Study of polysaccharides of the grass Hemlock spotted (Conium maculatum L.)

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RESUME

Polysaccharides have wide range pharmacological activity: anti-inflammatory, immune stimulating, wound healing, softening, cholagogue, encapsulating and antitumorigenic. Polysaccharides were isolated from Conium maculatum L. grass growing on the territory of the Republic of Bashkortostan. Using thin-layer chromatography after acid hydrolysis 5 monosaccharides were identified by comparison with library spectrums: glucose, maltose, rhamnose, galactose and saccharose. Using HPLC method water soluble polysaccharides with molecular mass 1359 and 9.5 kDa were identified.

The results can be used for preparation of normative documents describing Conium maculatum L. and preparations produced from it.

Keywords: Conium maculatum L., water-soluble polysaccharides, chromatography, monosaccharides.

SUMMARY

Polysaccharides have a wide spectrum of pharmacological activity: anti-inflammatory, immunostimulating, wound healing, emollient, choleretic, enveloping and antitumor. A study was carried out to isolate and study the component composition of the polysaccharide complex of the grass hemlock spotted growing on the territory of the Republic of Bashkortostan. Using the method of thin layer chromatography, after acid hydrolysis, the presence of monosaccharides: glucose, maltose, rhamnose, galactose and sucrose was preliminarily established. By GC-MS, 5 monosaccharides were identified in the composition of water-soluble polysaccharides (WSPP) by coincidence with the library spectra. Based on the studies carried out using HPLC, it was established that

The results obtained can be used for the development of regulatory documentation for the grass hemlock spotted and preparations obtained on its basis.

Key words: spotted hemlock, water-soluble polysaccharides, chromatography, monosaccharides.

Introduction

Among the large class of natural compounds, the attention of researchers is attracted by polysaccharides, which have long been considered a group of related substances. Polysaccharides are used in medical practice for the prevention and treatment of a number of diseases of various etiologies. They have a wide spectrum of pharmacological activity - they have anti-inflammatory, wound-healing, emollient, choleretic, enveloping and antitumor effects. They increase the general resistance of the body by stimulating the reticuloendothelial system, and although a number of polysaccharides inhibit the growth of Ehrlich's tumors and spontaneous breast cancer, only sarcomas undergo effective regression until the animals recover completely [2, 3, 4].

It has been proven that polysaccharides are able to stimulate cellular and humoral immunity, to exhibit immunomodulatory properties in immunodeficiency states.

In addition, polysaccharides potentiate the immunostimulating effect of flavonoids. Mixtures of polysaccharides containing D-glucose, D-galactose, L-arabinose, D-glucuronic acid are immunostimulants that affect the immune response and phagocytosis [1]. However, in the available literature, we did not find data on the content and characteristics of polysaccharides in the herb of spotted hemlock.

The purpose of this study was to isolate a polysaccharide complex from the grass hemlock spotted, its quantitative determination, as well as study of the monomeric composition and molecular weight distribution of polysaccharides by chromatographic methods.

Materials and methods

The grass hemlock spotted was harvested during the flowering period from wild plants growing in various regions of the Republic of Bashkortostan in 2010–2014. Raw materials were stored in accordance with the requirements of regulatory documents (OST 64-4-143-75 and GF USSR XI ed.) At room temperature, in a dry, well-ventilated room, not infected with barn pests, without direct sunlight.

Isolation of water-soluble polysaccharides (WSPP) was carried out by double extraction with purified water, acidified with 0.5% hydrochloric acid solution to pH = 1-2 (heating for 1 hour; settling for 24 hours; then heating for 1 hour) and subsequent precipitation of polysaccharides with 95% ethyl alcohol, followed by filtration, dissolution of the precipitate in water and dialysis through a semipermeable membrane with a permeability of MWCO (Molecular weight cut-off) 3-6 kDa for 48 h in a 50-fold volume of purified water at room temperature and stirring on a magnetic stirrer, changing the water after 24 hours. After dialysis, the solution was frozen and lyophilized.

The quantitative content of WSP was determined gravimetrically after drying the sediment [5]. In the resulting fraction, the total content of uronic acids was determined by the carbazole-sulfur method; for the quantitative determination of protein impurities, the Lowry method was used in accordance with the State Pharmacopoeia of the USSR XI edition (1989) and according to T. Bitter et al. (1962).

To establish the monosaccharide composition, hydrolysis of VSP was carried out with a solution of trifluoroacetic acid (4 mol / l) at a temperature of 100 ° C for 5 hours [6].

