

PHYTOCHEMICAL STUDY OF HAIRLESS MALLOW RAW MATERIAL**Mukhamedova M.Sh.**

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Abstract

This study is devoted to the phytochemical investigation of hairless mallow (*Malva* species) raw material. The aim of the research was to comprehensively analyze the chemical composition of the plant and to identify the main groups of biologically active substances both qualitatively and quantitatively.

Chloroform, alcoholic, and aqueous extracts were prepared sequentially from the plant raw material. The presence of biologically active compounds was determined using standard qualitative reactions, while chromatographic methods were applied for identification and confirmation of individual components.

The results of the study confirmed the presence of coumarins, flavonoids, sugars, and tannins in the composition of hairless mallow. Chromatographic analysis revealed two compounds of coumarin nature with R_f values of 0.38 and 0.54. Flavonoids were identified by characteristic color reactions. The total sugar content was determined to be 19.3–20.0% before hydrolysis and 59.4–60.6% after hydrolysis, indicating a high content of polysaccharides. The tannin content was found to be 3.2% and mainly represented by condensed forms.

Anthracene derivatives and alkaloids were not detected, while fats were present in trace amounts. The obtained results are consistent with literature data and confirm the rich phytochemical composition of the plant.

In conclusion, hairless mallow represents a promising medicinal raw material, and its biologically active constituents provide a scientific basis for further standardization and development of pharmaceutical preparations.

Keywords

hairless mallow, phytochemical analysis, flavonoids, coumarins, tannins, sugars, chromatography

Introduction

At present, the in-depth study of biologically active substances obtained from medicinal plants is one of the most relevant scientific directions in pharmacy, medicine, and phytotherapy (Harborne, 1998; Wagner & Bladt, 2009). The effectiveness and safety of medicinal products based on natural sources are directly related to their chemical composition, the content of biologically active components, and quality indicators (European Pharmacopoeia, 2020). From this perspective, a comprehensive study of the phytochemical composition of medicinal plants, identification of active substances, and their standardization are of great importance.

Plants of the genus *Malva* have long been widely used in both traditional and scientific medicine (Trease & Evans, 2009; Bruneton, 1999). They possess anti-inflammatory, emollient, laxative, and protective properties and are mainly used in the treatment of respiratory diseases, gastrointestinal disorders, and skin diseases. These pharmacological effects are primarily explained by the presence of polysaccharides, flavonoids, coumarins, tannins, and other biologically active compounds in the plant (Dewick, 2009).

Hairless mallow is also one of the medicinal raw materials with high biological activity, and the in-depth study of its composition and pharmacological properties is of scientific and practical importance (Dewick, 2009). Literature sources indicate that this plant contains various carbohydrates, phenolic compounds, including flavonoids and coumarins, as well as tannins. However, their quantitative indicators and compositional characteristics may vary depending on environmental factors, harvesting time, and processing methods (Dewick, 2009).

Therefore, studying the chemical composition of hairless mallow raw material using modern analytical methods, identifying the main groups of biologically active substances, and determining their quantities is one of the important scientific tasks. The use of chromatographic, chemical, and physicochemical analysis methods ensures high accuracy and reliability (European Pharmacopoeia, 2020; Wagner & Bladt, 2009; Abdullabekova V.N. & Mukhamedova M.Sh.,2026).

The aim of this study is to comprehensively investigate the chemical composition of hairless mallow and to determine the main biologically active substances qualitatively and quantitatively.

Materials and Methods

To identify the main biologically active compounds in hairless mallow raw material, chloroform, alcoholic, and aqueous extracts were sequentially prepared. These extracts were analyzed using generally accepted qualitative reactions. The composition of the identified components was studied by chromatographic methods based on comparison with standard substances, and their quantitative indicators were determined (Kokate, 2010; Wagner & Bladt, 2009).

Determination of Main Groups of Biologically Active Substances

Determination of Sugars

For this purpose, 1 g each of roots and leaves was placed in a 100 ml volumetric flask, and 50 ml of water was added. Free acids were neutralized with saturated sodium bicarbonate using litmus. The mixture was heated at 80°C for 30 minutes. After cooling, 10 ml of 10% lead acetate was added, the volume was adjusted to the mark, and the mixture was filtered.

