COMPOSITION OF FLAVONOIDS OF PLANTAGO MAJOR L., GROWING IN UZBEKISTAN

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Abstract. *The work studied the composition of flavonoids of the great plantain (Plantago major L.), growing in the Republic of Uzbekistan. The initial raw materials were collected in the Chust district of the Namangan region in mid-September. From this raw material, 104.4 g (5.22%) of polyphenolic compounds were obtained by extraction with organic solvents. Next, by column chromatography, 3 fractions were obtained - diethyl ether (1-fraction), aqueous (2-fraction) and acetone (3-fraction). Substances from the fractions were separated using paper and high-performance liquid chromatography. The presence of 5 flavonoids in P. major L. was shown: rutin, luteolin, isorhamnetin, quercetin-3-O-β-D-galactopyranoside and 3,5,7,3'4'-pentaoxyflavone (quercetin). It is concluded that this type of plant is promising in terms of developing new medicines for the treatment of various pathologies.*

Keywords: *plantaginaceae, plantago major L., phenolic compounds, flavonoids.*

Introduction. There is great interest worldwide in the use of traditional herbal medicines. This is due to the fact that patients consider such drugs to be "natural" and take them without fear, unlike purely "chemical" drugs. In this regard, much attention is paid to both the search for rich plant raw materials and the study of their chemical composition. Of particular interest are compounds isolated from plants of the Plantaginaceae family, which are widely used in medical practice. In Uzbekistan, the genus Plantago has 3 species. Among them, Plantago major L., the great plantain, is of great interest to medicine today. It is found almost throughout the entire territory of the republic [1]. It is Plantago major that is most often used for a number of diseases in folk medicine in different countries [2, 3, 4]. Considering that the therapeutic effect is carried out mainly by phenolic compounds (flavonoids, tannins), the composition of flavonoids of Plantago major, growing in our republic, was of interest.

Purpose of the study. Isolation and identification of phenolic compounds from Plantago major, growing in the Republic of Uzbekistan.

Material and methods. The aerial parts of the P. major L. plant were collected in the Chust district of the Namangan region in mid-September and dried in accordance with GOST requirements. The dried (primary) raw materials were purified from compounds that give color to the plant and are of a lipophilic nature using gasoline, and secondary dry raw materials were obtained. The latter was additionally extracted with chloroform and a tertiary dry raw material was obtained. From this raw material, phenolic compounds were extracted with 40% ethanol; after distilling off the alcohol in a rotary evaporator, the aqueous residue was treated with ethyl acetate. The ethyl acetate fraction was dried, the concentrate was mixed with hexane and precipitated. The isolated precipitate was dried in a vacuum drying oven and 104.4 g (5.22%) of polyphenolic compounds were obtained. We took 20 g of the total polyphenols isolated from the P. major L.

plant, dissolved it in 150 ml of double-distilled water at a temperature of 40 ℃, and the volume was adjusted to 450 ml. Next, 120 g of gole powder was poured into 450 ml of double-distilled water for 45 minutes. shook himself on a shaker. Afterwards, the powder was squeezed out of the mixture and 450 ml of polyphenol extract was added and another 45 minutes. shaken on a shaker, then the powder with adsorbed substances was packed into a column measuring 4.5x100 cm. The column was washed first with diethyl ether (fraction 1), then with water (fraction 2), and then with a 60% aqueous solution of acetone (fraction 3). Next, the substances of the fractions were separated by paper chromatography in solvent systems “n-butanol-acetic acid-water” - 4:1:5 and “n-butanol-acetic acid-water” - 40:12:28. The work also used the high-performance liquid chromatography (HPLC) method. The conditions for the separation are given in the text below the figure.

Results. A study of the composition of polyphenols isolated from Plantago major L. using high-performance liquid chromatography (HPLC) revealed the presence of more than 25 substances (Fig. 1).

