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Comprehensive Study Of The Chemical Composition Of Crataegus Pontica L

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ABSTRACT

The object of research was Crataegus pontica L. A comparative study of dioecious nettle growing both in mountainous areas and in the suburbs of Tashkent was conducted. For research, the aboveground parts of the plant (leaves and stem) were used. The analysis of micro and macronutrients was performed by inductively coupled plasma mass spectrometry (ICP-MS). The quantitative content of the water-soluble and vitamins was studied by HPLC relative to standard samples. Chromatographic and structural studies of the aboveground part of the Crataegus pontica L. plant were performed on the content of phenolic compounds and flavonoids in them. Chromatography-mass spectrometry was used to determine the chemical structure of isolated individual compounds. The results of these analyses suggest the presence of the following classes of flavonoids in the plant Crataegus pontica L.: polyphenols, (+) - catechin, (-) - epicatechin.

KEYWORDS

Crataegus pontica L., macro- and microelements, fraction, two-dimensional paper chromatography, chromatography-mass spectrometry, polyphenols, catechin, epicatechin, essential elements.

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INTRODUCTION

The genus Crataegus L. is represented by 15 species, but many of them differ in very insignificant characteristics. The fruits of many species of hawthorn are used by the population to one degree or another, but the most important is the Pontic hawthorn (Crataegus. Pontica C. Koch) or "dulana" with large-fruited, up to 2.5-3 cm in diameter, juicy and strawberry aroma and often high quality fruits [1]. Hawthorn grows in the mountainous regions of our country at an altitude of 1000-1500 meters above sea level, as a rule, separately from other trees, but sometimes hawthorn thickets are also found. The height of the tree can reach 4-10 meters. In nature, there are up to 900 species of this plant. 10 of them grow on the territory of our country. The hawthorn blooms in May-June; fruits ripen at the end of September. A 25-30 year old tree gives up to 70-80 kilograms of harvest [2].

The value of hawthorn preparations lies in the fact that they contain a large number of biologically active substances that affect the cardiovascular system, increase blood circulation in the coronary vessels of the heart, participate in redox processes, and have the ability to reduce capillary permeability and fragility. Hawthorn fruits and flowers are used for functional disorders of cardiac activity, angioedema, hypertension, atrial fibrillation, paroxysmal tachycardia [3,4].

Doctors recommend using alcoholic tincture and hawthorn extract to patients with functional changes in blood arteries, heart disorders, hypertension, weak cardiac activity, and infectious diseases. Hawthorn is a component of cardiovalent drugs and improves cardiac muscle contraction, acting as a sedative and lowering blood cholesterol [5].

Hawthorn is widely used in the pharmaceutical industry in Mexico, Romania, France and other countries. In Russia, it is included in the list of homeopathic remedies. Currently, preparations are being produced that include hawthorn fruits: Cardiovalen, Krategin, Krateponin [6].

Hawthorn varieties contain essential oil. Hawthorn fruits are rich in flavonoids - quercetin, hyperoside, vitexin [7]. The fruits also contain organic acids - citric, oleanic, ursolic, crotegusic, caffeic, chlorogenic acids - flavanoids - [8]. Hawthorn fruits contain carbohydrates - glucose (2.02 mg / g), fructose (2.21 mg / g), sucrose (0.23 mg / g), arabinose (1.82 mg / g), xylose (3.88 mg / g), mannose (4.25 mg / g), galactose (1.31 mg / g). More than 150 substances have been identified - carotenoids, tannins, fatty oils, pectins, monoterpenoids, triterpene and flavonoid glycosides, -sitosterol , choline, sugars, vitamins, steroids, sesquiterpenoids, lignans, hydroxycinnamic acid, organic acids and nitrogen-containing substances, flavanocoumarins, crategusins A and B (2) [9].

