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# POLYPHENOLIC COMPOSITION OF PLANT LEAVES HIPPOPHAE RAMNOIDS L. (ELAEAGNACEAE)

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**ABSTRACT**
The isolated amount of polyphenols from the plant Hippophae rhamnoides L. growing in the Republic of Uzbekistan was divided into individual compounds by column chromatography, HPLC, UV, IR. Namely, phenolic acid: gallic acid, flavonols: quercetin-3-O-galactoside, kaempferol, hydrolyzable tannins: geranin, 1-O-galloyl-4,6-hexahydroxydiphenol-β-D-glucose and 1,4,6-tri-O. -galloyl-β-D-glucose.

**Keywords:** Polyphenols, extraction, Hippophae rhamnoides, tannins, extraction temperature**.**

## Introduction:

In recent years, natural polyphenolic compounds have attracted the attention of scientists not only as an object for determining their chemical structure, but also for the purpose of creating promising drugs with biological activity on their basis. In modern medicine, compounds of this group are used for colds, allergies, tumors, stomach ulcers, viruses, chronic diseases, heart diseases, as well as capillary-strengthening, atherosclerotic, antioxidant and hypoglycemic agents. In recent decades, sea buckthorn Hippophae Rhamnoides L. has become the object of numerous studies [1]. The increased interest in this crop is explained by the content of such biologically active substances (BAS) as fat- and water-soluble vitamins, carotenoids, minerals, flavonoids, polysaccharides and others in its fruits, leaves and bark. Hippophae rhamnoides fruits contain vitamins, fatty acids, organic acids, flavonoids, choline, sugars, triterpene compounds and other substances. It has been established that the leaves of the plant contain vitamin C, flavonoids and additives.

The fruits are used in the treatment of scurvy and stomach diseases, and also as a pain reliever. A decoction of the leaves is also used in the treatment of gout. Extracts obtained from various organs of sea buckthorn have high antioxidant, antibacterial, antimicrobial, anti-inflammatory, anticarcinogenic and antiradiation properties [2,3] A wide range of medicinal properties of various organs is associated with their chemical composition. All parts of the plant are a rich source of biologically active substances, especially flavonoids, carotenoids, phytosterols and others [4,5].

Flavonoids are characterized by antioxidant, anti-radiant, anti-carcinogenic, antimicrobial, antibacterial, anti-sclerotic, immunomodulatory and other activities [6,7]. In the last decade, the antioxidant action of flavonoids, their ability to occupy free radicals, which are the cause of many severe pathologies in humans, and remove them from the body have been of particular interest [8]. In this regard, the search for new plant sources of flavonoids is relevant. Derivatives of quercentin, kaempferol and isorhamnetin have been found in the leaves and fruits of sea buckthorn [9,10]. Chromatographic spectrophotometric method has established that the leaves of sea buckthorn growing in Azerbaijan contain flavonoids, which were isolated individually and identified as: quercetin, myricetin, isorhamnetin, quercetin-3-rutinoside (rutin) and isorhamnetin-3-rutinoside (narcissin). Rutin and narcissin are the main components of H.rhamnoides leaves [10].

**The purpose of the work** is to study for the first time the polyphenolic composition of the leaves of Hippophae rhamnoids L, growing in the Republic of Uzbekistan.

**Experimental part:** To study the content of polyphenols in Hippophae rhamnoids L., we used the dried aboveground part of the plant growing in the Namangan region, harvested at the end of the growing season. 1 kg of plant material was collected and dried and extracted with chloroform (grinding degree 5-7 mm), (ratio 1:8, by volume) at 45°C for 3 hours, in a water bath with reverse cooling, in order to remove lipophilic compounds, 3 times. The extracts were filtered, dried at room temperature until the chloroform was completely removed (48 hours). The raw material was then extracted with 70% aqueous acetone (1:8 by volume) at 45°C for 3 hours, 3 times. The extracts were filtered and the aqueous fraction was separated by distilling off acetone in a vacuum at 35-40°C. The aqueous fraction was extracted with ethyl acetate (1:4 by volume) to obtain an ethyl acetate fraction. This fraction was dried over anhydrous $Na_2SO_4$, filtered and extracted on a rotary evaporator to obtain a concentrate with ethyl acetate. Precipitation of the concentrate with chloroform in a ratio of 1:4 isolated 3.8% of polyphenols based on the dry weight of the plant.