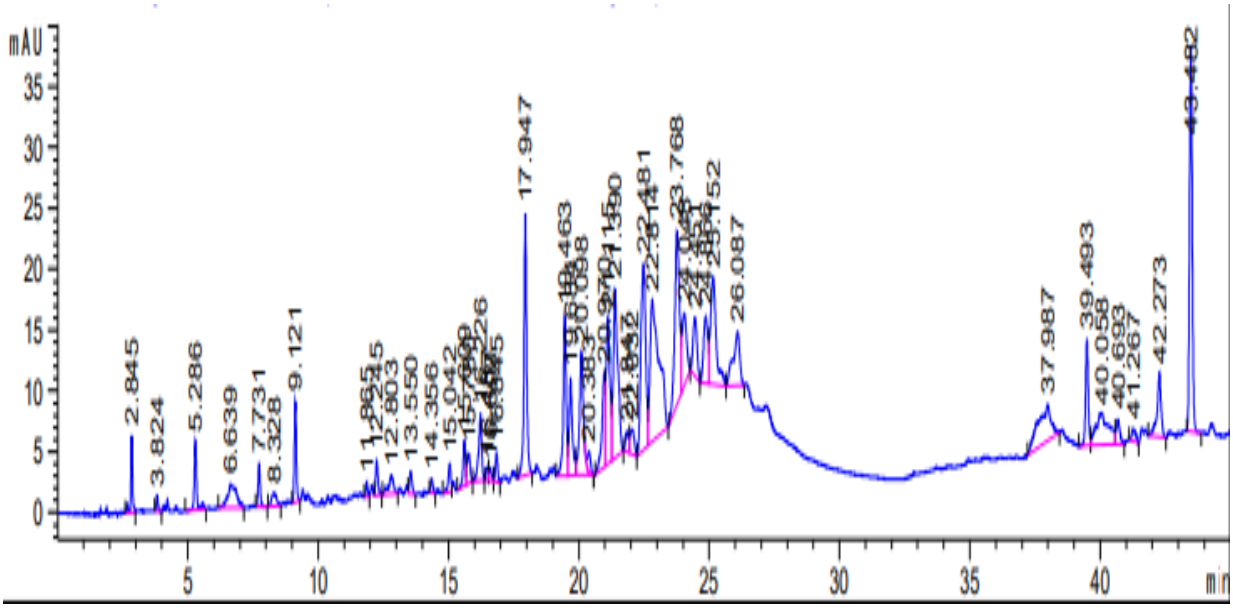


Fig. 1. HPLC analysis of total polyphenols isolated from the plant Plantago major L.

Solvents: C – acetonitrile, D – 0.1% trifluoroacetic acid buffer (pH=3). Concentration gradient of acetonitrile with buffer: 0–30 min – acetonitrile 5% (v/v), 30–33 min – acetonitrile 35% (v/v), 33–38 min – acetonitrile 55% (v/v), 38– 43 min – acetonitrile 70% (v/v), 43–45 min – acetonitrile 80% (v/v), 45–48 min – acetonitrile 5% (v/v). Flow rate – 1 ml/min. Absorption (wavelength) – 254, 269 nm.

It was established that the diethyl ether fraction (1.2 l) (**first fraction**) contains one substance. This fraction was evaporated in vacuum, the dry residue was dissolved in 15 ml of hot water and cooled at room temperature for 2-3 hours. The precipitate was filtered through a Schott funnel No. 4 and dried in a vacuum desiccator in the presence of P_2O_5 to obtain 123 mg of material. Using paper chromatography, it was found that the substance has $R_f = 0.51$, and the results obtained in the infrared spectrum are identical to the results obtained for pure gallic acid (Fig. 2). Therefore, the first fraction contains gallic acid.

Checking the composition of the extracted aqueous fraction (**second fraction**) by paper chromatography showed the presence of 5 compounds with values of Rf = 0.45, Rf = 0.58, Rf =

0.53, Rf = 0.35 and Rf = 0.64. Secondary testing of this fraction by HPLC also confirmed the presence of 5 compounds (Fig. 3).

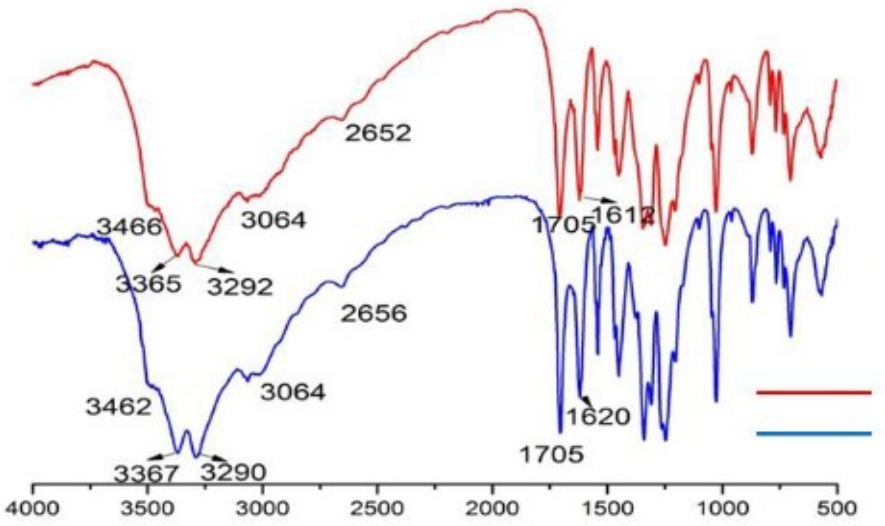


Fig. 2. IR spectrum of gallic acid

The red line is the IR spectrum of gallic acid, available in the device database, blue line - IR spectrum of a substance isolated from the plant Plantago major L.

