# STUDY OF PHENOLIC COMPOUNDS IN PLANTS INCLUDED IN THE PLANTAGINACEAE FAMILY

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***Abstract.*** *The growing demand for phytopreparations makes it possible to expand the range of medicinal plant materials, chemical and pharmacological studies of substances from poorly studied plant species, the creation of medicines based on them and their use in medical practice. In this regard, the purpose of our research is to study the chemical composition of Plantago plants from the Plantaginaceae family, to isolate flavonoids and hydrolysable tannins, to study their chemical structure and biological activity.*

***Keywords:*** *Plantaginaceae, Plantago major L., Plantago lanceolata L., chloroform extraction, acetone extraction, ethyl acetate fraction, flavonoids, hydrolysable tannins, ellagitannins, gallotannins.*

Today, the need of human for natural drugs based on medicinal plants is growing day by day. Main herbal drugs, unlike synthetic agents, are characterized by the fact that they can be used for a long time without side effects, showing the property of a wide spectrum of action on the human body.

The growing demand for phyto (herbal) medicines makes it possible to expand the range of medicinal plant materials, chemical and pharmacological studies of substances of poorly studied plant species, the creation of medicines based on them and their usage in medical practice.

In various scientific fields, including pharmaceutics and medicine, more attention worldwide is paid to the search for plant sources which have a huge amount of biologically active compounds and the study of their chemical composition. In particular, carbohydrates, flavonoids, iridoid glucosides, phenolcarboxylic acids and other compounds belonging to plants of the Plantaginaceae family have been isolated, as well as effective preparations based on them are widely used in medical practice.

It is important to study: the chemical composition of Plantago plants (plantains) from this family, the isolation of flavonoids and hydrolysable tannins, their chemical structure and biological activity.

Research studies worldwide have aim to reveal the relationship between the chemical structure and biological activity of polyphenols, their antioxidant and antiviral activities, as well as their mechanism of action, but there is not enough data on plant tannins and their biological activity.

There are more than 250 plantain species in the world, 30 species grow in Asia and 6 in Uzbekistan. Plantago major L. and P. lanceolata L., belonging to the Plantago family, grow in all regions of Uzbekistan, in fields, arable lands, meadows, forest edges, along streams [1]. In traditional medicine, the plant Plantago major L. is used for various purposes. The aerial parts of the plant mainly have wound healing, anti-inflammatory, analgesic, antioxidant, antibacterial, immunomodulatory, hypotensive, hepatoprotective effects. [2, 3, 4.]. The aerial parts of the plant have an anticoagulant effect on ulcers due to alcohol and aspirin. Psyllium extracts stimulate nitric

oxide and tumor necrosis factor (TNF-α), which resists infection and tumor growth. The main action of nitric oxide is - to inhibit the synthesis of DNA and ATP. Psyllium leaf extracts have antioxidant properties. Flavonoids isolated from plant components have high antioxidant activity [5, 6]

Baicalein isolated from the plant P. major L. has hepatoprotective properties, protects the liver of rats from pathogenic pests [8], has the ability to destroy carcinoma cells [9], slows down the growth of human hepatoma cells [10], and has a pronounced antiproliferative property. Scutallarein and baicalein are inhibitors of AIDS transcriptase in vitro. ($IC_{50}$ 2,5; 5,6 мМ).

**The purpose of the study.** Plantago major L. and P. lanceolata L. to determine the phenolic compounds of plants and their biological activity.

**Materials and methods.** Plants of P. major L. and P. lanceolata L. were collected during flowering. The dried raw material was extracted with chloroform to remove lipophilic compounds. Then, the raw material was dried at room temperature until no solvent residue remained, and was extracted three times with 70% aqueous acetone solution. Obtained water-acetone extracts were combined, condensed on a rotary evaporator, and the aqueous concentrate was treated several times with ethyl acetate.

The ethyl acetate fraction was concentrated on a rotary evaporator, dried over anhydrous sodium sulfate ($Na_2SO_4$) and the polyphenol fraction was precipitated with hexane.

When analyzing the polyphenol fraction by paper chromatography using the systems: n-butanol-acetic acid-water 4:1:5 (system 1), n-butanol-acetic acid-water 10:3:7 (system 2), n-butanol -acetic acid-water 4:1:2 (system 3), 15% aqueous solution of acetic acid (system 4) found the presence of 11 compounds in the plant, P. major L. and 10 compounds in P. lanceolata L. belonging to the class phenols.