**Chromatography conditions:** Chromatograph – HPLC 1260 Ultimate 3000, Thermo Fisher Scientific (USA), equipped with an automatic sampler,
- column Hypersil GOLDaQ 100 mm x 2.1 mm, particle size 1.9 µm;
- mobile phase: A-acetonitrile, B-0.1% trifluoroacetic acid buffer (pH=3). Buffer concentration gradient with acetonitrile: 0-15 min - acetonitrile 15% (v/v), 15-27 min - acetonitrile 30% (v/v), 27-42 min - acetonitrile 95% (v/v), 42-45 min - acetonitrile 15% (v/v), flow rate - 0.1 ml/min. UV: 220, 254, 280 nm. Thermostat (column) temperature 30°C. Separation of the sum of polyphenols. 5 g of the sum of polyphenols of the ethyl acetate fraction were dissolved in 100 ml of distilled water having a temperature of 40°C. Then the extract was cooled to room temperature, and the total volume was brought up to 500 ml with water. 7.5 g of bald powder was poured with 90 ml of distilled water and the mixture was shaken on a shaker for 20 minutes, then the bald powder was squeezed out and mixed with 500 ml of the obtained solution of polyphenols and shaken again on a shaker for 45 minutes. Then the bald powder with the substances adsorbed on it was placed in a chromatographic column with a size of 4.5x100 cm and washed with diethyl ether, then with distilled water until a negative reaction for phenolic compounds. Elution was continued successively with pure acetone and 60% aqueous acetone. The progress of elution was checked by TLC (toluene-acetone-formic acid 6:6:1).

**Discussion of Results:**

When studying the sum of isolated polyphenols by two-dimensional paper chromatography (system 1: n-butanol-acetic acid-water 4:1:5, system 2: n-butanol-acetic acid-water 10:3:7, respectively), it was found that the plant surface contained more than 10 compounds belonging to the class of phenolic substances. Three fractions were separated by washing the polyphenol aggregate on a silica gel column in a chloroform-methanol solvent system (17:3, 17:4, 17:5). The first fraction was evaporated in a vacuum, the remaining dry precipitate was dissolved in water, and upon cooling the solution, it was found that a substance with $R_f$ 0.51 had precipitated. As a result of comparing and analyzing the parameters of this substance with literary data, it was proven that this substance is gallic acid [10]. As a result of qualitative reactions (ammonia vapor, 5% $Na_2CO_3$ solution) and two-dimensional chromatography applied in systems 1 and 2, it was established that the second fraction contains substances related to the class of flavonols with $R_f$ 0.79, 0.82. To separate the second fraction into individual compounds consisting of flavonols, the polyamide was placed in a column, washed with a solution of methanol: acetone: water 7: 2: 1 (system 3) and a solution of methanol: acetone: water 5: 3: 2 (system 4). As a result of

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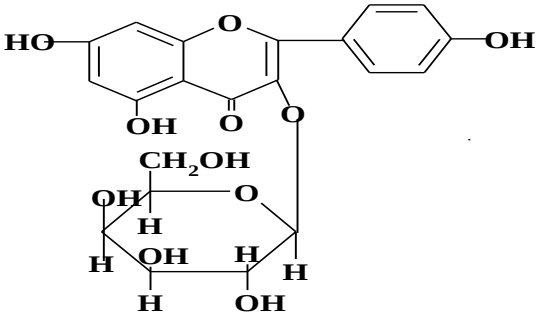
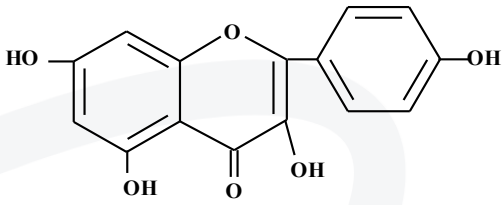
comparing the physicochemical parameters of the isolated substances with literary data, it was confirmed that these compounds are quercetin-3-O-galactoside, kaempferol.

