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

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При дослідженні фармакологічної активності ВРПК та пектинових речовини, виділених з трави анісу звичайного було встановлено, що пектинові речовини є практично нетоксичними і виявляють виражену послаблюючу дію за якою не поступаються препарату порівняння «Сенадекс».

Мета роботи. Вивчення моносахаридного складу водорозчинних полісахаридних комплексів і пектинових речовин, виділених з трави анісу звичайного.

Матеріали та методи. Для аналізу використовували траву анісу звичайного, заготовлену влітку 2019 року в м. Харків. Дослідження проводили методом рідинної хроматографії на рідинному хроматографі Agilent 1290, детектування – рефрактометричне.

Результати та обговорення. ВРПК, виділені з трави анісу звичайного містять два моносахариди – глюкозу і рамнозу. Рамноза з вмістом 215.5 мг/г є домінуючим цукром, глюкоза присутня в значно меншій кількості – 17.5 мг/г. Вміст глюкози в ПР приблизно такий самий – 12.3 мг/г. При цьому в пектинах за відсутності рамнози встановлена наявність галактози та арабінози в кількості 59.8 мг/г і 69.5 мг /г, відповідно.

Висновки. Методом рідинної хроматографії встановлена наявність двох моносахаридів в ВРПК і трьох моносахаридів в пектині, виділеному з трави анісу звичайного

Ключові слова: біополімери, ВРПК, ПР, аніс звичайний, послаблюючий ефект, препарат «Сенадекс», трава, моносахариди, кількісне визначення, тонкошарова хроматографія, рідинна хроматографія

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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. Bioavailable polysaccharides include carbohydrates of the class of water-soluble polysaccharides (WSP) and pectic substances (PS), as they play the most important role in plant development and are responsible for the specific activity of drugs derived from medicinal raw materials [2].

Polysaccharides are a group of carbohydrates formed from monosaccharide units connected by a glycosidic bond (α or β -configuration). These compounds are mainly obtained from various natural sources, such as bacterial, fungal, marine organisms and plants [3].

Polysaccharides perform three important types of biological functions in living organisms, acting as an

energy reserve, structural components of cells and tissues or protective substances.

Pectins are structural polysaccharides of the cell wall of plants, consisting mainly of units of galacturonic acid with differences in composition, structure and molecular weight. This polysaccharide is often associated with other components of the cell wall, such as cellulose, hemicellulose and lignin [4].

Pectin can be found in almost all plants, but most commercial pectins are derived from citrus fruits such as orange, lemon, grapefruit and apples [5, 6].

Together with other components of plant cell walls, pectins provide their strength and elasticity, protect plants from drying out, provide drought resistance and frost resistance, play a protective role against phytopathogens, help eliminate damage and release the plant from stress. Their macromolecules are crucial in seed germination and plant growth, in ripening and storage of vegetables and fruits, and the structure can change significantly during plant growth and development. They are characterized by an irregular type of structure and are considered as one of the most complex and dynamic in structure class of biopolymers [7].

Polysaccharides are widely used in the pharmaceutical industry as a leavening agent, filler and in drug delivery systems specific to a particular area [8].

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), «Alginatol» (hemostatic agent for topical use), «Algisorb» (complexing agent (antidote)), «Adaptovit» (general tonic), «Plantagluclid» (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 "Senadex" [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.

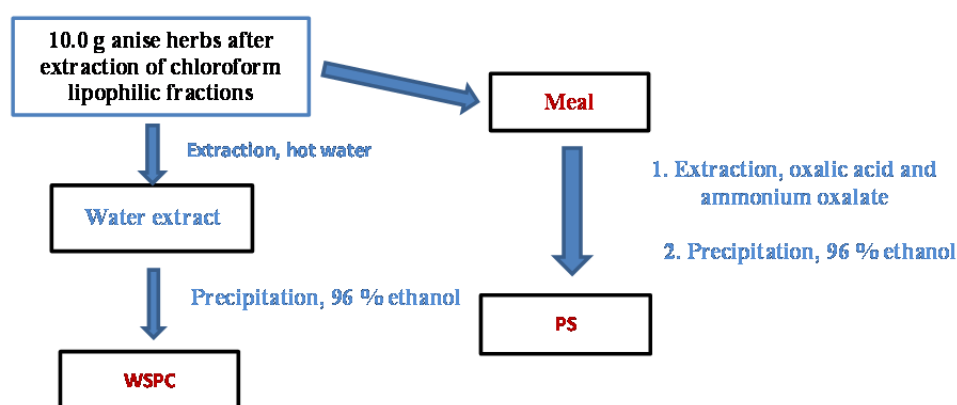


Fig. 1. The algorithm for obtaining WSPC and PS

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-creamy 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). 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:

$$X = \frac{S_i m_0 25}{S_0 100} 1000, \quad (1)$$

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
The yield of WSPC and PS from *Pimpinella anisum* herbs

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.