Fehling's reaction: 2 ml of filtrate was mixed with Fehling's I and II solutions (1:1) and heated. A red precipitate formed.

Molisch reaction: To 2 ml of filtrate, 1–2 drops of 20% alcoholic β -naphthol solution and 1 ml of concentrated sulfuric acid were added. A red-violet ring appeared between the layers.

Cobalt nitrate reaction: 5% cobalt nitrate and 10% sodium hydroxide were added to the filtrate, resulting in a violet color.

These results confirmed the presence of sugars in the roots and leaves of hairless mallow. Sugar content was determined according to the Fresenius method (State Pharmacopoeia of the Republic of Uzbekistan, 2021).

In determining the amount of sugars before hydrolysis in the roots and leaves of hairless mallow, 10 ml of aqueous extract obtained from 1.0 g of raw material was placed into a 250 ml conical flask, and 10 ml each of Fehling 1 and Fehling 2 solutions were added. The mixture was boiled for 3 minutes and quickly cooled under a stream of cold water, after which 20 ml of 10% potassium iodide solution and 15 ml of sulfuric acid ($d = 1.11$) were added. The released iodine was immediately titrated with 0.1 M sodium thiosulfate solution in the presence of a starch indicator.

In this case, 24.5 ml of 0.1 M sodium thiosulfate solution was consumed for the roots and 24.7 ml for the leaves, and the amount of sugars before hydrolysis was determined using Table 1.

To determine the amount of sugars after hydrolysis, 50 ml of the extract obtained by the above method was placed into a 100 ml volumetric flask, and 5 ml of concentrated hydrochloric acid was added, and the mixture was heated at a temperature of 68–70°C for 7 minutes with constant stirring. After cooling, it was neutralized with saturated sodium bicarbonate solution using litmus paper until a slightly alkaline medium was obtained. Then the mixture in the flask was brought to the mark with water and filtered.

10 ml of filtrate was taken and placed into a 250 ml conical flask, and 10 ml each of Fehling 1 and Fehling 2 solutions were added. The mixture was boiled for 3 minutes and quickly cooled under a stream of cold water, after which 20 ml of 10% potassium iodide solution and 15 ml of sulfuric acid were added. The released iodine was immediately titrated with 0.1 M sodium thiosulfate solution in the presence of a starch indicator.

In this case, 18.5 ml of 0.1 M sodium thiosulfate solution was consumed for the roots and 16.5 ml for the leaves, and the amount of sugars after hydrolysis was determined using Table 1.

Table 1
Calculation table of invert sugars by iodometric method

1/10 number in normal iodine solution 1/10- см ³ соди										
	0	1	2	3	4	5	6	7	8	9
	grams of invert sugar in 1L									
0	0	0,04	0,1	0,1	0,2	0,2	0,3	0,3	0,3	0,4
1	0,4	0,5	0,5	0,5	0,5	0,6	0,6	0,6	0,7	0,7
2	0,7	0,8	0,8	0,8	0,9	0,9	0,9	1,0	1,0	1,0
3	1,1	1,1	1,1	1,2	1,2	1,2	1,2	1,3	1,3	1,3
4	1,4	1,4	1,4	1,5	1,5	1,5	1,6	1,6	1,6	1,7
5	1,7	1,7	1,8	1,8	1,8	1,9	1,9	1,9	2,0	2,0
6	2,0	2,1	2,1	2,1	2,2	2,2	2,2	2,3	2,3	2,3
7	2,4	2,4	2,4	2,4	2,5	2,5	2,5	2,6	2,6	2,6
8	2,7	2,7	2,7	2,8	2,8	2,8	2,9	2,9	2,9	3,0
9	3,0	3,0	3,1	3,1	3,1	3,2	3,2	3,2	3,3	3,3
10	3,3	3,4	3,4	3,4	3,5	3,5	3,5	3,6	3,6	3,6
11	3,7	3,7	3,7	3,8	3,8	3,8	3,9	3,9	3,9	4,0
12	4,0	4,0	4,1	4,1	4,1	4,2	4,2	4,2	4,3	4,3
13	4,3	4,4	4,4	4,4	4,5	4,5	4,5	4,6	4,6	4,6
14	4,7	4,7	4,7	4,7	4,8	4,8	4,8	4,9	4,9	4,9
15	5,0	5,0	5,0	5,1	5,1	5,1	5,2	5,2	5,2	5,2