To separate the polyphenol fraction into individual compounds, the fraction was washed in a column filled with a specially prepared target powder with a solvent system: diethyl ether, water, and 60% aqueous acetone solution. As the result we got three factions.

After paper chromatography with diethyl ether fraction of both plants, a new substance whose Rf was 0.51; 0.72 was found (systems 1 and 2, respectively). Distilling off the ether fraction under vacuum, the dry residue was dissolved in a small amount of warm water. As a result, a white crystalline substance precipitated with a melting point of 239°C. This substance was identified by gallic acid.

**Results.** As a result of qualitative reactions (ammonia vapor, sodium carbonate solution), it was found that the aqueous fractions contain compounds belonging to the class of flavonols. According to two-dimensional paper chromatography (systems 2, 4), 5 flavonol compounds were found in plants P. major L. and P. lanceolata L.

It was found that the 60% water-acetone fraction of the P. major L. plant (systems 1, 2) contains 5 compounds, while the P. lanceolata L. plant contains 4 compounds belonging to the class of tannins.

Individual compounds were isolated by rechromatography of aqueous fractions isolated from both plants in chloroform-methanol (9:1; 8:2) systems on a polyamide column.

Comparing the results of physicochemical analysis with the data given in the literature, it was found that these substances are quercetin-3-rutinoside (P. major L. and P. lanceolata L.), 5,7,3', 4'-tetrahydroxy - flavone (P. major. L.), isorhamnetin (P. major L.), quercetin-3-ObD-

galactopyranoside (P. major L., P. lanceolata L.), 3,5,7,3'4' - pentaoxyflavone (P. major L., P. lanceolata L.), 3,5,7,4'-tetraoxyflavone (P. lanceolata L.), ramnetin (P. lanceolata L.).

*Identification of known tannins of P. major L., P. Lanceolata L. plants.* A 60% water-acetone fraction was rechromatographed on a silica gel column in a diethyl ether-ethyl acetate solvent system (in ascending order of ethyl acetate concentration) and separated into individual compounds. The separated fractions were analyzed by TLC and similar fractions were added to each other. As a result, 5 fractions containing individual substances were isolated. Their structure was established by physicochemical methods.

1,2,3,4,6-penta-O-galloyl-β-D-glucose (P. major L.) - brown amorphous powder, Rf 0.68 (system 2), temp. sq. 278-2800C (with decomposition). UV spectrum (MeOH, λmax, nm): 265, 221. Hydrolysis products in the presence of 5% HCl contain glucose [Rf 0.35 (n-butanol-pyridine-water 6:4:3) System 5, Rf 0.21 (methyl ethyl ketone: acetic acid: methanol, 55:5:2 for TLC) 6-system [1-developer (aniline phthalate reagent)] and gallic acid [Rf 0.51 (system-1)]. Quantitative analysis of hydrolysis products (glucose content was checked by the ferrocyanide method, gallic acid content by colorimetric method) showed that glucose and gallic acid are formed in a ratio of 1:5. Comparing the results with data presented in the literature, this substance was identified as 1,2,3,4,6-penta-O-galloyl-β-D-glucose.

1,2,3-tri-O-galloyl-β-D-glucose (P. major L., P. lanceolata L.) - brown amorphous powder, Rf 0.36 (system 2), temp.pl. 267-2690C (decomposed), [a]20D-70.60 (c 0.1; acetone). UB-(MeOH, λmax, nm): 218, 279. Acid hydrolysis in the presence of 5% HCl leads to the formation of glucose and gallic acid in a ratio of 1:3. Based on the obtained results and literature data, this substance was identified as 1,2,3-tri-O-galloyl-β-D-glucose.

1,3,4,6-tetra-O-galloyl-β-D-glucose (P. major L.) - brown amorphous powder, Rf 0.31 (system 2), temp. sq. 273-2750C (with decomposition), [a] 20D-57.30 (s 0.2; EtOH). UV spectrum (EtOH, λmax, nm): 265, 283. IR spectrum (KBr, ν, cm-1): 3345-3350 (OH), 1710-1730 (ester bond), 1510-1620 cm-1 (aromatic ring), 1010-1020 (carbohydrate part). The products of acid hydrolysis with 5% HCl solution were glucose and gallic acid in a ratio of 1:4. Analysis of the results of chemical and spectral studies and comparison with the literature data revealed that the substance is 1,3,4,6-tetra-O-galloyl-β-D-glucose.