Of polyphenols identified epicatechin, procyanidin,hyperoside, isoquercetin and chlorogenic acid. In fruits Crataegus oxyacantha β-sitosterol-3-O-β-D-glucopyranoside, lupeol, β-sitosterol, betulin, betulinic, oleanolic acids, chrysin - [10]. Hawthorn flowers contain salts K, Fe, S, I. They also contain a large amount of I, vitamins B1, B2, PP, C, E. Flowers and leaves of hawthorn contain oligomeric and polymeric procyanidins. Hawthorn leaves contain flavonoids: vitexin-4 '' - Oglycoside and vitexin - 2 '' - O-rhamnoside, biphenyl-5-ol-3-O-β-D-glucoside, 3,4'-dimethoxy-biphenyl-5- ol-4-O-β-D-glucoside, (E) -6- (benzoyloxy) -1-hydroxyhex-3-ene-2-O-β-D-glucoside,

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chanienoside, eriodectyl, and 2 β-O- rhamnosil vitexin. The content of flavonoids in leaves is 0.25 - 0.29%, in fruits 0.12 - 0.14% - [11].

Based on the above, the study of the mineral and chemical composition of the vegetative organs of the plant Crataegus pontica L. is an urgent task.

The aim of this work is to comprehensively study the chemical composition of the plant Crataegus pontica L.

For research, we used both the whole plant and its vegetative part, and its constituent components. Raw materials were analyzed according to generally accepted methods. The extraction of raw materials was carried out according to the method [12].The quantitative content of water-soluble vitamins was studied by HPLC in relation to standard samples. Calculations were made to extract vitamins from the product with an efficiency of 95% [13]. The data obtained are shown in Table 1.

Table 1

Vitamin composition of the aerial part of C. pontica

Vitamins	Water extraction mg / g	Alcohol extraction mg / g
Thiamin. Vitamin B_1	0,079	0,031
Riboflavin. Vitamin B_2	0,004	0,005
A nicotinic acid. Vitamin PP	0,098	0,043
Vitamin C.	8,864	2,654

From the data given in Table 1, it can be seen that in the aqueous extract of the aerial part of the Pontus hawthorn plant, the following vitamins were identified and determined: thiamine, riboflavin, nicotinic and ascorbic acids. The relative highest content of nicotinic acid is 0.098 mg/g and ascorbic acid is 8.864 mg/g. And in alcoholic extraction, their content is relatively small. According to the literature, the blood-red hawthorn contains 0.05 mg /g of thiamine, and 12 mg/g of ascorbic acid [14]. Since the vitamin composition of Pontic hawthorn has not been thoroughly studied, our study was based on the literature data of blood-red hawthorn, with which we further compared the data obtained in Pontic hawthorn. This is clearly seen in diagrams 1 and 2:

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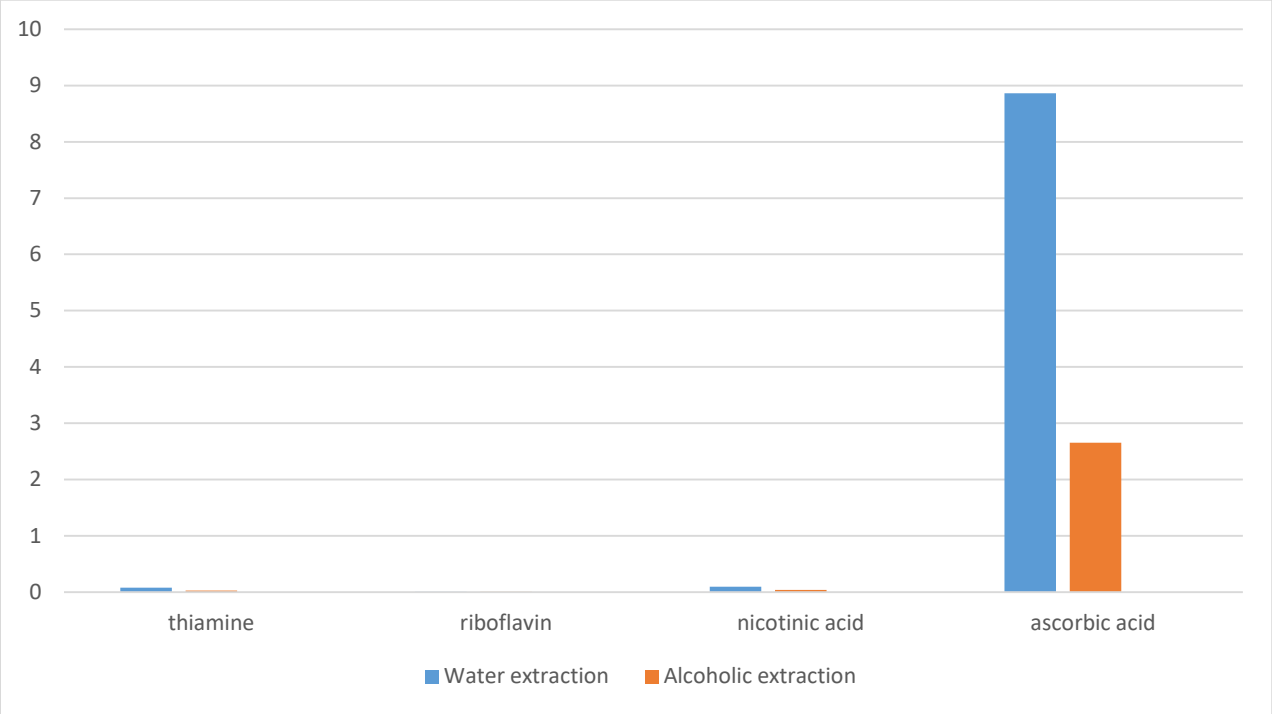