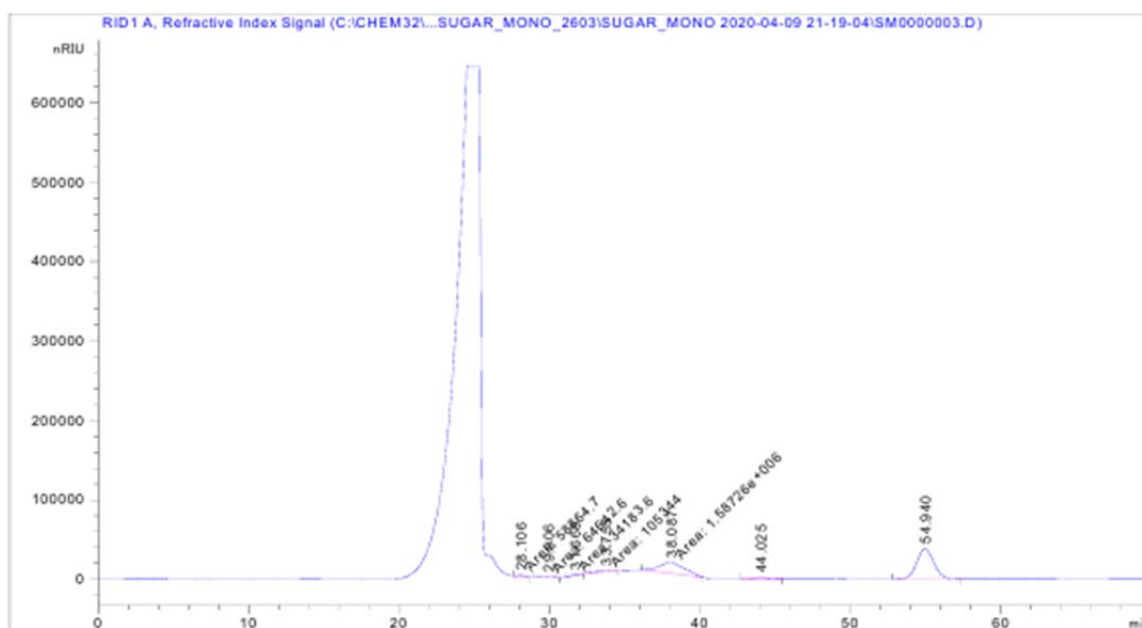


Fig. 2. Chromatogram of WSPC from Pimpinella anisum herbs

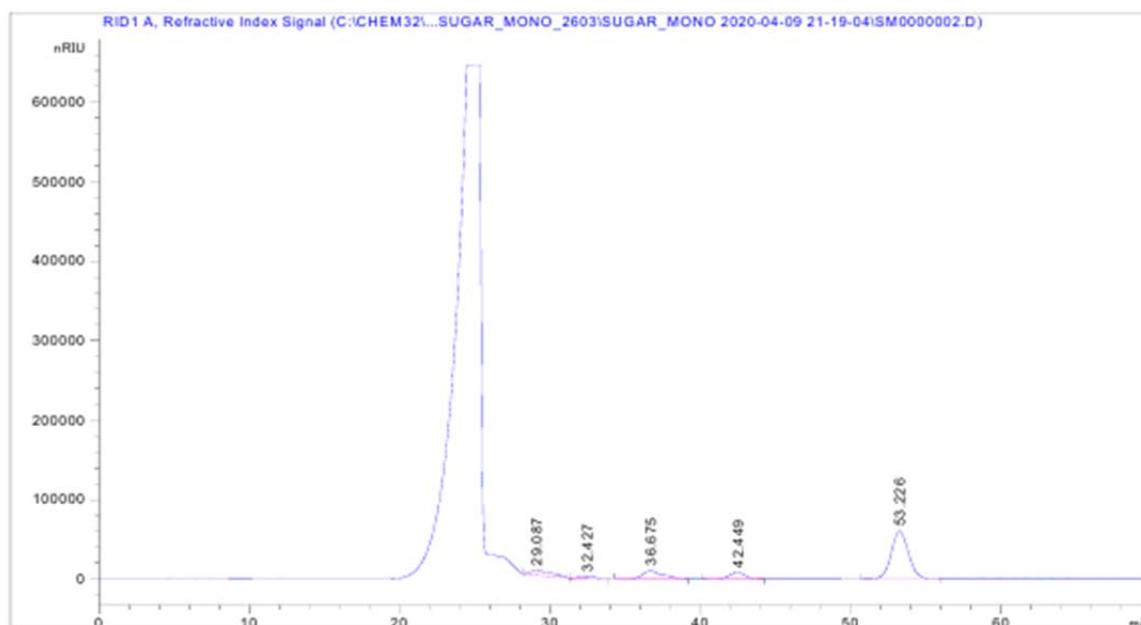


Fig. 3. Chromatogram of PS from Pimpinella anisum herbs

Table 2

The content of monosaccharides in WSPC and pectic substances

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

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.

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 SPhU 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.

Table 3

The content of monosaccharides in WSPC from seeds of *Pimpinella anisum*

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 pharmacological tests for other types of activity.

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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