

Chemical Composition and Content of Polysaccharides from the Yellow Iris (*Iris pseudacorus* L.) Rhizomes

Tikhomirova EA^{1,*}, Sorokina AA¹, Bubenchikova VN², Kostikova EN¹, Zhilkina VYu³, Bessonov VV⁴

Tikhomirova EA^{1,*}, Sorokina AA¹,
Bubenchikova VN², Kostikova
EN¹, Zhilkina VYu³, Bessonov VV⁴

¹Sechenov First Moscow State Medical
University, 8, Trubetskaya St., Moscow,
119991, RUSSIA.

²Kursk State Medical University, 3, Karl Marx
str., Kursk, 305041, RUSSIA.

³Peoples' Friendship University of Russia
(RUDN University), 6, Miklukho-Maklaya
Street, Moscow, 117198, RUSSIA.

⁴Federal Research Center of Nutrition,
Biotechnology and Food Safety, 2/14,
Ustyinsky pr., Moscow, 109240, RUSSIA.

Correspondence

Tikhomirova EA

Sechenov First Moscow State Medical
University, 8, Trubetskaya St., Moscow,
119991, RUSSIA.

E-mail: tekatal@rambler.ru

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ABSTRACT

Background: The aim of the present study was to examine the composition and content of the polysaccharide complex and polysaccharide fractions from yellow iris (*Iris pseudacorus* L.) rhizomes growing in Russian Federation (Moscow region). **Materials and Methods:** Traditional pharmacognostic methods were used in the analysis of polysaccharides as biologically active substances. The total amount of polysaccharides was determined by a gravimetric procedure with alcohol precipitation. Fractions of polysaccharides were isolated by sequential changing of solvents. The content of monosaccharides was determined by the method of high-performance liquid chromatography with refractometric detection (HPLC-RID) after acid hydrolysis. HPLC-RID procedure was performed using Chromatograph Agilent 1260 Infinity, Sugar-Pak column (300×6.5 mm), and isocratic elution mode. **Results:** Polysaccharide complex was isolated, the total content of polysaccharides in the rhizomes of *I. pseudacorus* was determined, as well as their distribution by fractions (water-soluble polysaccharides, pectic substances, hemicellulose A, hemicellulose B), the composition of individual sugars (glucose, xylose, galactose, mannose, rhamnose, arabinose) was determined for each fraction. **Conclusion:** In our experiment polysaccharide complex was first identified and described in detail for *I. pseudacorus* grown in Moscow region.

Key words: Yellow iris, *Iris pseudacorus* L., Polysaccharides, Hemicellulose, Water-soluble Polysaccharides, Pectins.

INTRODUCTION

Yellow iris (*Iris pseudacorus* L.) is a perennial herbaceous rhizomatous plant distributed widely throughout the world (Figures 1 and 2). The plant grows in the swamps, damp rivers, and lakes, in shallow waters, often forms small thickets or groups, sometimes found on flood meadows in the European part of Russia. The original habitat of *I. pseudacorus* is Europe. The main distribution of *I. pseudacorus* includes almost the entire middle and southern part of Europe, the European part

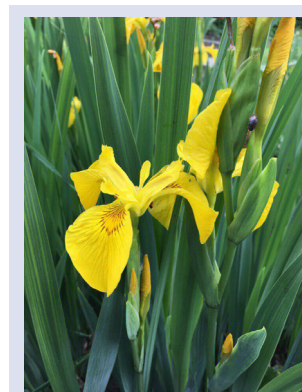


Figure 2. *Iris pseudacorus*, flowering plant.



Figure 1. *Iris pseudacorus* L. in the Botanical Garden of Sechenov University.

of the Russian Federation, Siberia, and the Far East. Due to its vitality, *I. pseudacorus* was able to enter the flora of the Caucasus, Iran, Turkey, Syria, China, Japan, North and South America.^{1,2} *I. pseudacorus* is used in traditional medicine as an antimicrobial, expectorant, diuretic, hemostatic agent. In Russian official medicine, it is included in the antitumor herbal collection named Zdrenko M.N.³

Different parts of *I. pseudacorus* accumulate various groups of biologically active substances: phenolic compounds⁴, flavonoids (isoflavonoids)⁵, triterpenoids (iridals)⁶, organic acids⁷, essential oil⁸, hydroxybenzoic acids⁹ and others. Two isoflavones irisolidone and irigenin together with an isoflavone glycoside iridin were isolated from the rhizomes of