**1-substance**. Kaempferol-3-galactoside - $C_{21}H_{20}O_{11}$, yellow needle crystal, $R_f$ 0.60 (TLC, system 1), liquid part 176-178°C, UV spectrum (EtOH, λmax, nm): 380, 275, IR-(ν,, $cm^{-1}$) spectrum: 3425, 3180 (OH), 1665 (>S=O), 1610, 1575, 1510, 1455 (Ar), 1095, 1055, 1030, 902, 885 (sugar part), 815, 835 (p-state in ring "B").




**2-substance.** Kaempferol - $C_{15}H_{10}O_6$, Mm 286, light yellow finely crystalline substance, $R_f$ 0.82 (TLC, system 1), liquid part 273-275°C ($CH_3OH$), UV spectrum (EtOH, $\lambda_{max}$ max, nm): 266, 366; IR spectrum (ν,$cm^{-1}$): 3340 (OH), 1660 (>C=O), 1615, 1570, 1515 (Ar), 810, 840 (para-substituted "B" ring)




**3-substance**. It was found that the third fraction contains substances related to class 3 hydrolyzable tannins with $R_f$ 0.21, 0.34, 0.40. The sum of substances isolated from this fraction was soaked in holly powder, then placed in a column, washed in a system of solvents 6, 7, 8, 9, 10 -, rechromatographed and individual substances were isolated. The physicochemical parameters of the isolated substances were studied and compared with literary data. As a result, the presence of geraniin, 1-O-galloyl-4,6-hexahydroxydiphenol-β-D-glucose and 1,4,6-tri-O-galloyl-β-D-glucose was found in the composition of the plants.

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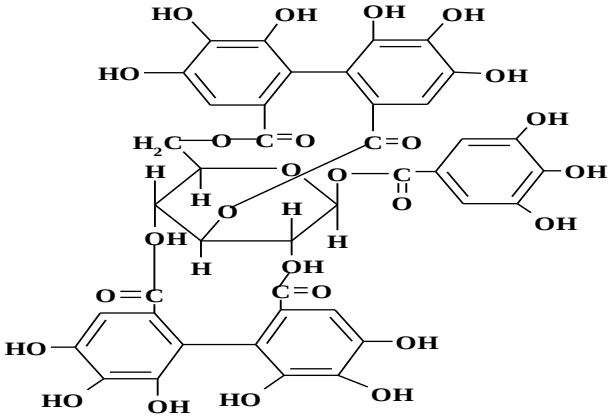
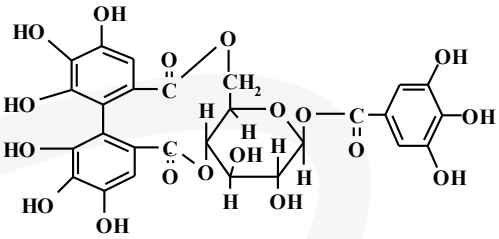
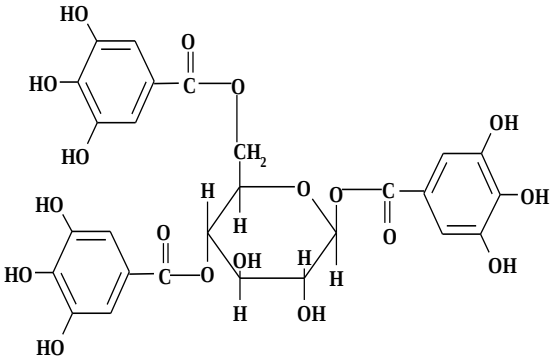
**4-substance**. Geraniin is a hygroscopic yellow powder. $C_{41}H_{28}O_{27}$, Mm= 952, $R_f$ 0.40 (system 2), UV spectrum (EtON, λmax, nm): 224, 285. PMR spectrum (δ, 400 MHz, acetone-d6, J=Hz, m.u.): 5.40 (1H, d, J=8, gluc. H-1), 3.40 (1H ,t, J=9, gluc. H-2), 3.69 (1H, t, J=10, gluc. H-3), 4.77 (1H, t, J=10, gluc. H-4), 3.92 (1H, m, J=5, gluc. H-5), 5.15 (2H, dd, J=6, 13, gluc. H-6 ), 6.97, 7.02, (2H, galloyl, gr. H-2, H-6), 6.98, 6.57 (2H, hexahydroxydiphenol, gr. H-3, H-3`



**5-substance**.1-O-galloyl-4,6-hexahydroxydiphenol-β-D-glucose is a white amorphous powder, $C_{27}H_{22}O_{18}$, liquid part 209-210°C (with decomposition), $R_f$ 0.34 (BC, system 2), IR spectrum (ν, $cm^{-1}$): 3300-3400 (OH), 1620-1610,1450 (Ar), 1320 (-C-OH), 1250, 1045 (-C-O-C), 1080-1070 (C-O), 1040, 1010 (sugar part).