Diagram 1. Vitamin composition of C. pontica

Next, we analyzed the content of macro- and microelement composition of the leaves of Pontic hawthorn, collected in April 2019. The quantitative analysis of the composition of macro- and microelements, essential elements and heavy metals was studied by the method of optics-emission spectrometry with inductively coupled argon plasma (ICP OES). The data obtained are shown in Table 2.

Table 2

The content of macro- and microelements in the leaves and flowers of C.pontica

№	Elements	Quantitative content
		mg/kg
1	K	24198,320
2	Ca	9986,874
3	Mg	4103,895
4	Na	348,728
5	Fe	322,807
6	B	60,035
7	Mn	29,126

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8	Si	239.438
9	Co	0,816
10	Cu	21.479
11	Se	0.335
12	Cr	3.828
13	Zn	34.038

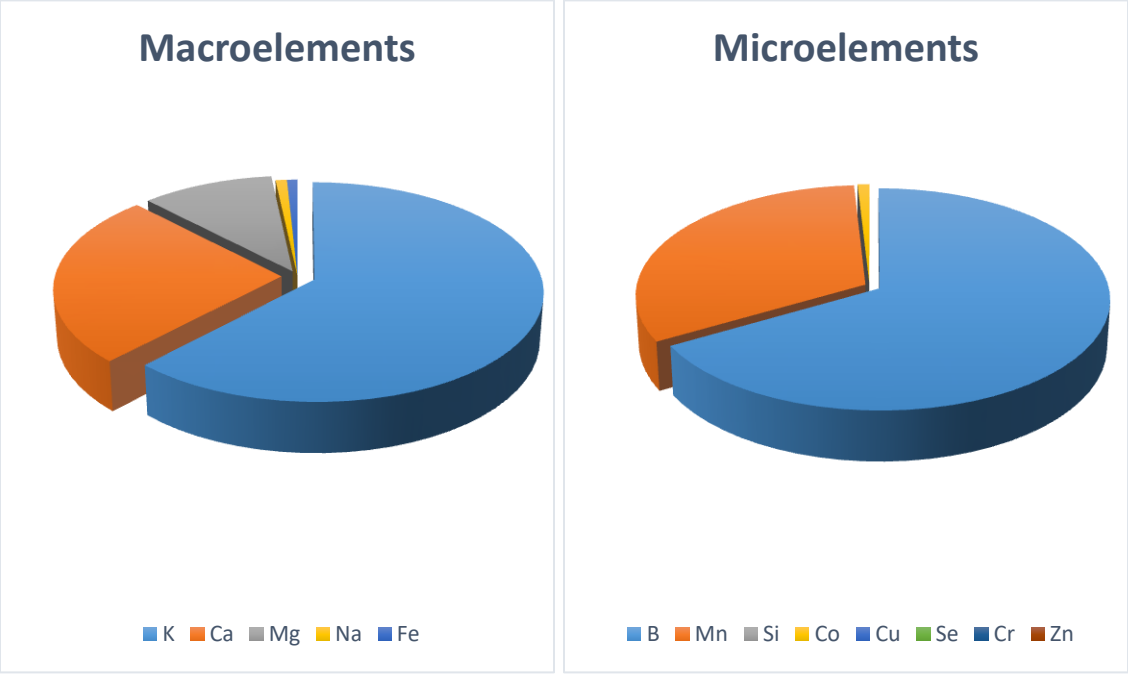

Diagram 2. Macro- and microelements of *C. pontica*

From the data shown in table 2, it can be seen that the macronutrients with the highest content index are 24198 320 mg/kg and 9986 874 mg /kg, which provides the highest content of the trace elements is 239 438 mg/kg. Apparently, such a discrepancy in the amount of macro- and microelements depends on climatic conditions. The literature contains data on the study of macronutrients in the leaves of the plant Crataegus pontica L.

