STUDY OF THE MONOSACCHARIDE COMPOSITION OF WATER-SOLUBLE POLYSACCHARIDE COMPLEXES AND PECTIC SUBSTANCES OF PIMPINELLA ANISUM HERBS

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1. Introduction

Biopolymers, as a mandatory component of any living cell, attract the attention of researchers in various fields of science. Biopolymers include various classes of compounds, including protein (proteins), carbohydrates (polysaccharides), phenolic (melanins, tannins, humic and fulvic acids) and mixed nature (lipopolysaccharides, glycoproteins, etc.) [1].

The bulk of organic matter on the planet are polysaccharides, which account for most of the dry weight of terrestrial plants, providing the skeletal functions of the body and cells - cellulose, hemicellulose and pectin. Another important function of polysaccharides is to ensure the energy supply of plant organisms by switching to monosaccharides. Biopolymers, as a mandatory component of any living cell, attract the attention of researchers in various fields of science. Biopolymers include various classes of compounds, including protein (proteins), carbohydrates (polysaccharides), phenolic (melanins, tannins, humic and fulvic acids) and mixed nature (lipopolysaccharides, glycoproteins, etc.) [1].

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It is known that higher plant polysaccharides exhibit various pharmacological effects, such as antitumor, immunomodulatory, antioxidant, hepatoprotective, antiulcer and other activities [9–11].
Pectins have a number of advantages as additives to food and pharmaceuticals. Pectins have the necessary stability in acidic conditions, even at higher temperatures, which makes them a suitable candidate for use in the drug delivery system. Due to the high gelling ability of pectins in the presence of divalent cations, they are carriers for the delivery of biologically active substances [12].

Dietary fiber, especially pectin, is one of the most important components of the human diet, as the lack of dietary fiber in everyday food can adversely affect human health, increasing the risk of serious diseases [13–15]. It was found that pectin has a beneficial effect on the gastrointestinal tract [16], and also has antioxidant [17], antihypertensive [18], cytoprotective [19], immunomodulatory [20], hypocholesterolemic, hypoglycemic, prebiotic and other activities [21, 22].

It should be noted that on the basis of polysaccharides were successfully obtained drugs such as «Algipor», «Algimaf» (for the treatment of wounds and burns), «Algimadrin» (hemostatic agent for topical use), «Algisorb» (complexing agent (antidote)) , «Adaptovit» (general tonic), «Plantaglucid» (used in hypoacid gastritis), «Laminarid» (mild laxative), «Mucaltin» (expectorant). In turn, pectin is part of drugs such as Medetopect (antidote for heavy metal poisoning), Pepidol (antibacterial, sorption – detoxification, antidiarrheal and antiemetic effect), Zosterin-ultra (enterosorbent, hemosorbent and immunomodulator).

Anise (Pimpinella anisum L.) is a medicinal plant of the Umbelliferae family. The exact origin of anise is unknown, but it is common in Egypt, Syria, Cyprus, Greece, Crete, and Turkey [23]. It has antibacterial [24], antiviral [25], anticonvulsant [26], antitoxic [27], antioxidant [24], antiulcer [28] and muscle relaxant effects [29].

Previously, we studied the pharmacological activity of WSPC and pectin substances isolated from the Pimpinella anisum herbs and found that pectin substances are virtually non-toxic, show a pronounced laxative effect, which is not inferior to the comparison drug "Sedanex" [30].

The aim of the work was to study the monosaccharide composition of water-soluble polysaccharide complexes and pectin substances isolated from the Pimpinella anisum herbs.

2. Research planning (methodology)

An analysis of the scientific literature showed that the chemical composition of the Pimpinella anisum herbs has not been sufficiently studied, and no information on its carbohydrate composition has been found in available sources.

The study of the carbohydrate composition of plants is a promising direction, since this class of compounds makes up about 80 % of the dry weight of plants, which has become our object of study.

Based on this, we set a goal to study the monosaccharide composition of water-soluble polysaccharide complexes (WSPC) and pectin substances (PS) by liquid chromatography, since this method is one of the most optimal for the analysis of these objects.

To achieve this goal, the first stage was to isolate WSPC from the meal of Pimpinella anisum herbs after separation of lipophilic fractions with chloroform. The second stage was to obtain the PS from the meal remaining after separation of the WSPC. The third stage involved the establishment of qualitative and quantitative monosaccharide composition of WSPC and PS by liquid chromatography.

The goal can be easily achieved, since during the experiment there are no difficulties and the study is almost safe. The experimental design is presented below Fig. 1.