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I. pseudacorus; rapid and specific RP- HPLC procedure for irigenin analysis was developed.^{10,11}

A distinctive feature of perennial herbaceous plants is the ability to store polysaccharides in underground organs. According to results of identification reactions (precipitation in the decoction of Iris rhizomes after addition of 96% ethanol) rhizomes of the *I. pseudacorus* contain a sufficient amount of PCHs. According to chemical classification, several PSC fractions are distinguished: water-soluble polysaccharides (WSPSCs), pectic substances (PSs), hemicelluloses (HC A and HC B). Pectic substances are plant heteropolysaccharides with a molecule formed by the main chain of galacturonic acid residues linked by glycosidic bonds with rhamnopyranose residues. PSs are part of the plant cell wall, along with cellulose, hemicelluloses, and lignin, and are involved in maintaining the ionic and water balance of plant tissues. In pharmacy, it is used as a sorbent due to the formation of complexes with heavy metals.^{12,13} Hemicelluloses (HCs) in plant objects are also among the components of the cell wall. Hemicellulose molecules are complex, branched, but have a lower molecular weight than cellulose.^{14,15}

Monosaccharides (MSs), like their polymers, perform important functions in plant organisms: polysaccharides mostly perform the storage function, carbohydrates serve as the main source of energy for all the vital processes of a plant. It was established experimentally that the germination of perennial herbaceous plants depends on the content and percentage ratio of MSs / WSPSCs. PSCs also perform cryoprotective and signaling functions in the plant cell: the WSPSCs maintain the native protein conformation in winter low temperatures. Phloem carbohydrates are capable of triggering the expression or repression of organelle and nucleus genes. Monosaccharides are involved in the expression of genes responsible for the entire plant life cycle.¹⁶

PSCs can have a variety of pharmacological effects in the human or animal body. For pharmacopoeial plant raw material containing a significant amount of PSCs, the following therapeutic effects are established: expectorant (marsh-mallow roots, large plantain leaves), antiseptic and anti-inflammatory (three-part beggartick herb), laxative (flax seeds, laminaria thallus), diuretic (greater burdock roots), diaphoretic (lime tree flowers).¹⁷ The results of experimental analysis of raw materials new types confirm the growing potential of plant polysaccharide complexes determination. It has been established that PSCs influence on the circulatory and immune systems (when injected in animals, PSCs accelerate hematopoiesis in the bone marrow and spleen); PSCs have a beneficial effect on metabolic processes (fern-leaf dropwort PSCs stimulate the regeneration of hepatocytes when exposed to damaging factors); antitumor (delayed growth of sarcoma cells in experimental rats under the action of laminaria) and anti-ulcer effect ("Plantaglucidum" drug produced from plantain leaves is successfully used in gastrointestinal diseases), as well as many others.¹⁸

This research aims to determine the total content of PSCs in the *I. pseudacorus* rhizomes, analyze the main fractions and establish their monosaccharide composition.

MATERIALS AND METHODS

Plant material

The object of the study was rhizomes of *I. pseudacorus* from the Moscow region of the Russian Federation collected in 2018. Raw materials were dried in natural shady conditions to air-dried state.

Total amount of polysaccharides extraction

First of all, the amount of PSC was determined by the method of the State Pharmacopoeia of the Russian Federation XIV edition (Pharmacopoeial monograph PM.2.5.0032.15 "Plantain leaves").¹⁹ A

precise sample of the *I. pseudacorus* crushed rhizomes was filled with hot purified water; the resulting mixture was heated on a plate with reflux refrigerator for 30 minutes. After the time the liquid was drained and the extraction was repeated twice. The resulting aqueous extracts were combined and filtered through several layers of gauze, and then through a paper filter into a volumetric flask. The solution was adjusted to the mark; an aliquot portion was taken from the resulting solution and a threefold excess of ethanol of 96% was added, the mixture was stirred and heated on a water bath for 30 minutes. The contents of the flask were filtered through a filter paper, pre-dried and weighed. The filter cake was successively washed with 96% ethanol and a mixture (1: 1) of ethyl acetate and ethanol 96%. The filter with the precipitate was dried first in air and then in a drying chamber at a temperature of 100-105 °C to constant weight and weighed. The content of the sum of PSCs in absolutely dry raw materials was determined by the formula:

$$X = \frac{(m_2 - m_1) \cdot 500 \cdot 100 \cdot 100}{a \cdot 25 \cdot (100 - W)} = \frac{(m_2 - m_1) \cdot 2000000}{a \cdot (100 - W)}$$

where m_1 – weight of the filter, g; m_2 – weight of the filter with sediment, g; a – weight of raw material, g; W – humidity of the raw material, %.