The obtained data show that of the macronutrients were potassium-24198.320 mg/kg, calcium-9986.874 mg/kg, magnesium-4103.895 mg/kg, sodium -348.728 mg/ g and iron-322.807 mg/kg, as well as from trace elements manganese-29.126 mg/kg, bromine-60.035 mg/kg, silicon-239.438 mg/ g, cobalt-0.816 mg/kg, copper -21.479 mg/kg, selenium-0.335 mg/kg, chromium-3.828 mg/kg, zinc-34.038 mg/kg [15].

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Table 3

The content of essential elements in the leaves and flowers of C. pontica

№	Elements	Quantitative content mg/kg
1	Cu	21,479
2	Zn	34.038
3	Co	0.816
4	Cr	3.828
5	Li	0.129
6	V	0.156

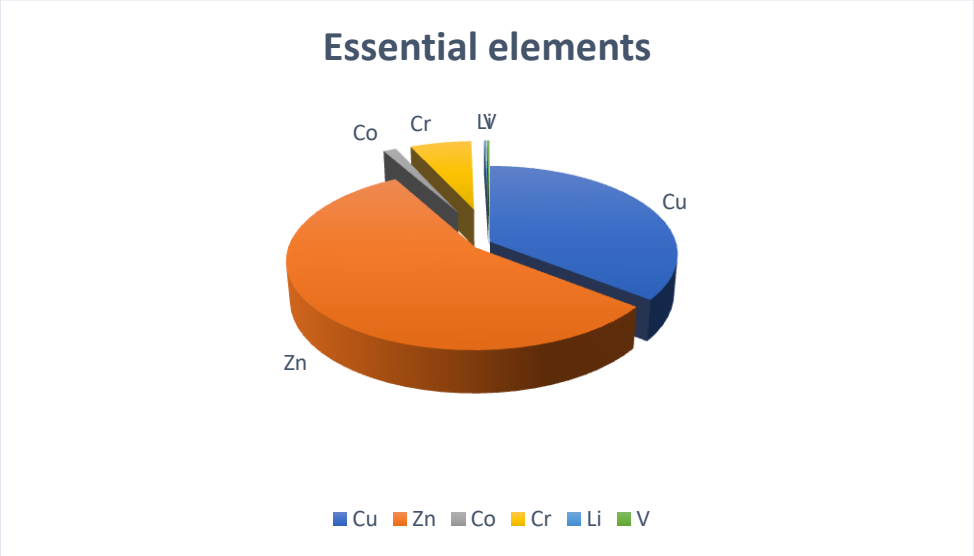

Diagram 3. Essential elements of *C.pontica*

The salts of toxic heavy metals were also determined, such as: copper-21.479 mg / kg, zinc-34.038 mg / kg, cobalt-0.816 mg / kg, chromium-3.828 mg / kg, lithium-0.129 mg / kg, vanadium-0.156 mg / kg. It was shown that in the studied sample of medicinal plant raw materials the content of salts of heavy elements does not exceed the permissible norm.

In the raw material of Pontic hawthorn, for a number of elements, exceeding the maximum permissible concentrations (established for vegetables and greens) for the content of copper, cobalt, zinc, nickel and chromium was noted. Inorganic compounds, along with organic substances, also play a significant role in the manifestation of the pharmacological effect. Research indicates the need to control

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the content of not only toxic heavy metals in the raw material, but also the number of trace elements, which in high concentrations can become hazardous to human health [16]. The results of studies to determine the content of heavy metals in the leaves of the studied plant Pontic hawthorn are given in the table 4.