Monosaccharides were identified by gas chromatography-mass spectrometry (GC-MS) in the form of the corresponding trimethylsilyl (TMS) derivatives of monosaccharides after preliminary hydrolysis with 4 M trifluoroacetic acid. Acid hydrolysis was carried out as follows: an exact weighed portion of the polysaccharide fraction (10 mg) was placed in a 10 ml ampoule, and 2 ml of 4 M trifluoroacetic acid was added. The ampoule was sealed and kept in an oven at a temperature of 100 ° C (5 hours). After cooling, the contents of the ampoule were transferred to a flask and evaporated on a rotary evaporator, adding three times 0.5 ml of methyl alcohol to free the residues of trifluoroacetic acid. The dry residue was dissolved in 95% ethanol, filtered, and transferred into a clean flask, in which the solution was dried to constant weight at a temperature of 50 ° C.

To obtain TMS derivatives, anhydrous pyridine (100 μL) and N-trimethylsilylimidazole (30 μL) were added to the residue of monosaccharides obtained after acid hydrolysis, covered tightly with a lid and left for 25 minutes in an oven (75 ° C), then cooled and 1 ml of hexane was added, stirred, and the upper layer was taken for GC analysis.

Separation of TMS samples was carried out on an Agilent 7890A gas chromatograph (USA) on a 30 m HP-1MS column consisting of polydimethylsiloxane, the inner diameter of the capillary was 0.25 μm, the carrier gas (He) flow rate was 1 ml / min, in a temperature gradient : 70 deg. - 2 minutes, then 10 degrees per minute (up to 300 degrees), injector temperature 280 degrees, detection was carried out on an Agilent 5975S mass spectrometer (USA), electron impact ionization, scanning m / z 33-600, ion source temperature 120 degrees ...

The analysis of the molecular weight distribution was carried out on an Ultimate 3000 liquid chromatograph (Dionex, Germany) equipped with a refractometric detector. Chromatography conditions: TSK GMPWXL column, 300 × 78 mm, 13 μm, mobile phase - water, flow rate 1 ml / min, column oven temperature - 30 ° C. Refractometric detection, the temperature of the detector cell is 40 ° C. When calculating the molecular weight distribution, a calibration plot was used, built using dextran standards in the range of 1 - 2000 kDa (Sigma).

results

The results of the yield of water-soluble polysaccharides (WSPC), the content of uronic acids and protein impurities in them are given in table. 1. According to the data obtained (Table 1), the VRPS fraction contains a fairly high amount of impurities of protein compounds, which must be taken into account in further studies related to the isolation and purification of polysaccharides from medicinal plant materials. According to the literature, a significant content of uronic acids in the polysaccharide complex (in the VRPS - 20.5 ± 0.3%) can cause a therapeutic effect associated with anti-inflammatory and antimicrobial activity [5, 6].

Table 1

Withretention of proteins and uronic acids in the VRS from the hemlock grass spotted Wow

Fraction polysaccharides	The content of the damage acids,%	Protein content,%
VRPS	20.5 ± 0.3	5.74 ± 0.06

The composition of monosaccharides was preliminarily determined after acid hydrolysis by chromatography on paper in the solvent system ethyl acetate - acetic acid - formic acid - water (18: 3: 1: 4) and in a thin sorbent layer (TLC) on a Silufol UV-254 plate in the system solvents butanol - acetone - water (4: 5: 1) with reliable samples of monosaccharides. After drying in air, the chromatograms were treated with aniline phthalate reagent and heated in a drying oven at a temperature of 100–105 ° C. Monosaccharides appeared as reddish brown spots. Glucose, maltose, rhamnose, galactose and sucrose were found in the polysaccharide complex.

After preliminary studies by thin layer chromatography, the monomeric composition of polysaccharides was studied using the GC-MS method of the obtained TMS derivatives (Fig. 1).

By coincidences with the library mass spectra, five monosaccharides were identified in the VRPS fraction (table. 2).

