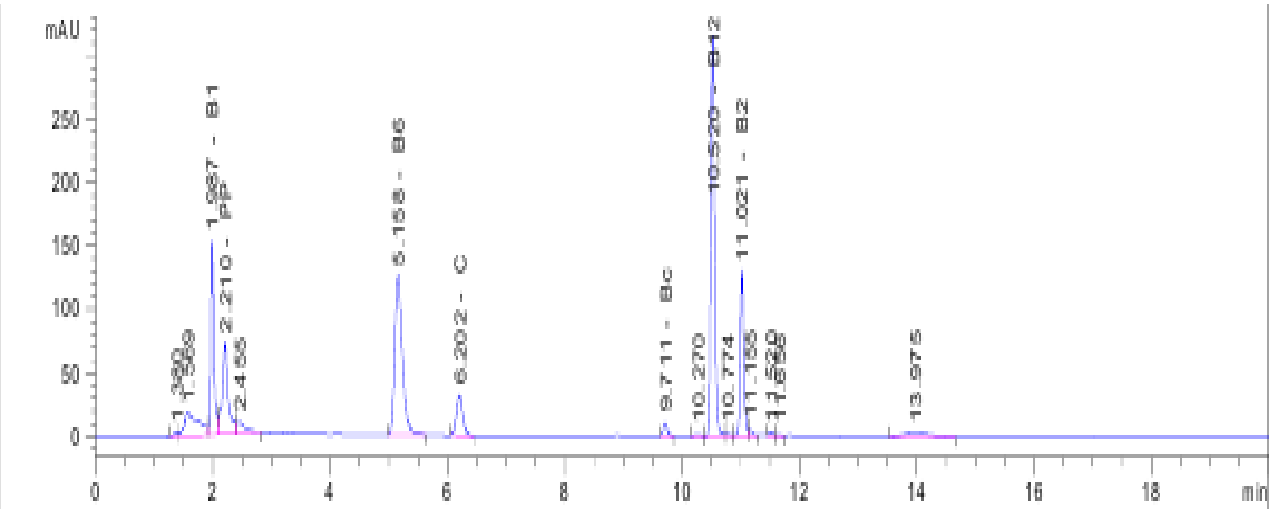

Fig 1. The chromatogram of standard samples of vitamins.

The analyses were carried out according to the method [14].

The following amino acids were found and identified in the fruits of the narrow-leaved oak tree: aspartic acid, glutamine, serine, glycine, asparagine, glutamine, cysteine, threonine, arginine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, histidine, trypilaninyltophan, phenylaniltophan, lysine.

As a result of the studies (see Table 3), macro- (K, Ca, P, Na) and microelements (Fe, Al, Mg, Cu, Zn, Pb, Ba, Sr, B, Mn, Ni, V, Cr, Zr, Ga, Be) 9 of which belong to essential (K, Na, Ca, Mg, P, Cu, Zn, Mn) were found in all studied varieties of raw materials. As can be seen from Table 3, the studied varieties of raw materials have a similar qualitative set of macro- and microelements, but differ in their quantitative content.

The study of the fatty acid composition showed that the quantitative content of oleic and linoleic acids was 91% of the total amount of fatty acids in the lipid fraction.

In the studied varieties of raw materials, potassium and calcium predominate from trace elements-magnesium, manganese, iron, aluminum.

Table 3

Quantitative determination of the content of micro- and macroelements by the method of optics emission spectrometry with inductively coupled argon plasma.

№	element	Pulp											
		№1	№2	№3	№4	№5	№6	№7	№8	№9	№10	№11	№12
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
1	Ag	-	-	-	-	-	-	-	1,547	-	-	-	-
2	Al	1229.23	1330,41	1255,208	23,903	670,208	28,598	20,072	46,350	13,039	28,608	13,004	30,183
3	As	-	-	-	78,556	124,712	103,713	116,306	120,355	137,558	122,268	129,693	130,209
4	Ba	7,59925	6,2	7,3671	1,816	1,297	1,515	2,229	1,109	1,219	1,361	1,977	1,655
5	Be	-	-	-	12,129	48,233	7,879	10,574	-	29,488	-	-	-
6	Bi	-	0,00056	0,0016	2,499	--	-	0,006	-	-	0,078	0,003	0,082
7	Ca	1122,89	1337,3	1146,36	986,54	548,806	820,696	689,456	992,140	674,874	559,896	685,562	653,456
8	Cd	-	0,0084	0,0720	0,339	0,296	0,284	0,084	0,084	0,184	0,156	0,214	0,141
9	Co	0,83601	0,96	0,5289	0,821	0,508	0,850	0,635	0,331	0,408	0,444	0,389	0,491
1	Cr	2,843	2,298	2,4894	1,562	1,236	0,987	1,117	1,896	1,213	0,897	1,201	1,363