When studying toxic heavy metals, it is important to study their content for the safety of medicinal plant materials that have a toxic effect on the human body. And in the pharmacopoeial articles "Medicinal herbal raw materials" in the I part of the SP XII, section 24.2 (OFS 42-0059-07) "Heavy metals" is included, according to which maximum permissible concentrations (MPC) of the total content of salts of heavy metals are provided: lead, mercury, cadmium , silver, copper, bismuth, tin, as well as iron not more than 0.05% (not more than 50 mg /%)

Table 4

The content of heavy metals in the composition of leaves of C. pontica

№	Elements	Quantitative content
		mg/kg
1	Al	103.478
2	Pb	0,0753
3	Cd	0.060
4	As	0.107
5	Ag	0.589
6	Hg	0.038

From the data given in Table 4, it can be seen that the highest content of heavy metals is in aluminum, its content is 103.478 mg /kg, and the lowest content is in mercury - 0.038 mg/kg. The toxic elements cadmium and mercury are found in very small quantities. Arsenic 0.107 mg/kg is observed in insignificant quantities, and the lead content is 0.0753 mg/kg. Silver-0.589mg/kg was also found. This content may be due to the fact that in the soil where the plant Crataegus pontica L. grows, there are lead salts, which eventually accumulate in the roots and rhizomes. "Salts of heavy metals", which provides for the maximum permissible concentration (MPC) of salts of heavy metals: lead, mercury, cadmium, silver, copper, bismuth, tin and iron should not exceed 0.05% (no more than 50 mg/%). Iron, iodine, copper, zinc, cobalt, chromium, molybdenum, nickel, vanadium, selenium, manganese, arsenic, fluorine, silicon, lithium are essential (vital) elements. Among the essential elements, iron has a relatively higher amount of 21.479 mg/kg, while in the literature it is 0.3 mg/kg [17].

The next stage of work is the study of polyphenols in leaves of the Crataegus pontica plant. Qualitative reactions to phenolic compounds were carried out according to the generally accepted method [18].

The general scheme for the isolation of phenolic compounds from the plant Crataegus

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pontica is shown in Fig. 1 [19]. The raw material crushed to 2-4 mm was dried under a canopy.