The separated aqueous fraction with a volume of 5.7 l was evaporated in a rotary evaporator under vacuum at a temperature of 65 °C, 920 ml of aqueous concentrate was isolated, which was treated several times with ethyl acetate. As a result, 2.7 liters of ethyl acetate extract was obtained. The isolated concentrate was reduced to 520 ml by evaporation on a rotary evaporator. The concentrate was precipitated with 2.2 L of hexane, obtaining 2.02 g of a yellow precipitate. The dry residue was dissolved in 30 ml of methanol and mixed with 25 g of polyamide powder for 5 min, then dried at room temperature until the solvent evaporated.

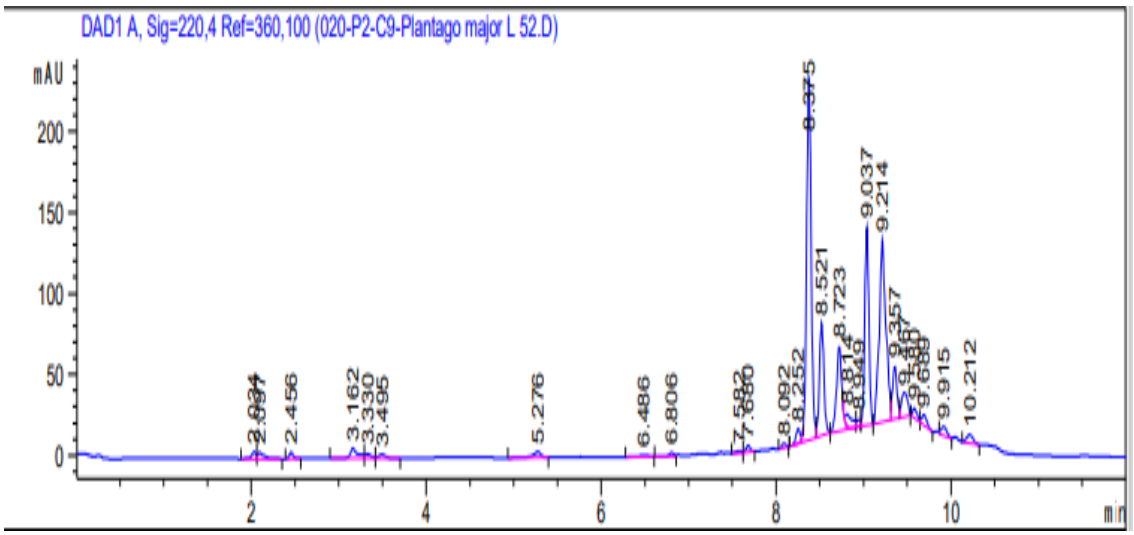


Fig. 3. HPLC chromatogram of the composition of the aqueous fraction of flavonoids from P. major L.

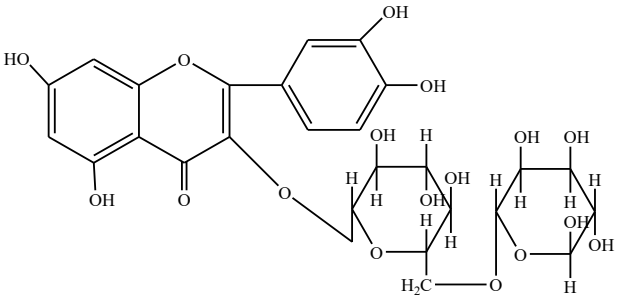
Solvents: C – acetonitrile, D – 0.1% trifluoroacetic acid buffer (pH=3). Buffer concentration gradient with acetonitrile: 0–3 min – acetonitrile 70% (v/v), 3–6 min – acetonitrile

80% (v/v), 6–9 min – acetonitrile 85% (v/v), 9 - 12 min – acetonitrile 90% (v/v), 12–14 min – acetonitrile 70% (v/v). Flow rate – 0.6 ml/min. Absorption (wavelength) – 254, 269 nm.