PSCs fractions isolation

Subsequently, polysaccharide complexes were isolated from the rhizomes of *I. pseudacorus* in accordance with the N.K. Kochetkov successively in fractions: water-soluble polysaccharide complexes (WSPSCs), pectin substances, hemicellulose A and hemicellulose B.^{20,21}

Water-soluble polysaccharide complex isolation

Isolation of WSPC from the dried raw material of the *I. pseudacorus* was carried out after preliminary removal of phenolic compounds. Iris raw materials (precise sample) were placed in a 2000 ml flask and poured with 70% ethanol in the ratio of 1:20. The resulting mixture was heated in a water bath under reflux refrigerator for 1 hour. The hot solution was filtered through several layers of gauze, the raw material was pressed. After that, the raw materials were twice subjected to re-extraction under the same conditions. The resulting solutions were poured together and filtered through paper filter after cooling. These solutions were not involved in the further isolation of PSCs, but can be used to analyze compounds extracted with ethanol (including phenolic compounds). The remaining schroth was extracted with hot purified water at a ratio of 1:20 when heated in a water bath for 2 hours with occasional stirring for isolating WSPSCs. The extraction of the WSPSCs was repeated two more times, reducing the amount of the extractant and the extraction time by 2 times. The resulting extracts were combined and filtered through gauze, followed by squeezing the raw material, and then through filter paper. Purified extracts were concentrated in a water bath using a vacuum compressor (-1 atm) to 150 ml. Three times the volume of 96% ethanol was added to the combined extracts and left for 12 hours. The water-soluble PSCs formed bulk dark, loose sediments in the solution, which were filtered under vacuum, then washed on the filter with 96% ethanol until the filtrate was decolorized to prevent resinification by the accompanying substances and dried.

Pectin substances isolation

Pectic substances were isolated from the schroth of *I. pseudacorus* rhizomes after separation of WSPSCs. For extraction of the PSs it was used a mixture of 0.5% solution of oxalic acid and 0.5% solution of ammonium oxalate in equal amounts (20-fold excess). The raw material was heated with the reaction mixture in a water bath for 2 hours at a temperature of 80-85 °C. The extraction was repeated twice in the ratio of raw material and extractant 1:10 for 1 hour. After filtration the resulting extracts were poured together and concentrated in vacuo. To evaporated filtrate it was poured 5-fold volume of 96% ethanol. Under

such conditions, the PSs formed a light fluffy precipitate and were separated by filtration under vacuum. On the filter, they were washed with 96% ethanol; the final precipitates were dried and ground.

Hemicellulose A and B isolation

Hemicelluloses isolation was carried out after separation of PSs. A 5-fold volume of a 10% aqueous solution of sodium hydroxide was added to the remaining schroth and left for 12 hours at room temperature. After the filtration a double volume of glacial acetic acid was added to the obtained alkaline extraction. After some time precipitated hemicellulose A was formed and was separated by filtration under vacuum, washing with 96% alcohol. To the filtrate was added 2-fold volume of 96% ethanol, precipitated hemicellulose B was formed and was separated by filtration under vacuum in the same condition, dried and weighed.

Determination of the composition and content of monosaccharides in carbohydrate fractions

MSs analysis was performed by direct-phase HPLC with refractometric detection as in study.²² The content of MSs was determined in terms of the corresponding standard sample (Sigma-Aldrich, USA): D-(+)-xylose (CAS 58-86-6, ≥99%), D-(+)-mannose (CAS 3458-28-4, 99%), D-(-)-arabinose (CAS 10323-20-3, ≥98%), D-(+)-glucose (CAS 50-99-7, ≥99.5%), D- (+)-galactose (CAS 59-23-4, ≥99%), L-rhamnose (CAS 10030-85-0, ≥99%). For each MS 3 standard solutions were prepared with a concentration of 0.1; 0.25 and 0.5 mg/ml. MSs solutions were prepared by dissolving a 50.0 ± 0.1 mg sample at 25 °C in a 100 ml volumetric flask, followed by dilution to the required concentration. Chromatograph Agilent 1260 Infinity, software – ChemStation (ver. A.09.03). Refractometric detector 1260 RID (G1362A). Autosampler 1260 ALS (G1329B). Column thermostat 1260 TCC (G1316A). Chromatographic column for HPLC Sugar-Pak (WATERS, USA), length 300 mm and internal diameter 6.5 mm, filled with microcrystalline cation-exchange gel in calcium form. For free sugars, the elution mode is isocratic (mobile phase – purified water with the addition of Ca-EDTA 0.05 mg/ml). The flow rate is 0.5 ml/min, the temperature of columns is 80°C. The volume of the injected sample is 10 µl. The identification of MS entering into the polysaccharide complex was carried out after complete acid hydrolysis with 2 mol/l trifluoroacetic acid when heated for 5 hours at 100 °C in a sealed ampoule.²³ An excess of trifluoroacetic acid was removed by repeated evaporation to dryness with methyl alcohol, the residue was dissolved in purified water. The resulting solution was placed in centrifuge tubes and centrifuged for 15 minutes at a rate of 4500 rpm. The supernatant (purified solution) was placed in vials for chromatography.