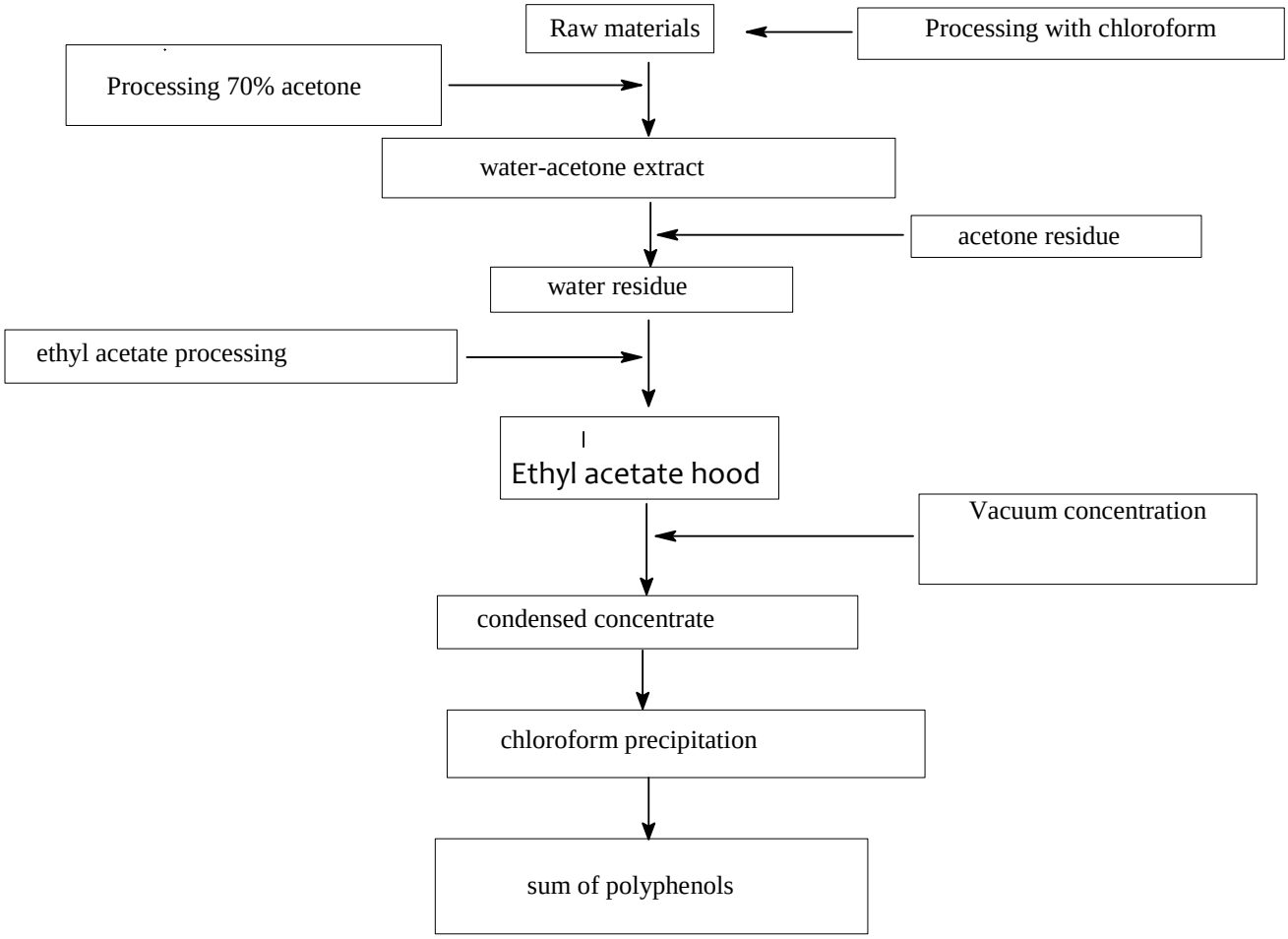

Fig. 1. Scheme of obtaining polyphenols from C. pontica

The dried raw material was treated with chloroform to remove resinous substances, pigments and other related impurities. The material thus treated was dried under air draft to remove residual solvent, and the dried material was extracted three times with 70% acetone at a ratio of 1:10. The obtained extracts were combined, concentrated in vacuo to a small volume, and the remaining aqueous residue was additionally treated with chloroform, then several times with ethyl acetate (aqueous residue: ethyl acetate - 2: 1). The combined ethyl acetate extracts were dried over freshly calcined anhydrous sodium sulfate and concentrated under vacuum in a nitrogen flow at 40-450C [20, 21]. From the concentrated ethyl acetate extract (1.5 L), polyphenols were precipitated by the addition of a fivefold amount of hexane. The flocculent precipitate that formed was filtered off on a glass filter, redissolved in absolute ethanol, concentrated and reprecipitated. The precipitate was filtered through a Schott funnel and dried in a vacuum drying oven.

The resulting amount of polyphenols from the ethyl acetate fraction of hawthorn leaves is an amorphous powder of light brown color, astringent taste. With ferric chloride, the sum gives a greenish-blue color (developers 2 and

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3), with a 1% solution of vanillin in concentrated hydrochloric acid - bright red (developer 1).

During the chromatographic study of the isolated fractions, it was found that the polyphenols of the ethyl acetate fraction are represented mainly by monomeric catechins with a small impurity.

Two-dimensional chromatography on paper in solvent systems 1 and 2 showed that the composition of the total polyphenols of the ethyl acetate fraction contains catechins, namely (+) - catechin, (-) - epicatechin.

5 g of the preparation of the sum of polyphenols from the ethyl acetate fraction was repeatedly ground in a mortar with wet diethyl ether (total volume 1000 ml). In this case, only monomeric catechins, completely freed from condensation products, checked by paper chromatography, pass into the ether. The ether solution was chromatographed on a column (4.5x70 cm) with silica gel (100 g) using peroxide-free water-saturated diethyl ether (system 4, 5) as an eluent. The separation was monitored using paper chromatography in system 1. As a result, flavan-3-ols were isolated in the individual state. The isolated flavan-3-ols are identified as, (+) - catechin, (-) - epicatechin.

(+) - catechin - 5,7,3 ', 4'-tetraoxyflavan-3-ol. Mole mass 290, so pl. 172-173°C, Rf 0.64 (system 1), λ_{max} = 280 (in ethanol), $[\alpha]_D$ -16.9° (ethanol, c 1.05).