Then 310 g of polyamide was taken, mixed with 510 ml of petroleum ether and placed in a chromatographic column measuring 4.5x90 cm. The adsorbent was compacted by gentle beating and the adsorbent with the impregnated substance was placed on top of the main adsorbent. The column was washed with a chloroform-methanol solvent system (v/v, 17:1 to 4:3) in increasing order of methanol concentration. Fractions isolated from the column were checked by thin layer paper chromatography and similar fractions were combined.

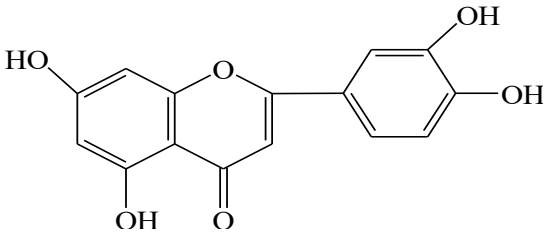
As a result, fractional extracts containing 5 individual substances were obtained. To extract substances from this fractional composition, it was evaporated in vacuum and the substances were extracted. The extracted substances were dissolved in 30, 40, 50, 55 and 80% aqueous solutions of ethyl alcohol and left at room temperature for 4-5 hours. As a result, it was noticed that yellow crystals had formed in the vessel. The resulting crystal Schott funnel was filtered, dried in P_2O_5 or a vacuum desiccator, and pure substances were obtained.

From **fraction 1** (1220 ml) 187 mg of yellow crystalline substance was isolated. As a result of analysis of the results of chemical and spectral studies and comparison with literature data, this substance was identified with quercetin-3-rutinoside (rutin).



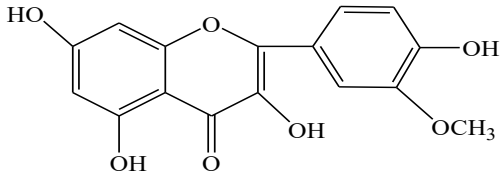
Rutin

Fraction 2 (540 ml) yielded 98 mg of a yellow crystalline substance. Based on the obtained chemical and spectral results, this substance was identified as 5,7,3',4'-tetrahydroxyflavone (luteolin).



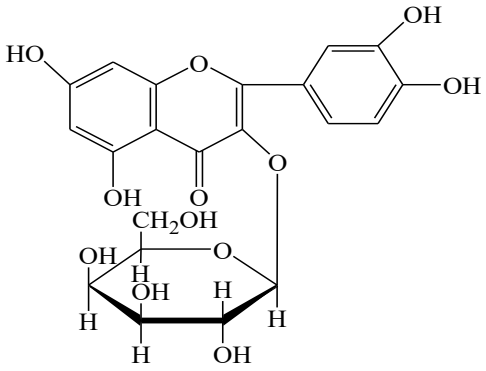
Luteolin

Fraction 3 (610 ml) yielded 87 mg of a yellow crystalline substance. Analysis showed that the substance was isorhamnetin.



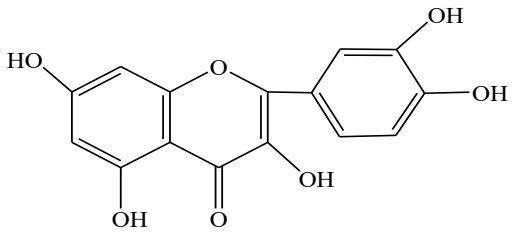
Isorhamnetin

Fraction 4 (780 ml) yielded 124 mg of a yellow crystalline substance. Analysis showed that this substance was quercetin-3-O-β-D-galactopyranoside.



Quercetin-3-O-β-D-galactopyranoside

From **fraction 5** (925 ml) 143 mg of yellow crystalline substance was isolated. Analysis showed this substance to be 3,5,7,3'4'-pentaoxyflavone (quercetin).



3,5,7,3'4'-pentaoxyflavone (quercetin)

Conclusion. Thus, the results obtained indicate the presence in the plant Plantago major L., growing in Uzbekistan, of such flavonoids as rutin, luteolin, isorhamnetin, quercetin-3-O-β-D-galactopyranoside and 3,5,7,3'4 '-penta-hydroxyflavone (quercetin). Therefore, this type of plant has promise in terms of developing new medicines for the treatment of various pathologies.

Conflict of Interest: The author declares that there is no conflict of interest regarding the study.

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