RESULTS

The total content of PSCs was determined for Iris raw materials of varying degrees of grinding to determine the best conditions for quantitative determination and extraction into the dosage form. In the course of the experiment, it was found that the best values for the isolation of PSCs were obtained for raw materials finesse 0.4 mm and 1 mm. The differences in quantitative content for these two fractions are insignificant, however, it is technically more convenient to perform the analysis with the raw material passing through a sieve with a hole diameter of 1 mm, since the particles of rhizomes with size 0.4 mm are too small (Table 1).

During the analysis of polysaccharide fractions from *I. pseudacorus* rhizomes, the yield of water-soluble polysaccharide complexes was 3.02 ± 0.09%. Extracted WSPSC complex was a dark dense film on the filter, after drying became brittle, when grinding turned into a dark brown coarse powder. Pectic substances were isolated from the acidic solution by the precipitation of alcohol, their content was 2.91 ± 0.06%; they were a thin light brown film, easily crushed into powder for further analysis. The content of hemicelluloses was determined after separation of PSs. After successive addition of sodium hydroxide solution and glacial acetic acid solution, a HC A precipitate formed. Adding a double excess of ethanol to supernatant liquid precipitated HC B. Content of hemicelluloses A and B was 1.98 ± 0.07% and 0.87 ± 0.03% respectively. Light beige flakes of HC A after grinding formed a light beige powder. HC B after extraction did not require additional grinding and was transferred from the filter immediately in the form of brown powder. The results of the analysis of PSCs fractions are presented in Table 2.

As a result of HPLC analyses with standard samples, the monosaccharide composition of each fraction of *I. pseudacorus* PSCs was established. In process the standard samples of main plant monosaccharides were used: glucose, xylose, galactose, mannose, rhamnose, arabinose. A preliminary HPLC analysis of the standard samples mixture in the established standard concentration was carried out to determine the retention parameters (Figure 3, Table 3).

Then it was conducted the study of hydrolysates of PSC individual fractions. It was found that both five- and six-carbon monosaccharides are present in the WSPSCs composition: galactose and rhamnose predominate, and mannose and arabinose present in smaller quantities (Figure 4).

According to the literature, PSs consist mainly of mono-residues of galactose and galacturonic acid, the product of its oxidation. HPLC analysis of this fraction confirmed the predominance of galactose and the presence of other monosaccharides (Figure 5).

Table 1: Quantitative determination of PSCs in the *I. pseudacorus* rhizomes of varying degrees of grinding.

No	Size of raw material particles, mm	Total content of PSCs, %*
1	0.4	11.69 ± 0.08
2	1	11.56 ± 0.12
3	2	9.17 ± 0.25
4	3	9.07 ± 0.18

*moisture content of raw materials was 6.07%

Table 2: Distribution of polysaccharide complexes in *I. pseudacorus* rhizomes by fractions.

No	Fraction	Content, %
1	WSPSCs	3.02 ± 0.09
2	PSs	2.91 ± 0.06
3	HC A	1.98 ± 0.07
4	HC B	0.87 ± 0.03

Table 3: The retention time standard samples of monosaccharides.

