OLIGO- AND POLYSACCHARIDES FROM THE AERIAL PART OF PLANTS EREMURUS FUSCUS

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ABSTRACT:

Background: For the first time, the carbohydrate composition of the above-ground part of E.fuscus plants growing in Kyrgyzstan was studied; fructosans, water-soluble polysaccharides, pectin substances and hemicellulose were isolated.

Aim: To establish the type of link between hexoses, glucomannan was methylated according to the Hakomori method. The completeness of methylation was checked by double repetition. 2,3,6-tri-O-Me-D-mannose (67.5%), 2,3,4,6-tetra-O-Me-D-mannose (13.5%) and 2,3-di-O-Me-D-glucose 4.0%.

Materials and Methods: The crushed dry aerial parts of plants were treated with ethyl alcohol (96%) at a ratio of 1:8, and heated in a water bath for 1 hour. The extract was filtered, the raw material was extracted twice with 82% ethanol. All alcoholic extracts were combined and evaporated to half the volume, treated with activated carbon at 60-65ºC for 10 minfiltered several times. The clear solution was evaporated at 40-45ºC to a thick syrup. The syrup was treated several times at room temperature in a ratio of 1:8 with concentrated isopropyl alcohol, then acetone, and ether. Oligosaccharides were stored in a vacuum desiccator, which is a slightly yellowish powder, easily spreading in the air.

Results: As a result of extraction with ethanol, oligosaccharides, fructosans, were isolated from the aerial part of E. fuscus, and the quantitative ratio of fructose and glucose was determined. A water-soluble polysaccharide isolated from the remains of raw materials, consisting of mannose and glucose, which is glucomannan, is a slightly yellowish powder soluble in water. According to gel filtration, the molecular weight is 33500, the ratio of glucose and mannose is 1:3.5.

Conclusion: In the IR spectrum of glucomannan there are absorption bands 815cm^{-1}-hexapyranose ring, 840cm^{-1}-equatorial bending vibration of α-sugars, 1040cm^{-1}- are characteristic of bending vibrations of axial C-H bonds, 1240cm^{-1}-ester bonds, 1635cm^{-1} - adsorption water.

Keywords: Oligosaccharides, Fructosans, Polysaccharides, Aerial part, Pectin substances.

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INTRODUCTION

Plant polysaccharides, including glucomannans, fructosans, and pectins, are important biopolymers that are part of almost all plants and perform many different vital functions and are characterized by a wide range of biological effects [1–4]. Polysaccharides are promising raw materials for the pharmaceutical, medical, food, microbiological, chemical and printing industries. They can become an additional source for obtaining new drugs with antibiotic, antiviral, and antitumor activity [5–7].

Recently, glucomannan has attracted more attention due to its good biocompatibility, biodegradability and hydrophilic ability. The results show that glucomannan is well tolerated, has a beneficial effect on total cholesterol, triglycerides, body weight, and therefore is proposed as an effective over-the-counter weight loss supplement [8-11].

The synthetic way of obtaining glucomannans, fructosan and pectins requires the use of expensive chemical reagents. In this regard, the task of finding among the wild medicinal plants growing on the territory of Kyrgyzstan, those that would be easily accessible, cheap and renewable annually and would contain a large amount of carbohydrates, is very urgent.

Plants of the genus Eremurus are the richest in glucomannan and fructose-containing plants [12-15].

The study of the biological, physiological and chemical properties of plants of the genus Eremurus was devoted to the research of several scientists. So, for the first time, the study of the chemical properties of plants of the genus Eremurus was started under the guidance of B.N. Stepanenko, and then continued by Z.F. Ismailov. The study of biology, physiology and chemistry of plants of the genus Eremurus was carried out by A.P. Khokhryakov and D.A. Rakhimov. The properties, structure and dynamics of eremuran accumulation were studied by V.D. Sherbukhin and R.K. Rakhmanberdiyeva and they also outlined the ways of practical use of these plants.

Eremurus is a genus of perennial plants of the Asphodelaceae subfamily. Eremurus or Shiryash, characteristic of the flora of Central Asia. The name of the Eremurus is interpreted in different ways, for example the Kyrgyz call them “chyrych” or “kulun zhal”, “shiryach” or “siresh” is in Uzbek, “shires” in Kazakh and “shyrysh” in Tajik.