![Fig. 1. The algorithm for obtaining WSPC and PS](image)

3. Materials and methods

Anise herbs, harvested in the summer of 2019 in Kharkiv, were used for analysis. To obtain water-soluble polysaccharide complexes (WSPC) used air-dry meal of anise herbs after extraction of chloroform lipophilic fractions.

10.0 g of meal was extracted with 200 ml of hot water by heating to 95 °C for 1 h with constant stirring. Re-extraction was performed in a ratio of raw material-extractant 1:10. The obtained extracts were combined and evaporated to 1/5 of the initial volume. Polysaccharides were precipitated with three times the amount of
96 % ethanol. The precipitate was filtered off, washed successively with 96 % ethanol, acetone, ether, dried to constant weight. Received a fraction of WSPC.

Pectins were isolated from the meal remaining after receiving WSPC. Extraction of raw materials was performed twice with a mixture of 0.5 % solutions of oxalic acid and ammonium oxalate (1:1) in a ratio of raw material-extractant 1:20 at a temperature of 80–85 °C for 2 hours. The obtained extracts were combined, concentrated and precipitated five times the amount of 96 % ethanol. The precipitate formed was filtered off, washed with ethanol and dried to constant weight.

WSPC isolated from Pimpinella anisum herbs is an amorphous powder of light gray color, when dissolved in water forms an opalescent solution (pH of a 1 % aqueous solution is in the range of 5–6), it also dissolves in aqueous solutions of acids and alkalis and does not dissolve in organic solvents. The polysaccharide complex gives a positive precipitation reaction with alcohol, acetone, a reaction with the Feling reagent after acid digestion of polysaccharides.

The pectin substances of the Pimpinella anisum are an odorless light-cream crystalline powder, with a sour taste, with a moisture content of not more than 10 %, without extraneous impurities visible to the naked eye. It is soluble in water with the formation of viscous solutions (pH of a 1 % aqueous solution is in the range of 3–4). Pectin substances precipitate from an aqueous solution of 1 % aluminum sulfate solution to form pectates [31].

For a preliminary study of the qualitative composition of the isolated polysaccharide complexes, thin layer chromatography (TLC) was used. Acid hydrolysis to determine the monosaccharide compositions of WSPC and PS was carried out with sulfuric acid (0.5 mol/dm³). Quantitative determination of polysaccharide complexes, thin layer chromatography (TLC) was used. Acid hydrolysis to determine the monosaccharide compositions of WSPC and PS was carried out with sulfuric acid (0.5 mol/dm³). Chromatographic study of monosaccharides was carried out by the method of ascending chromatography in the system n-butanol – acetic acid – purified water (4:1:2) in parallel with reliable samples. Chromatograms after drying in air were treated with an aniline phthalate reagent and heated in an oven at a temperature of 100–105 °C.

### Quantitative determination

The tests are performed by liquid chromatography (SPhU *; 2.2.29, 2.2.46) [32].

**Test solution:** 400 mg of plant extract (an exact amount sample) is washed with 20.0 ml of ethyl alcohol 96 % on a paper filter "blue tape", then the washed extract is placed in a volumetric flask with a capacity of 50.0 ml, dissolved in 10 ml of hydrochloric acid (2:5) and refluxed for one hour. The flask was cooled to room temperature and then 10.0 ml of sodium hydroxide solution (7.9 %) was added. The solution is quantitatively transferred to a 25 ml flask with 15 ml of water, the volume of the solution is adjusted to the mark with water, mixed and filtered through a membrane fluoroplastic filter with a pore size of 0.45 μm, discarding the first 0.5 ml of filtrate.

**Reference solution:** The exactly indicated amount sample of SPhU RSs of saccharide is placed in a volumetric flask with a capacity of 100.0 ml, dissolved in 60 ml of water, stirred until the sample is dissolved, the solution volume is adjusted to the mark with water and mixed.

**The solution to test the suitability of the chromatographic system:** 5 ml of the reference solution is placed in a 50 ml volumetric flask and the volume of the solution is adjusted to the mark with water.

Chromatograph 10 μl of the solution to test the suitability of the chromatographic system on an Agilent 1290 liquid chromatograph, obtaining from 2 to 6 chromatograms of SPhU RSD, respectively.

Chromatograph 10 μl of the reference solution, and the test solution obtaining the number of chromatograms not less than for the solution to test the suitability of the chromatographic system.

**Chromatography conditions:**

- a column measuring 300×7.8 mm Aminex HPX-87C, or a similar validated column for which the requirements of the test "Testing the suitability of the chromatographic system";
- detection – refractometric (1 sec);
- the speed of the mobile phase – 1.0 ml/min;
- column thermostat temperature – 30 °C;
- mobile phase A: Water;
- mobile phase B: acetonitrile for chromatography R.