No	Monosaccharides	Chemical formulas	Peak retention time, min
1.	Glucose	$C_6H_{12}O_6$	9.45
2.	Xylose	$C_5H_{10}O_5$	10.38
3.	Galactose	$C_6H_{12}O_6$	10.58
4.	Mannose	$C_6H_{12}O_6$	10.75
5.	Rhamnose	$C_6H_{12}O_5$	11.58
6.	Arabinose	$C_5H_{10}O_5$	11.89

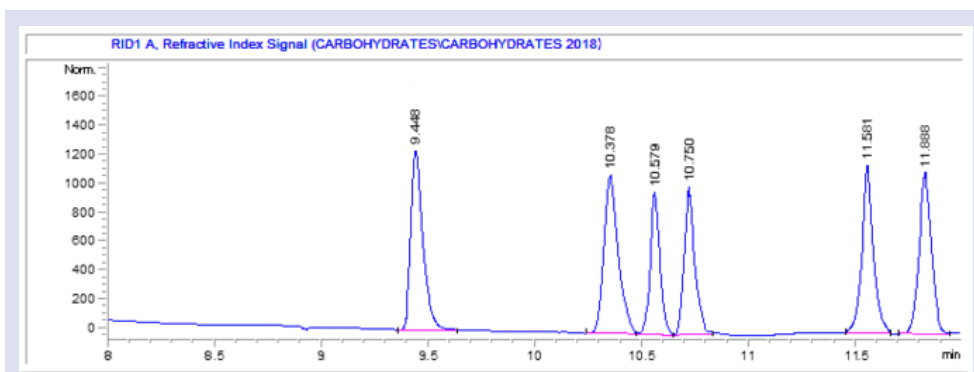


Figure 3: HPLC-RID chromatogram of a standard samples mixture of MS with a concentration of 0.25 mg/ml. 1 – glucose; 2 – xylose; 3 – galactose; 4 – mannose; 5 – rhamnose; 6 – arabinose.

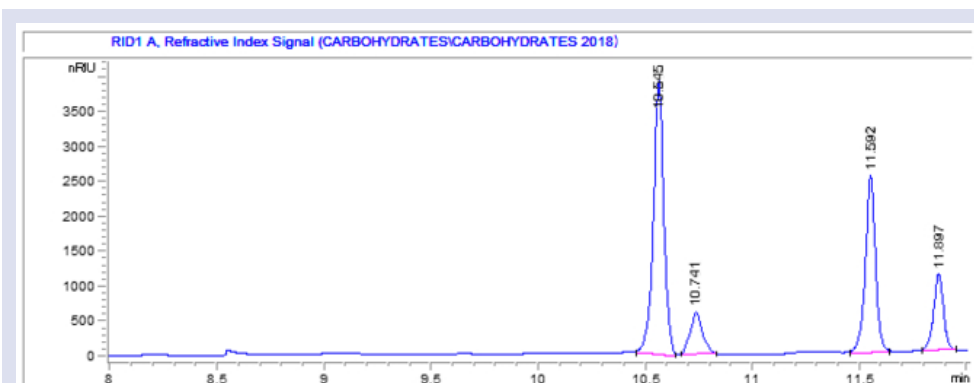


Figure 4: HPLC-RID chromatogram of the WSPSC hydrolyzate. 1 – galactose; 2 – mannose; 3 – rhamnose; 4 – arabinose.