(-) - epicatechin - (5,7,3 ', 4'-tetraoxiflavan-3-ol), Mol. weight 290, so pl. 235°C, Rf 0.56 and 0.30 (systems 1 and 2), λ_{max} = 276 nm (in ethanol), $[\alpha]_D$ -60° (acetone-water 1: 1, s 1.22).

The remaining compounds were separated on a polyamide column using a mixture of water-ethanol in various ratios as eluent, and 4 compounds were isolated. The structures of these compounds were established using physicochemical methods. To determine the chemical structures of the isolated individual compounds, the method of chromatography-mass spectrometry was used.

Compound 1-white crystals from water, so pl. 221-223°C, R_f 0.51 in system 1 (n-butanol-acetic acid-water 4: 1: 5 - upper phase). Identified with gallic acid.

Compound 2- (eluted with 30% ethanol) $C_{21}H_{30}O_{16}$, mp. 190–192°C (from CH_3OH), R_f 0.45 in system 2 (n-butanol-acetic acid-water 4: 1: 2). UV spectrum (C_2H_5OH, λ_{max}, nm) 256, 264, 355 nm. During the severe acidic hydrolysis of 10% H_2SO_4, quercetin and rutinose are formed (mp 187–188°C). Acid hydrolysis of 1% H_2SO_4 (stepwise hydrolysis) produces quercetin (mp 312–313°C), D-glucose, L-rhamnose, which was confirmed by thin layer chromatography with reliable witness samples. Identified as rutin (quercetin-3-rutinoside).

Compound 3 - (eluted with 55% ethanol) $C_{21}H_{20}O_{12}$, mp. 232-234°C (from CH_3OH), R_f 0.35 (system 2). UV spectrum (C_2H_5OH, λ_{max}, nm) 361, 257, 265. During acid hydrolysis of the substance (1% H_2SO_4), quercetin (mp 312–313°C) and D-galactose are formed. Comparing the obtained data with the literature, the substance was identified as quercetin-3-O-β-D-galactopyranoside (hyperoside).

Compound 4 - (eluted with 80% ethanol) $C_{15}H_{10}O_7$, m.p. 310-312° C (from CH_3OH), UV spectrum (C_2H_5OH, λ_{max} , nm) 372, 264, 256. R_f 0.64 (system 2). IR spectrum (KBr, υ, cm^{-1}) 3380, 3300 (OH), 1665 (> C = O), 1615, 1565, 1515 (Ar), 815, 840 (p-substitution in ring "B"). Alkaline melting produces phloroglucinol, protocatechuic acid. The substance was identified as 3, 5, 7, 3 ', 4'-pentaoxflavone (quercetin).

Experimental Part

Object of study. The objects of the study were hawthorn leaves (Crataegus pontica) collected at the end of April 2019. Place of sampling - Tashkent region, Republic of

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Uzbekistan. For research, we used both the whole plant and its vegetative part, and its constituent components. Raw materials were analyzed according to generally accepted methods. Extraction of raw materials was carried out with 40% ethyl alcohol [22].

10 g of the crushed sample was placed in a 500 ml flat-bottomed flask and filled with 250 ml of 40% ethanol solution. The mixture is stirred with a magnetic stirrer with vigorous stirring for 1 hour at the boiling point of the solution and then stirred at room temperature for 2 hours. The process was repeated 3 times and the extracts were depleted and concentrated to a residue of 200 ml. The residue was centrifuged for 10 minutes at 7000 rpm, separated the needed part from the sediment. HPLC analysis was performed from the solution. Chromatography conditions:

chromatograph - Agilent-1200 (with autosampler)

column - Exlipse XDB S18, 5mkm, 4.6x150mm

detector - diode-matrix (DAD), (detection at 204 nm, 245 nm, 254 nm, 290 nm).

flow rate - 1 ml / min

eluent (gradient) - acetate buffer: acetonitrile: 0-5 min 96: 4, 5-8 min 90-10, 8-15 min 80-20, 15-22 min 96: 4.

thermostat temperature - 25 0C.

Working standard solutions of water-soluble vitamins with a concentration of 1 mg/ml were used.

Calculations for the extraction of vitamins from a product with an efficiency of 95% [22].

The quantitative analysis of the composition of macro- and microelements, heavy metals was studied by the method of optics – emission spectrometry with inductively coupled argon plasma (ICP OES) [22]. The analysis was performed on an ICP OES Optima-2400 DV (Perkin Elmer, USA) instrument using multi-element standard samples.