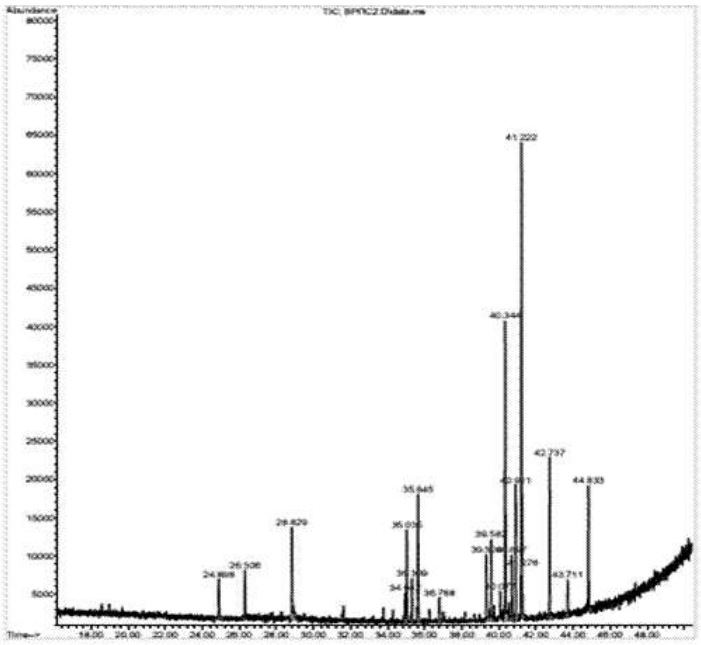


Fig. 1. Chromatogram of trimethylsilyl derivatives of monosaccharides VRPS grass hemlock spotted (along the axis abscissa - retention time in min., ordinate - intensity of ion current in Abundance).

table 2

Monomeric composition of the VSPR of the grass hemlock spotted (GC-MS results)

Fraction polysaccharides	Glucose, %	Galactose, %	Arabinose, %	Mannose, %	Galacturonic acid,%
VRPS	16.22 ± 0.74	59.35 ± 2.7	10.39 ± 0.45	8.50 ± 0.32	5.54 ± 0.17

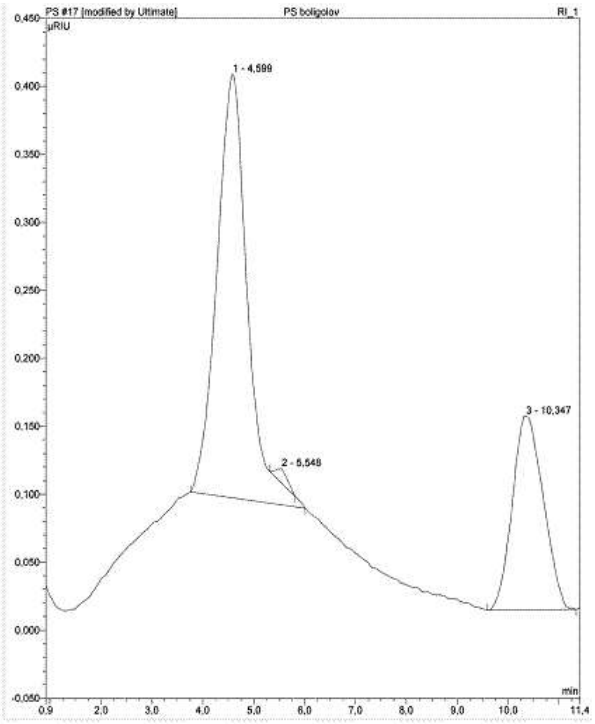
According to the data obtained, the polysaccharide complex

VRPS as macrocomponents contains glucose, galactose, arabinose, mannose and a minor monosaccharide - galacturonic acid (Table 2).

When examining the HPLC gram of the VSPV of the hemlock grass, it was found that the VSPR has a wide molecular weight distribution; two main peaks with retention times of 4.599 min are detected on the chromatogram. and 10.347 minutes. and consist of polysaccharides with a molecular weight of 1359 kDa and 9.5 kDa with a relative content of 59.34 and 39.79%, respectively (Fig. 2).

Discussion

Thus, on the basis of the studies carried out, it was found that the fraction of water-soluble polysaccharides of the hemlock grass is a polysaccharide complex with an admixture of (5.74 ± 0.06%) protein. The content of uronic acids, in terms of galacturonic acid, according to the spectrophotometric method of determination, is 20.5 ± 0.3%. The VRPS contains major components - glucose, galactose, arabinose, mannose and a minor monosaccharide - galacturonic acid. Analysis of the molecular weight distribution by high-performance size exclusion chromatography showed that the complex of VSPR of the grass hemlock spotted has a wide distribution of polysaccharides in molecular weight from 1359 kDa (main fraction - 59.34%) to 9.5 kDa (39.79%).



Rice. 2. Size exclusion chromatogram of a sample of the VSPL of the grass hemlock spotted (on the abscissa - the retention time in min., ordinate - refractive index in RIU).

conclusions

1. A complex of water-soluble polysaccharides has been isolated from the grass hemlock.

2. The spectrophotometric method established the content of uronic acids in it in terms of galacturonic acid 20.5 ± 0.3%.

3. It was found that the polysaccharide complex consists of the following monosaccharides: glucose, galactose, arabinose, mannose, galacturonic acid.

4. Based on HPLC analysis, it was found that the complex of water-soluble polysaccharides of hemlock herb spotted consists of two polysaccharides with a molecular weight of 1359 kDa and 9.5 kDa.

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