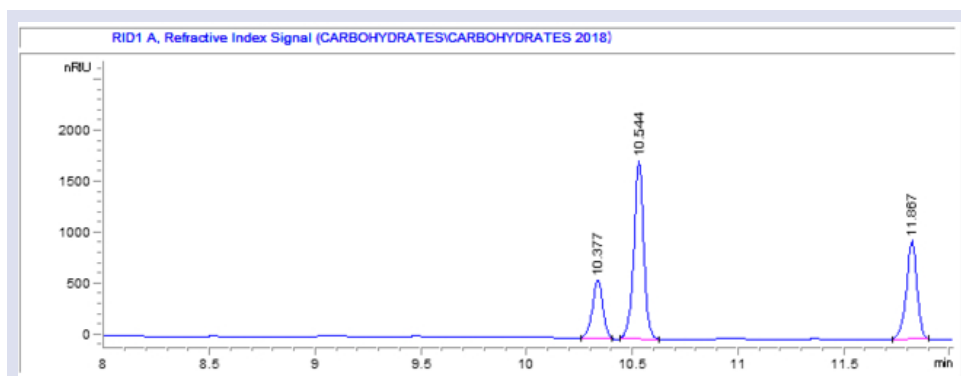
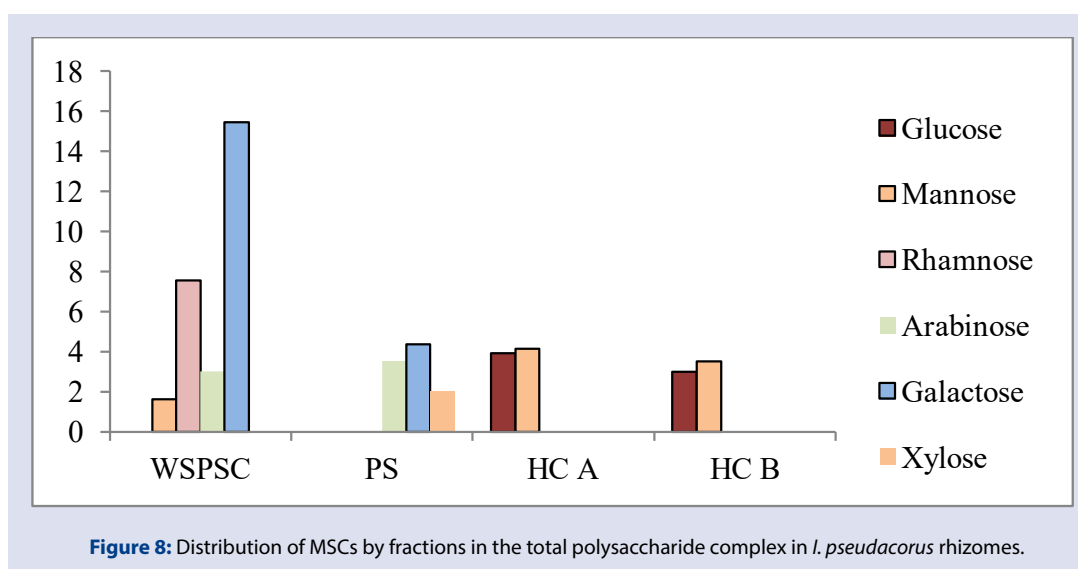
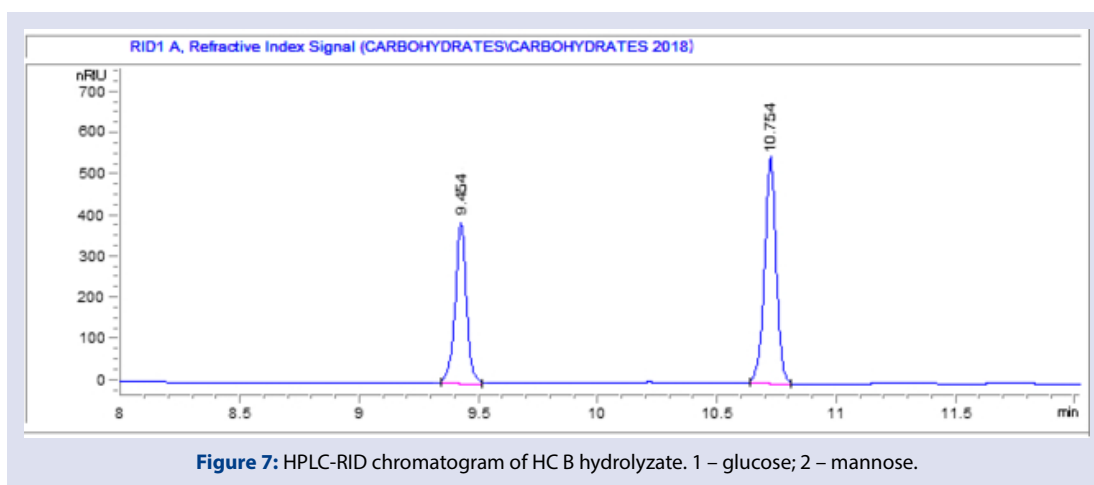
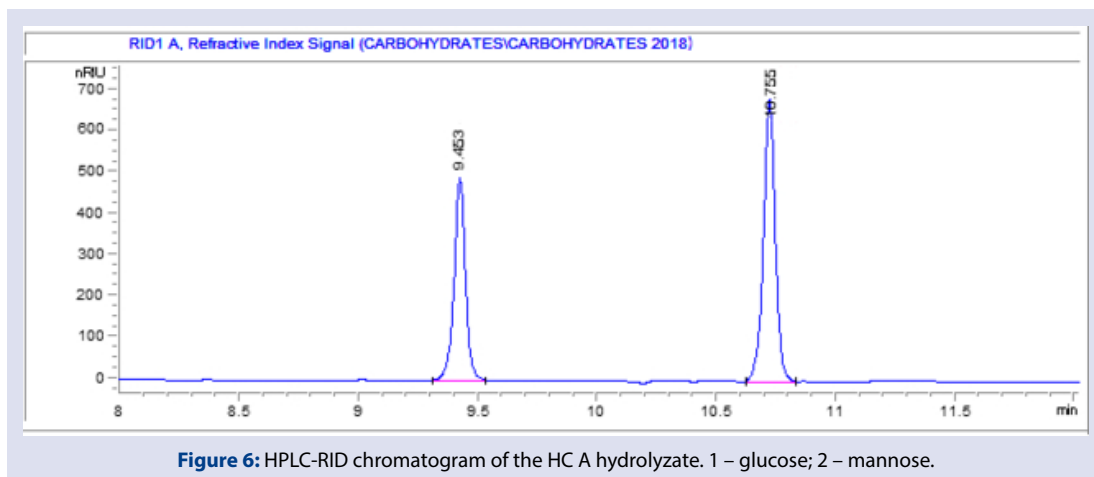