Thus, it was determined that the content of sugars was 19.3–20.0% before hydrolysis and 59.4–60.6% after hydrolysis.

Determination of coumarins.

1. Lactone test. In order to determine the presence of coumarins in the plant raw material, a chloroform extract (1:10) was prepared from the raw material and 2 ml was placed into two test tubes. To one of the test tubes, 0.5 ml of a 10% sodium hydroxide solution was added, and both test tubes were heated in a water bath until boiling and then cooled. Then, 4 ml of purified water was added to both test tubes. In the test tube to which the alkaline solution was added, a clear yellow color appeared. Then a few drops of diluted hydrochloric acid solution were added to the same test tube, as a result of which the clear yellow solution lost its color and became slightly turbid.

2. Diazo reaction. 2 ml of chloroform extract was placed into a test tube, a 10% alcoholic solution of sodium hydroxide was added, and then freshly prepared Pauly reagent was added. As a result, a reddish color characteristic of coumarin nature appeared.

3. Chromatographic analysis. In order to carry out chromatographic analysis of coumarins in the raw material, the chloroform extract was applied to the start line of a "Silufol" plate using a capillary glass tube. Then the chromatographic plate was placed into a chamber containing a mixture of petroleum ether: ethyl acetate (9:1) for 30 minutes. After 30 minutes, the plate was removed from the chamber and dried in air for 10 minutes. The plate was sprayed with a 10% alcoholic solution of sodium hydroxide and heated in a drying oven at a temperature of 110–120°C for 2–3 minutes. Then freshly prepared Pauly reagent was sprayed. As a result, reddish spots with R_f values of 0.38 and 0.54, characteristic of coumarin nature (Wagner & Bladt, 2009), appeared. The obtained results confirmed the data presented in literature sources and showed that there are two substances of coumarin nature in the roots of the studied plant.

Determination of flavonoids.

In order to determine flavonoids in the plant raw material, a cyanidin reaction was carried out in the alcoholic extract. As a result of the reaction, a light pink color appeared. Flavonoids were identified by characteristic color reactions (Harborne, 1998).

When a 1% aluminum chloride solution was added to the alcoholic extract, a light-yellow color appeared. The results of the reactions showed that flavonoids are present in the raw material.

Preparation of Pauly reagent: a mixture consisting of 3.0 g of sulfanilic acid, 6.0 ml of concentrated hydrochloric acid, 14 g of n-butanol, and 180.0 ml of purified water is prepared.

Determination of tannins.

Tannins were identified qualitatively and quantified using pharmacopoeial titration methods (State Pharmacopoeia of the Russian Federation, 2018; European Pharmacopoeia, 2020). When ferric-ammonium alum was added to the extract, a dark green color appeared, indicating the presence of condensed tannins. This reaction indicates that condensed tannins are present in the raw material.

When 2 ml of 10% acetic acid and 1 ml of a 10% solution of neutral lead acetate were added to 1 ml of extract, a precipitate was formed.

Stiasny reaction. To 50 ml of extract, 10 ml of concentrated (1:1) hydrochloric acid and 15 ml of a 40% formalin solution were added. Then the flask was connected to an air condenser and heated. As a result, the condensed group of tannins present in the extract precipitated in the form of a brown precipitate.

The results of qualitative reactions showed the presence of tannins in the raw material.