Analysis conditions:

Generator power (for plasma) 1300-1500W

Argon flow (plasma) -12 L/min

Nebulizer -0.8 l/min

Peristaltic pump - 1.2 ml/min

Overview - axial

Sample preparation of raw materials was carried out by microwave decomposition (wet ashing) on a Berghoff device with MWS-3 + software (Germany). 0.0500-0.5000 g of an accurately weighed sample of the raw material under study is placed in DAP-60+ Teflon autoclaves for wet ashing. Then the samples are filled with 3 ml of nitric acid (c/h) and 2 ml of hydrogen peroxide (c/h). Decompositions are carried out on a Berghoff apparatus with MWS-3 + software (Germany). After decomposition, the contents in the autoclaves are quantitatively transferred to volumetric flasks and the volume is adjusted to the mark with 2% nitric acid. The determination of the elemental composition is carried out on an ICP OES Optima-2400 DV device (Perkin Elmer, USA) or on a similar device using a multi-element standard (for OES) and a standard - Hg (for OES).

Isolation of polyphenols: air-dry crushed (up to a size of 2-4 mm) leaves of Crataegus pontica (150 g) are pre-extracted with chloroform to remove lipophilic substances. For this, the raw material was placed in a 3 L flask equipped with a reflux condenser and extracted with 2 L chloroform in a water bath at a temperature of 40–45 ° C for 2 h. After three times processing, the extraction of the raw material was continued with 70% aqueous acetone, in a ratio of 1:10. The extraction was repeated three times. Then the obtained water-acetone extracts were evaporated under vacuum on a rotary evaporator to the

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remainder of the aqueous portion. The purified aqueous extract was fractionated with ethyl acetate in a separatory funnel, in a ratio of 1: 5. The ethyl acetate fraction was concentrated and treated with a 5-fold volume of hexane, a flocculent precipitate formed. The yield of the sum of polyphenols from the ethyl acetate fraction was 4.2% of the air-dry mass of the raw material. For the extraction of plant raw materials, solvents were used from JSC "Himreaktivkomplekt" (Uzbekistan), all other reagents were produced by Reakhim (Russia). UV spectra of polyphenols were recorded in an alcohol solution on an EPS-3T instrument (Hitachi, Japan); IR spectra were recorded on an IRTracer-100 instrument (Shimadzu, Japan) in the region of 400-3800 cm – 1. Separation of polyphenols was carried out by column chromatography on polyamide and silica gel LS 100/40 (Czechoslovakia). To identify and determine the homogeneity of substances, BC (Filtrak paper for chromatography) and TLC (on a plate) were used.

The following solvent systems were used to separate and study the composition of polyphenols:

1). n-butanol-acetic acid-water (40:12:28);

2). 2% acetic acid;

3). n-butanol-acetic acid-water (4: 1: 5);

4). diethyl ether-ethyl acetate (7: 3);

five). diethyl ether-ethyl acetate (4: 6);

The following reagents were used as developers for spraying chromatograms:

1). 1% solution of vanillin in concentrated hydrochloric acid;

2). 1% aqueous and alcoholic solutions of FeCl3;

3). A mixture of 1% aqueous solutions of FeCl3 and K3 [Fe (CN) 6];

4). Catechin reagent (1% picric acid solution in 95% ethanol and 5% KOH solution in 80% ethanol).

CONCLUSIONS

1. The chemical composition of the plant Crataegus pontica L growing in the mountainous regions of the Republic of Uzbekistan was investigated. The following water-soluble vitamins were identified in the composition of the Pontic hawthorn plant: thiamine, riboflavin, nicotinic and ascorbic acids. The obtained data are compared with the literature data.
2. The content of macro- and microelement composition of the aerial part of the plant Crataegus pontica L was studied, and it was shown that it contains more than 25 elements, of which the content of essential elements reaches up to 24%, of the total content of elements.
3. The qualitative and quantitative composition of the polyphenols of the plant Crataegus pontica L was studied, the chemical structures of individual polyphenols, such as catechin and epicatechin, were determined by the methods of BC and TLC, as well as chromatography-mass spectrometry, the content of which depends on the time and period of collection.

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