Figure 5: HPLC-RID chromatogram of the PS hydrolyzate. 1 – xylose; 2 – galactose; 3 – arabinose.

It is known that HCs consist mostly of five- and six-carbon monosaccharides. During the HPLC analysis of the isolated HC A (Figure 6) and HC B (Figure 7) fractions, the presence of glucose and mannose in their composition was established in similar quantities, and mannose slightly predominates in both fractions.

The results of the analysis of PSC fractions obtained from *I. pseudacorus* rhizomes are presented in Table 4 and in the diagram (Figure 8). In the composition of WSPSCs and PSs, galactose is contained in larger quantities, while HCs give mannose and glucose residues during hydrolysis.



DISCUSSION

Polysaccharides in plant objects can be located in any parts of the plant: leaves, seeds, fruits, underground organs. Rhizomes are modified shoots and perform storage and vegetative function, therefore the accumulation of polysaccharide complexes in them should be significant. So, it was expected good yield of polysaccharides from *I. pseudacorus* raw material. The quantitative precipitation reaction was confirmed by the presence of PSCs previously.

The total amount of polysaccharides was 9.07-11.69% depending on the degree of grinding of the raw material. The results obtained for Iris rhizomes are comparable with the content of polysaccharides in other PSCs-containing objects of the State Pharmacopoeia of the Russian Federation XIV edition. In greater burdock roots the amount of PSC is standardized at least 8%, in large plantain raw materials at least 12%, in flax seeds and three-part beggartick herb at least 7% and 3.5% respectively. On the basis of this, the raw material of the *I. pseudacorus* can be considered as a source of polysaccharides along with the pharmacopoeial objects.

Polysaccharide fractions were sequentially extracted, their quantitative content was determined. Water polysaccharides make a greater contribution – $3.02 \pm 0.09\%$. Similar amount is determined in pectic substances – $2.91 \pm 0.06\%$. Hemicelluloses A and B had lower quantities – $1.98 \pm 0.07\%$ and $0.87 \pm 0.03\%$ respectively. After separation of the fractions, they were turned into fine powders that are easily amenable to further study after sample preparation by acid hydrolysis.

Analysis of the fractions by HPLC with the standards of monosaccharides showed that they contain well-known monosaccharides. It was determined that galactose is the dominant monosaccharide in water-soluble fraction. So it can be considered the marker for the entire polysaccharide complex of *I. pseudacorus*. In other fractions the difference was not so significant: arabinose, galactose and xylose were found at similar concentrations in PSs; monosaccharides in hemicelluloses are mainly represented by mannose and glucose. Thus, the data obtained in the course of this study are the scientific basis for the development of regulatory documentation in the Russian Federation.²⁴

CONCLUSION

For the first time, the polysaccharide complex from raw material of *I. pseudacorus* growing in the Moscow region was investigated, polysaccharide fractions were identified, its quantitative and qualitative monosaccharide composition were established.

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CONFLICTS OF INTEREST

None.