The amount of tannins was determined according to the pharmacopoeial method. For this purpose, about 2 g of accurately weighed hairless mallow roots crushed to 3 mm size were placed into a 500 ml flask. 50 ml of hot water was added, and the mixture was heated on an electric plate for 30 minutes, connected to a reflux condenser and periodically stirred. After cooling to room temperature, 100 ml of liquid was filtered through cotton into a 250 ml flask.

From the extract, 25 ml was taken using a pipette, and the polysaccharides in it were precipitated with 50 ml of 96% ethanol. The precipitate was separated under vacuum through a Büchner funnel. The filtrate was poured into a 750 ml flask, and 350 ml of water and 25 ml of indigosulfonic acid solution were added. The mixture was titrated with 0.02 mol/L potassium permanganate solution until a golden-yellow color appeared while constantly stirring.

In the control experiment, 25 ml of indigosulfonic acid solution was mixed with 500 ml of water and titrated.

Since qualitative reactions showed that tannins in the roots belong to the condensed group, 1 ml of 0.02 mol/L potassium permanganate solution corresponds to 0.00582 g of tannins.

Tannins were identified qualitatively and quantified using pharmacopoeial titration methods (State Pharmacopoeia of the Russian Federation, 2018).

$$X = \frac{(V - V_1) \cdot 0.00582 \cdot 250 \cdot 100 \cdot 100}{M \cdot 25 \cdot (100 - W)}$$

where:

V – volume of potassium permanganate solution used for titration of the extract, ml;

V₁ – volume used in the control experiment, ml;

0.00582 – amount of tannins corresponding to 1 ml of potassium permanganate solution,

g;

M – weight of raw material, g;

W – moisture loss during drying, %;

250 – total volume of extract, ml;

25 – volume of extract taken for titration, ml.

As a result, it was determined that the tannin content in the roots is 3.2%.

Determination of anthracene derivatives.

Borntrager reaction. In order to determine anthracene derivatives in the plant raw material, an aqueous extract was prepared from it and treated with diluted hydrochloric acid to bring it into an acidic medium, and then shaken with ether. The ether layer was separated and 2–3 drops of a 10% ammonium hydroxide solution were added to it. As a result of the reaction, no changes were observed.

Determination of alkaloids.

For this purpose, an extract was prepared from the plant raw material in an acidic medium according to the Yurashevsky method. For this, 1 g of plant raw material was weighed, placed into a flask, and 10 ml of 1% acetic acid was added, and heated in a water bath for 5 minutes. Then the extract was filtered, and one drop was placed onto a watch glass, and reactions were carried out with general precipitating reagents used to determine the presence or absence of alkaloids. In this case, no precipitate was formed.

Qualitative reactions carried out for anthracene derivatives and alkaloids did not give results characteristic of these substances.

Determination of fats.

In the chloroform extract obtained from the hairless mallow plant, the acrolein reaction gave a positive result. This indicates that fats are present in the raw material in residual amounts and confirms the data presented in the literature (Dewick, 2009).

Conclusion

As a result of the conducted studies, the chemical composition of the hairless mallow plant was comprehensively investigated, and the main biologically active substances in its composition were identified. Chloroform, alcoholic, and aqueous extracts were obtained from the plant raw material, and analyses were carried out using chemical and chromatographic methods.

According to the results of the study, the presence of coumarins, flavonoids, sugars, and tannins in the plant composition was confirmed. In particular, as a result of chromatographic analysis, two substances belonging to the coumarin nature were identified. Flavonoids were confirmed by qualitative reactions. The sugar content was determined to be 19.3–20.0% before hydrolysis and 59.4–60.6% after hydrolysis, which indicates a high content of polysaccharides in the plant composition. The tannin content was 3.2%, and it was determined that they mainly belong to the condensed type.

At the same time, it was determined that anthracene derivatives and alkaloids are not present, while fats are found in small amounts. The obtained results correspond to the data presented in the literature and confirm that the hairless mallow plant has a rich phytochemical composition.

In conclusion, this plant is promising as a medicinal raw material, and the biologically active substances in its composition expand the possibilities of its use in the development of pharmaceutical preparations and in phytotherapy. The obtained results serve as a scientific basis for the standardization of this plant and the creation of new medicinal products.

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