3-O-galloyl-4,6-hexahydroxydiphenol-β-D-glucose (P. lanceolata L.), - white amorphous powder, Rf 0.68 (system 2), [a] 20D + 400 (c 0.9 ; acetone), UV spectrum (EtOH, λmax, nm): 220, 285. The decomposition reaction products in the presence of HCl were glucose, gallic and ellagic acids [Rf 0.20; 0.01; Systems 2 and 7 (2% aqueous acetic acid solution)]. Comparing the obtained results with the data presented in the literature, the substance was identified with 3-O-galloyl-4,6-hexahydroxydiphenol-β-D-glucose.

2,3-di-O-galloyl-β-D-glucose (P. lanceolata L.) - dark brown amorphous powder, Rf 0.25 (system 2), [a] 20D-1370 (c 0.5; EtOH). UV spectrum (EtOH, λmax, nm): 220, 280. As a result of acid HCl hydrolysis, glucose and gallic acid were obtained in a ratio of 1:2. Comparing the results with the literature data, the substance was identified with 2,3-di-O-galloyl-β-D-glucose.

**New plant compounds P. major L. and P. lanceolata L.**

*Diether of hexahydroxydiphenoyl-1-(O-2-O-galloyl-β-D-glucopyrano-zido)-1-(O-β-D-xylopyranoside) (1)*- isolated from P. major L., white amorphous powder, [α]20D -460 (c 0.5; EtOH), Rf 0.22 (2-system), UV spectrum (EtOH λmax, nm): 225, 283. IR (KBr, ν, cm-1) spectrum: 3345 -3350 (OH), 1710-1730 (C=O), 1510-1620 (Ar), 1010-1020 (sugar part).

The chemical structure of the diether was established from the data of 1H, 13C NMR spectra. The 1H NMR spectrum shows a signal of the anomeric proton H-1 of xylose in the region of 4.24 ppm. (J=7.7 Hz) in the form of a doublet, hence we can conclude about the β-configuration of this anomeric center. A strong downfield shift of the signal of the anomeric proton indicates the acylation of the OH group of the xylose located in the C-1 atom. Signals characteristic of other xylose protons and appearing at 3.03 (1H, m, J=9.3 Hz, H-2), 3.11 (1H, m, J= 6.2 Hz, H-3), 3 .26 (1H, m, J=9.3 Hz, H-4), 3.63 ppm (1H, m, J=8.5 Hz, H-5) indicate that the OH groups located in the corresponding positions are not galloized.

In the lower field spectrum, a signal characteristic of the H-1 proton of glucose is observed in the form of a doublet at 6.01 ppm. (J=8 Hz). This confirms that the anomeric center has the β-configuration. The downfield shift of the H-2 signal of the glucose proton (δ 4.01 ppm) indicates the galloing of the OH group in the C-2 carbon atom. At the same time, the signals of the remaining glucose protons do not change and appear at 4.60 (1H, t, J=8 Hz, H-3), 4.43 (1H, t, J=8 Hz, H-4), 4.64 (1H, m, J=12 Hz, H-5), 4.20 ppm (2H, d, J=12 Hz, H-6). In addition, in the low field spectrum, signals characteristic of the H-3 and H-6 protons of the haloyl group are observed at 7.08; 7.12 ppm as a single. At 6.62; 6.63 ppm signals of protons H-3, H-3` of the hexahydroxydiphenoyl group appear, in the form of a singlet.

These statements are confirmed by 13C NMR data. Under conditions of complete suppression of spin-spin interaction with protons, typical signals characteristic of carbon atoms of xylose, glucose, gallic and ellagic acids are detected.

Intense signals at 94.9 and 91.3 ppm refer to the C-1 carbon atoms of the sugar moiety of the compound. This indicates that ellagitannin contains two sugar residues, at which the anomeric centers have the β-configuration. At 114.6 and 114.4 ppm signals of C-1, C-1` carbon atoms located in the A and B rings of the hexahydroxydiphenoyl group were observed. The signals of C-7 carbon atoms containing carbonyl groups appear at 166.4-168.7 ppm. Strong signals at 110.0 ppm refer to the C-2 and C-6 carbon atoms of the haloyl group. The signals of carbon atoms C-3 and C-5 match and give relatively strong signals at 145.0 ppm. The C-4 carbon atom of this residue is screened, as a result of the diamagnetic shift it resonates at 139.1 ppm.

Pharmacological and toxicological properties and anti-inflammatory activity of some compounds isolated from plants Plantago major L. and Plantago lanceolata L. are currently being studied in the laboratory of pharmacology of the Institute of Bioorganic Chemistry.

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