ABBREVIATIONS

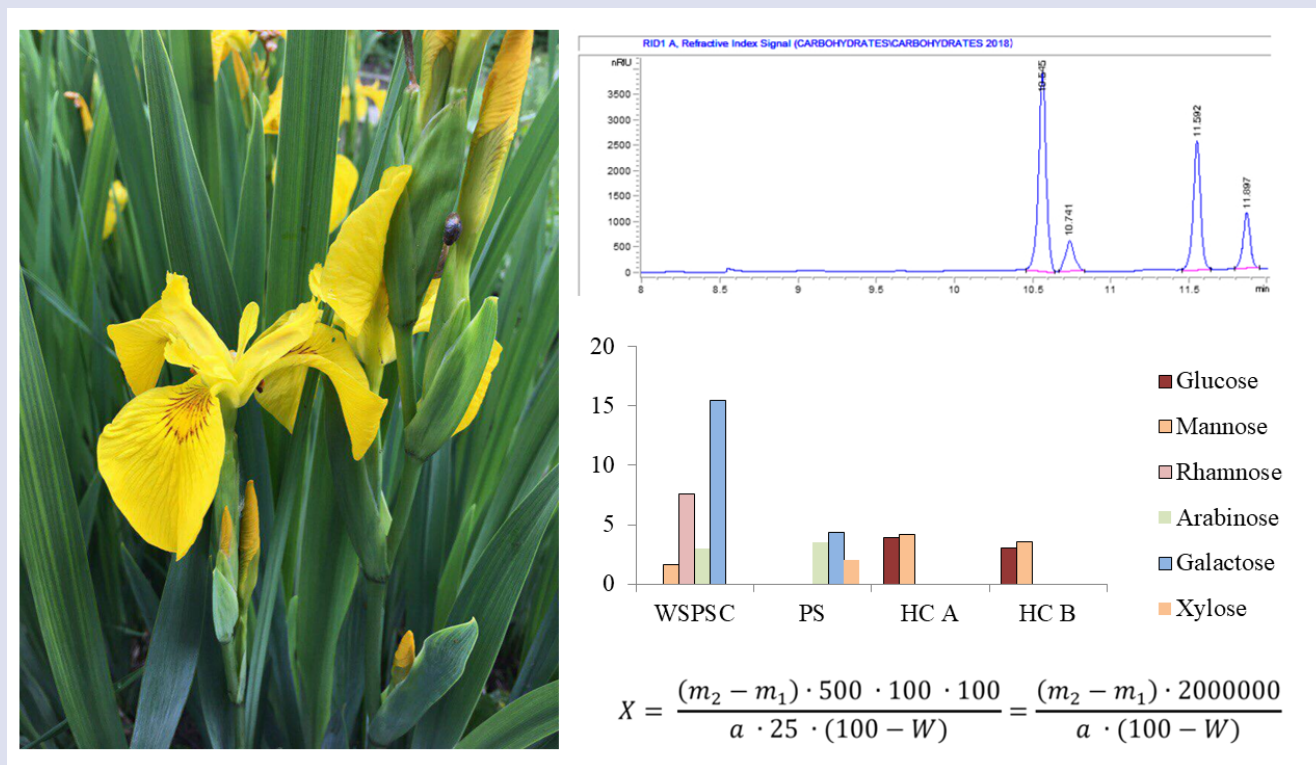
HPLC: high performance liquid chromatography; **PSCs:** polysaccharides; **PSs:** pectic substances; **HC A and HC B:** hemicellulose

A, hemicellulose B; **HCs:** hemicelluloses; **MSs:** monosaccharides; **WSPSCs:** water-soluble polysaccharide complexes; **HPLC-RID:** high performance liquid chromatography with refractometric detection.

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GRAPHICAL ABSTRACT



ABOUT AUTHORS

- **Tikhomirova Ekaterina Alekseevna:** PhD student, Pharmaceutical Natural Sciences Department, A.P. Nelyubin Institute of Pharmacy, Sechenov University.
- **Sorokina Alla Anatolyevna:** Doctor of Pharmaceutical Sciences (Dr.Sc.), Professor, Professor of Pharmaceutical Natural Sciences Department, A.P. Nelyubin Institute of Pharmacy, Sechenov University.
- **Bubenchikova Valentina Nikolaevna:** Doctor of Pharmaceutical Sciences (Dr.Sc.), Professor, Head of Department of Pharmacognosy and Botany, Kursk State Medical University.
- **Kostikova Elena Nikolaevna:** Candidate of Pharmaceutical Sciences (Ph.D.), Assistant of Pharmaceutical Natural Sciences Department, A.P. Nelyubin Institute of Pharmacy, Sechenov University.
- **Zhilkina Vera Yurievna:** Candidate of Pharmaceutical Sciences (Ph.D.), Associate Professor of Institute of Biochemical Technology and Nanotechnology, Peoples' friendship university of Russia (RUDN University).
- **Bessonov Vladimir Vladimirovich:** Doctor of Biological Sciences (Dr.Sc.), Head of Laboratory of Food Chemistry, Federal Research Center of Nutrition, Biotechnology and Food Safety.

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