In folk medicine, the roots of these plants were used for pain in the larynx, intestines, kidneys and bladder. When used externally, hernias, broken bones and malignant ulcers were cured. In modern medicine, shiryash roots are used as a diuretic, laxative and anti-inflammatory agent.

Many types of shiryash are used as food and dye plants. All parts of plants of this genus contain dyes, and therefore, since ancient times, they have been used to dye natural fibers of fabrics and leather in yellow and orange.

From the roots of the eremurus, a “shiryash glue” is obtained, which is used in the national economy. A patch is made from dried and powdered plant tubers.

21 out of more than 60 species of eremurus known in the world grow on the territory of Kyrgyzstan. Eremurus species growing in Kyrgyzstan are distributed throughout the republic, as well as in mountainous areas at an altitude of 700-2800 m above sea level.

Complexes of previous experimental works were devoted to the study of the carbohydrate composition of E. cristatus [18-20].

The species E. fuscus growing in Kyrgyzstan has not yet been studied in terms of chemical composition and properties. E. fuscus plants are one of the interesting and promising raw material sources, as they are the richest in terms of the content of water-soluble polysaccharides-glucomannan, fructosan and pectin substances.

The purpose of this work is to study the carbohydrate composition of Eremurus fuscus plants and the isolation of glucomannans from plant materials.

MATERIALS AND METHODS

Study design: Extraction method

The crushed dry aerial part of the plants was treated with ethyl alcohol (96%) at a ratio of 1:8, and heated in a water bath for 1 hour. The extract was filtered, and the raw material was extracted twice with 82% ethanol. All alcoholic extracts were combined and evaporated to half the volume, treated with activated carbon at 60-65ºC for 10 min, and filtered several times. The clear solution was evaporated at 40-45ºC to a thick syrup. The syrup was treated several times at room temperature in a ratio of 1:8 with concentrated isopropyl alcohol, then acetone, and ether. Oligosaccharides were stored in a vacuum desiccator, which is a slightly yellowish powder, easily spreading in air.

The monosaccharide composition of the oligosaccharides was determined by paper chromatography in the system n. butanol-pyridine-water (6:4:3) using an acid aniline phthalate developer. In addition to fructose, glucose and sucrose, 8 more spots were found. Acid hydrolysis was performed to determine the monomeric composition. A portion of 0.05 g of oligosaccharides was dissolved in 5 ml of 0.5%

hydrochloric acid and hydrolyzed at 90–95°C in a water bath for 45 min. The hydrolyzate was neutralized with barium carbonate, filtered and concentrated in vacuo. Chromatography of the hydrolyzate was carried out (BC) in the system n.butanol-pyridine-water (6:4:3). The chromate grams showed glucose and fructose, as well as traces and spots of other substances. According to Kolthoff, the ratio of fructose and glucose is: fructose 86% and glucose 14%, the molecular weight of fructosan is 1245. The rest of the raw material (after isolation of oligosaccha rides) was extracted at 75ºC to 80ºC for 45 minutes.

The extraction of raw materials with water was repeated twice. The mixture was filtered, the aqueous extracts were combined and purified from proteins by the Sevag method (benzene:methanol 1:1). The precipitated proteins were separated by centrifugation, and the polysaccharide solution was precipitated (1:2) with ethyl alcohol. The precipitate was then separated by filtration through a Buchner funnel and dried in air.

To determine the monomer composition, the polysaccharide was subjected to acid hydrolysis with 2.5% HCl at 100ºC for 3 hours, followed by neutralization with $BaCO_3$. Paper chromatography of the hydrolysates was carried out in the system: acetone-n.butanol-water (7:2:1), and acid aniline phthalate was used as a developer. Apart from glucose and mannose, no other monosaccharides were found on the chromatograms.