0		43											
11	**Cu**	-	0,000174	0,0043	1,286	0,892	1,095	1,025	0,665	0,775	0,831	1,649	1,624
12	**Fe**	18,1644	16,943	17,9174	16,54	12,512	13,258	15,854	17,865	14,854	15,745	14,869	15,636
13	**Ga**	-	-	-	0,184	-	-	-	-	-	-	-	-
14	**In**	1,63938	-	-	2,325	-	0,410	0,273	3,101	-	1,212	0,854	0,781
15	**K**	7046,60	6892,45	7074,93	8252,314	7897,854	8102,365	7325,251	8369,521	7458,695	7685,562	7785,326	7653,364
16	**Li**	0,2418	0,1949	0,2150	30,606	30,606	153,030	30,606	61,212	-	30,606	61,212	-
17	**Mg**	354,472	383,32	327,293	203,673	125,237	148,792	199,259	92,839	86,940	144,652	261,843	167,481
18	**Na**	1329,71	1365,2	1319,105	157,493	176,144	130,921	172,593	141,353	157,270	233,330	239,268	293,925
19	**Mn**	2,5086	2,247	2,4828	0,085	0,049	0,074	0,087	0,0454	0,0366	0,062	0,044	0,029
20	**Ni**	0,1977	0,224	0,2858	1,026	0,684	0,937	0,802	0,508	0,615	0,625	0,549	0,689
21	**Rb**	3,5188	3,227	3,7324	2,545	3,213	1,541	4,144	1,627	1,497	3,552	6,517	3,099
22	**Se**	10,2917	9,685	10,0041	53,821	88,876	74,811	83,756	87,510	100,778	86,727	94,625	35,132
23	**Sr**	13,9774	11,974	14,0028	2,779	3,295	1,926	4,939	1,493	1,372	4,015	7,885	3,402

24	**Tl**	14,7791	13,222	13,3698	1,464	-	-	0,004	-	-	0,047	0,017	0,112
25	**U**	-	-	-	0,235	0,032	0,049	0,028	0,021	0,010	0,014	0,015	0,028
26	**V**	1,65364	1,5368	0,8423	1,456	1,498	1,654	1,214	1,686	1,326	1,326	1,412	1,289
27	**Zn**	5,9555	5,1416	6,9491	0,588	0,403	0,411	0,419	0,228	0,248	0,306	0,210	0,321
28	**Pb**	0,09684	0,0525	0,08444	-	-	-	0,007	-	-	0,054	0,028	0,126
29	**Cs**	-	-	-	1,005	-1,713	0,849	1,236	0,618	0,683	0,757	1,107	0,916

Conclusion:

- the data obtained indicate a high content of carbohydrates. The water solubility of this fraction, like water-soluble carbohydrates, indirectly implies their high bioavailability in the human body.
- vitamins in raw materials are represented by ascorbic acid (vitamin C), as well as vitamins B_6, B_1, B_C and B_2. It has been established that the fruits of the oleagus contain 2,323 mg/ml of folic acid and 0,687 mg/ml of B_6.
- by studying the amino acid composition of the plant *Elaeagnus angustifolia L.*, it was shown that the content of free 19 amino acids averages 0,90 mg/g, and among them the highest content was observed in proline (2,26 mg/g), cysteine (1,53 mg/g) and asparagine (1,098 mg/g).
- the fat-acid composition of seed oil consists of 7 fatty acids, and a large number of unsaturated fatty acids of the omega-6 series are oleic (23,6%) and linoleic (67,5%) acids.
-it should be noted that the narrow-leaved goose contains all vital elements: macroelements (K, Na, Ca, Mg, Si), essential trace elements (Fe, Cu, Zn, Mn, Cr, Se, Mo, I, Co, F), conditionally essential trace elements (As, Br, Li, Ni, V, Cd, Hg), brain elements (Al, Au, Sn, Ta, Te, Ge, Ga). The studied varieties of raw materials contain significant amounts of macronutrients, in particular potassium and calcium, which are vital elements. Among the trace elements, iron, magnesium, aluminium, silicon, manganese and to a lesser extent copper, zinc, barium, strontium, prevail.

Thus, a quantitative assessment of biologically active substances contained in various organs of the narrow-leaved oak tree growing in different climatic conditions of the Republic of Uzbekistan is given.

The results of this work can be used to develop regulatory documents for the production of food and pharmaceutical products from this raw material.

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