The content of saccharide C (mg/1 g) is calculated by the formula:

$$x = \frac{S \times m_0 \times 25}{S_0 \times 100 \times 1000},$$

where $S_i$ is the average value of the areas of the saccharide peaks calculated from the chromatograms of the test solution; $S_0$ is the average value of the areas of the saccharide peaks calculated from the chromatograms of the reference solution; $m_0$ is mass of the sample SPhU RSs saccharide, mg.

#### 3.1. Testing the suitability of the chromatographic system

The chromatographic system is considered suitable if the following conditions are met:
- the efficiency of the chromatographic column calculated from the chromatograms of the reference solution must be not less;
- the coefficient of symmetry of the saccharide peaks from the chromatograms of the reference solution should be from 0.8 to 1.5.

### 4. Results

The yield of WSPC and PS from the Pimpinella anisum herbs is given below (Table 1).

**Table 1**

| Compound | Yield, g |
|----------|---------|
| WSPC     | 0.4370±0.02 |
| PS       | 1.5254±0.03 |

Chromatograms of WSPC and PS of anise herbs are presented in Fig. 2, 3, respectively. The results of the research are shown in the Table 2.
WSPC isolated from the Pimpinella anisum herbs contain two monosaccharides – glucose and rhamnose. Rhamnose with a content of 215.5 mg/g is the dominant sugar, glucose is present in a much smaller amount – 17.5 mg/g. The glucose content in the PS is approximately the same – 12.3 mg/g. In the absence of rhamnose, the presence of galactose and arabinose in the amount of 59.8 mg/g and 69.5 mg/g, respectively, was established in pectins. Taking into account the results of studies of the pharmacological properties of WSPC and PS, isolated from the Pimpinella anisum herbs, we can assume that the presence of these substances causes a high laxative effect of PS.

### Table 2

| Compound                     | Content, mg/g | Retention time |
|------------------------------|---------------|----------------|
| Water-soluble polysaccharide complex |               |                |
| glucose                      | 17.5±0.04     | 32.427         |
| rhamnose                     | 215.5±0.02    | 38.087         |
| Pectic substances            |               |                |
| glucose                      | 12.3±0.02     | 32.427         |
| galactose                    | 59.8±0.04     | 36.675         |
| arabinose                    | 69.5±0.03     | 42.449         |

Fig. 2. Chromatogram of WSPC from Pimpinella anisum herbs

Fig. 3. Chromatogram of PS from Pimpinella anisum herbs
5. Discussion
Using the method of thin-layer chromatography, the presence of glucose and arabinose was revealed in WSPC and PS of Pimpinella anisum herbs. The glucose spot was brown, and the arabinose spot was pink.

The results obtained are reliable, because the selected liquid chromatography method is accurate and the study was performed using standard samples of SPiU RSs.

On the other hand, the results indicate specific compounds. In fact, the WSPC and PS obtained by us are complex compounds, due to the presence of hydroxyl and carboxyl groups in their structures, they can combine with phenolic compounds, amino acids, organic acids, and further pharmacological studies in terms of these compounds will be inappropriate, since concomitant substances will greatly affect the action of the isolated substances.

The monosaccharide composition of WSPC and PS differs depending on the type of raw material. The proof of this is the study of WSPC in the seeds of Pimpinella anisum by gas chromatography-mass spectrometry by researchers from Tunisia in 2019 [33]. The result of the study is shown below (Table 3).

As can be seen from the table, galactose (334.7 mg/g) and mannose (182.1 mg/g) predominate in the composition of the WSPC of Pimpinella anisum seeds, and the content of rhamnose (215.5 mg/g) is higher in the composition of the WSPC from herbs of Pimpinella anisum. No studies have been conducted on the study of PS of Pimpinella anisum seeds.

6. Conclusions
The presence of two monosaccharides in WSPC and three monosaccharides in pectin isolated from the Pimpinella anisum herbs was established by liquid chromatography. The results of this study expand the information about the biologically active substances of the Pimpinella anisum herbs and can be taken into account in the development of new herbal remedies based on them.

Conflict of interests
The authors declare that they have no conflicts of interest.

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Table 3
| Monosaccharides | Content, mg / g |
|-----------------|----------------|
| galactose       | 334.7          |
| mannose         | 182.1          |
| fructose        | 29.8           |
| glucose         | 14.3           |
| arabinose       | 6.7            |

Limitations of the research. The study requires additional study of WSPC and PS of the Pimpinella anisum herbs in different phases of vegetation.

Prospects for further research. A significant amount of arabinose and galactose in PS, rhamnose in WSPC, indicates the need for biological and pharmacological tests for other types of activity.
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