**6-substance**. 1,4,6-tri-O-galloyl-β-D-glucose - brown amorphous powder $C_{27}H_{24}O_{18}$, liquid. 203-205° C, $R_f$ 0.45 (BX, system 1), $R_f$ 0.44 (BX, system 2), PMR spectrum (δ, 400 MHz, acetone-d6, J=Hz, m.u): 6.34 (1N, d, J= 3.5 glut. H-1), 3.86 (1H, dd, J=3.5, 10 glut. H-2), 4.24 (1H, t, J=10 glut. H-3), 5.34 (1N, t, J= 3.5 glut. H-4), 4.33 (1H, m, J=3.5 glut. H-5), 4.46 (1H, dd, J=2, 12.5, glut. H-6). 13S NMR spectrum (d, 100 MHz, acetone-d6, m.u.): 62.9 (gluc. C-6), 71.2 (gluc. C-5), 71.5 (gluc. C-4), 72.2 (gluc. C-2), 72.5 (gluc. C-3), 92.9 (gluc. C-1), 109.8, 110.1, 110.2 (2C, galloyl (gal) C-2,6), 120.9, 121.0, 121.3 (gal C-1), 138.8, 139.0, 139.2 (gall C-4), 145.9, 146.0, 146.1 (2C, gall C-3,5), 165.6, 166.2 166.5 (gal C-7).



## Conclusion

The polyphenol composition of the leaves of the plant Hippophae rhamnoides L. was studied and several polyphenols were isolated from it, the structure of which was established by physicochemical methods of study. Such substances as gallic acid, kaempferol and hydrolyzable tannins: geranine, 1-O-galloyl-4,6-hexahydroxydiphenol-β-D-glucose and 1,4,6-tri-O. -galloyl-β-D-glucose.

## References

1.Chai Q., Xiayan G., Zhao M.,WemminH.,Giang Y.The experimental studies on the cfrdiovascular pharmacology of seabuckthorn extract from Hippophae Rhamnoides L.//Proceedings of the International Symposium on Seabuckthorn.China, 1989.Pp 398.

2.Irwandi J.,Dedi N.,Reno F.H.,Fitri O.Carotenoids; Sources,medicinal properties and their application in food and nutraceutical industry// J.Med Plants Res.2011. №5.Pp.7119-7131.

3.Kumar S., Sagar A.Microbial associates of (Hippophae Rhamnoides L)//Plant Pathol J.2007. №6 Pp.299-305.

4.Mukhamedyarova M.M.,Chumbalov T.K.,Polyphenols of H.Rhamnoides leaves//Chemistry of Natural Compounds.1977.Vol.2.Pp 281-282.

5. Novruzov E.N. Pigmenty reproduktivnykh organov rasteniy i ikh znacheniye.//Pigments of the reproductive organs of plants and their significance. Baku, 2010, Pp 308.

6. Lutsenko S.V., Fel'dman N.B., Bykov V.A. //Plant flavonolignans. Biological activity and therapeutic potential. Moscow, 2006, Page 236.

7. Jamyansan D. Biological active substances of the fruit of NMR seabucthorn and their commercial use. Author' s Abstract, Moscow, 1973, Pp 23 .

8. Kallio H., Yang B.R., Tahvonen R., Hakala M. Proceeding of International Symposium on Sea Buckthorn (IWS), Beijing, China, 1999, Pp 17–23.

9. Novruzov E.N. Seabuckthorn (Hippophae L.) a multipurpose plant. Delhi, 2000, Pp177–196
10. Novruzov E.N., Mamedov Z.G., Mustafayeva L.A., Miryusifova H.M., Zeynalova A.M.// Composition and content of flavoinods in the leaves of hippophae rhamnoides l. growing in Azerbaijan. Chemistry of plant raw materials. 2018. No. 3. Pp. 209–214.