For further studies, gel chromatography determined the homogeneity of glucomannan (GM). The monomeric composition and the ratio of monosaccharides are presented in the table. The rest of the raw material after isolation of oligosaccharides and water-soluble polysaccharides was treated with a mixture of 0.5% solutions of oxalic acid and ammonium oxalate (1:1) at 70ºC for 6 hours, filtered, the extraction of the meal was repeated again. The extracts were combined, evaporated to half the volume, dialyzed with water, and concentrated into a syrup. Pectin substances (PS) were precipitated with ethyl, isopropyl alcohol, or acetone in a ratio of 1:2, after 24 hours the precipitate was filtered off, washed with acetone and dried. Acid hydrolysis was performed to determine the monomeric composition.

A sample of 0.1 g of HP was hydrolyzed in 5 ml of 2.5% H_2SO_4 in a boiling water bath for 72 h. After hydrolysis, the solution was cooled, neutralized with calcium carbonate to pH 6.5, filtered, the filtrate was concentrated at 40ºC to a syrup. The syrup was analyzed by PC in the system n.butanol-pyridine-water (6:4:3). Fructose (Fru), glucose (Glu), rhamnose (Rham), arabinose (Ara), xylose (Xyl), galactose (Gal), and galacturonic acid (GalUa) were detected on chromatograms using true witnesses.

The ratio of sugars and monomeric composition is presented in the table.

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RESULTS

Hemicelluloses (HC) were isolated from the remains of raw materials. The raw material was extracted with 10% NaOH solution in the ratio of raw material-solvent 1:10 at room temperature for 24 hours with occasional stirring. After filtration, the extraction was repeated twice. Neutralization was carried out with acetic acid. The extract was dialyzed and concentrated, after precipitation with ethanol after 24 hours it was filtered, washed and dried.

To determine the monomeric composition, acid hydrolysis was carried out with 2.5% H_2SO_4 for 6 hours. The results are shown in Table 1.

Table 1. Characterization of oligos and polysaccharides in the lower part of E. Lutsus plants.

Oligosaccharide types	Monosaccharides ratio							
	Fru	Glu	Gal	Ara	Mann	Xyl	Rham	GalUa
Fructosans	10.3	0.7	-	-	-	-	-	-
GM	-	0.6	-	-	11.4	-	-	-
PS	-	1.1	3.15	0.5	Res	1.2	4.1	6.6
HC	-	1.6	1.8	Res	Res	1.5	2.2	2.7

We used waste-free raw materials to isolate alcohol-soluble sugars, water-soluble polysaccharides, pectins and hemicelluloses.

The aerial part of E. Lutsus contains fructose and glucose in the alcoholic extract. Therefore, the oligosaccharide is fructosan and their ratio has been determined.

A water-soluble polysaccharide isolated from the remains of raw materials, consisting of mannose and glucose, which is glucomannan, is a slightly yellowish powder, soluble in water, and does not give color to iodine. According to gel filtration, the molecular weight is 33500, the ratio of glucose and mannose is 1:3.5. To establish the type of linkage between hexoses,

glucomannan was methylated according to the Hakomori method [14]. The completeness of methylation was checked by double repetition. 2,3,6-tri-O-Me-D-mannose (67.5%), 2,3,4,6-tetra-O-Me-D-mannose (13.5%) were found in paper chromatography hydrolyzate permethylate and 2,3-di-O-Me-D-glucose 4.0%. In the IR spectrum of glucomannan there are absorption bands 815cm^{-1}-hexapyranose ring, 840cm^{-1}-equatorial bending vibration of α-sugars, 1040cm^{-1}- are characteristic of bending vibrations of axial C-H bonds, 1240cm^{-1}-ester bonds, 1635cm^{-1} adsorption water.

Pectin substances isolated from the remains of raw materials (after isolation of glucomannan) consist of a set of sugars, with a predominant content of galacturonic acid, characteristic of pectin.

The resulting hemicellulose consists of a set of sugars contained in small amounts, while arabinose and mannose are present in trace amounts.

CONCLUSION

The aerial part of E. Lutsus plants was studied for the first time and oligos and polysaccharides consisting of fructosans, water-soluble polysaccharides, pectin substances and hemicellulose were isolated, their monomeric composition was determined. Fructosans consist mainly of fructose and glucose, water-soluble polysaccharides of glucose and mannose, pectins are a set of sugars with a predominant content of galacturonic acid, and hemicelluloses are a set of sugars.

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