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# Characteristic of Polysaccharide Complexes from *Centaurea scabiosa* L. and *Centaurea pseudomaculosa* Dobrocz.

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**Abstract**—We characterized polysaccharide complexes from *Centaurea scabiosa* L. and *Centaurea pseudomaculosa* Dobrocz. We proposed the technique of sequential selection of water-soluble polysaccharides and pectin substances from the aerial parts of studied objects. We have discovered that the content of water-soluble polysaccharides in the aerial parts of *C. scabiosa* was 2.8 times higher (2.7 ± 0.3%, $n = 3$) than in *C. pseudomaculosa* (0.97 ± 0.50%, $n = 3$). The content of pectin substances in the aerial parts of *C. scabiosa* was 2 times higher (7.6 ± 0.4%, $n = 3$) than in *C. pseudomaculosa* (3.9 ± 0.3%, $n = 3$). The residues of D-galacturonic acid, L-rhamnose, D-xylose, D-mannose, D-glucose, and D-galactose are the monomeric units of polysaccharide complexes from *C. scabiosa* and *C. pseudomaculosa*. Using ion-exchange chromatography, three polysaccharide fractions (molecular weights 667, 722, and 1027 kDa), whose monomer units are D-galacturonic acid, L-rhamnose, D-galactose, D-xylose, and D-glucose were isolated from the water-soluble polysaccharides of *C. scabiosa*.

*Keywords*: water-soluble polysaccharides, pectin substances, *Centaurea scabiosa* L., *Centaurea pseudomaculosa* Dobrocz., DEAE-cellulose, D-galacturonic acid

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## INTRODUCTION

Plants from the genus *Centaurea* (Asteraceae) include about 800 species. Sixteen of them are located in the territory of Siberia and the Russian Far East. The genus *Centaurea* is widely used in traditional and official medicine [1–3]. Within the last few years, the phytochemical and pharmacological studies of some species such as *Centaurea scabiosa* L., *Centaurea pseudomaculosa* Dobrocz., *Centaurea jacea* L., and *Centaurea phrygia* L. were conducted at the Department of Pharmaceutical Chemistry, Siberian State Medical University [4]. It is established that *C. scabiosa* and *C. pseudomaculosa* are most prospective. The water and water-ethanol extracts from *C. scabiosa* and *C. pseudomaculosa* have a pronounced antiopisthorchiasis and antioxidant effects [5–7]. Moreover, the pharmacological studies identified the presence of anticonvulsant, antihypoxic and hepatoprotective properties in extracts from *C. scabiosa* [8–10]. The study of the chemical composition of the extracts from these species showed that the phenolic compounds (flavonoids, hydroxycinnamic acids, coumarins, tannins), sesquiterpene lactones, and polysaccharides are the main groups of biologically active substances [11–14]. It should be noted that the interest in the study of plant polysaccharides is increasing in connection with their unique structure, unique biological functions and a wide range of physiological activity (anti-inflammatory, immunomodulating and antitumor, etc.) [15, 16]. Thus, the study of polysaccharide complexes from *C. scabiosa* and *C. pseudomaculosa* is actual.

The purpose of this investigation was the study of chemical composition of polysaccharide complexes from *C. scabiosa* and *C. pseudomaculosa*.

## MATERIALS AND METHODS

**Plant material.** The aerial parts of *C. scabiosa* and *C. pseudomaculosa* were collected in 2013 near Tomsk and Zavarzino (Tomsk oblast), respectively. The aerial parts of plants were harvested in the phase of mass flowering, and dried to a dry consistency.

**General analytical methods.** The identification of monosaccharides, belonging to the polysaccharide complexes was carried out after complete acid hydrolysis with 2 mol/L of trifluoroacetic acid (TFA) by

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heating for 5 h at 100°C in a sealed ampoule [17]. The excess of TFA was removed by repeated evaporation with methanol.

Monosaccharides from the hydrolyzate of polysaccharide complexes in the form of corresponding TMS monosaccharides were studied by GLC-MS method. TMS monosaccharides were obtained by the following procedure: 100 μL of anhydrous pyridine was added to a mixture of monosaccharides, obtained as a result of acid hydrolysis (after evaporation to dryness). The mixture was kept in an oven at 50°C for 20 minutes. After that, 25 μL of N-trimethylsilyl imidazole (Sigma-Aldrich Chemie GmbH, Germany) were added. The mixture was kept in an oven at 70°C for 40 minutes. One mL of hexane was added to the mixture of TMS derivatives. The mixture was vigorously shaken and left to separate. After that, the upper layer was sampled and analyzed by GLC-MS [18].

Chromatography-mass spectrometry of TMS-esters was carried out by an Agilent 7890A gas chromatograph (United States) equipped with an Agilent 5975C mass-selective detector, and an HP5MS capillary column (30 m × 250 μm × 0.25 μm). Carrier gas was helium (flow rate 1 mL/min).

The content of uronic acids in polysaccharide complex was established by the carbazole-sulfur method expressed as galacturonic acid [18]; the total protein content was established based on the reaction with the biuret reagent by the Flores method [19] using a Unico 2800 spectrophotometer (United States).

The molecular-mass distribution in the studied samples was determined using the SE-HPLC method according to retention time with the calibration values defined by standard samples of dextrans using an Ultimate 3000 Dionex liquid chromatograph with the refractive index detector (United States). The separation was performed on a TSK-gel $GMP_{XL}$ exclusion column 300 × 7.8 mm (Supelco, Japan). The eluent was water, 1.0 mL/min.

$^1$H NMR spectra were recorded using an AVANCE AV 300 Fourier spectrometer (Bruker, Germany). The working frequency was 300 MHz, the solvent was $D_2O$. Tetramethylsilane (TMS) was used as an internal standard. All chemical shifts were expressed in ppm (δ-scale) against TMS.

**Method of sequential separation of water-soluble polysaccharides (WSPs) and pectic substances (PSs) from raw materials.** The quantity of raw material (the aerial parts of *C. scabiosa* or *C. pseudomaculosa*) (50.0 g) was filled with 1000 mL of purified water (raw water ratio 1 : 20) and heated in a boiling water bath for 2 h with stirring. Then, the extraction was filtered, the raw material was pressed, separated from the extract, and filtered through a paper filter. The quantity of raw material was filled with 500 mL of purified water, heated in a boiling water bath under identical conditions for 1 h. The mixture was allowed to cool to room temperature and filtered through a multilayer fabric filter. The filtrate was evaporated in vacuo to 100 mL at a temperature of not more than 50°C. The solution was slowly poured into 300 mL of 96% ethanol and was left in a cool place for precipitation for 24 h. The settled solution was drained. The precipitate was filtered through a paper filter and washed with 96% ethanol, ethyl acetate, and acetone, sequentially. After that, the precipitate without drying was transferred from the filter into a glass tumbler and dissolved in 100 mL of purified water with rapid agitation on a magnetic stir bar at room temperature for 3 h. The solution was then centrifuged (4000 rpm, 30 min) and the supernatant was evaporated to a volume of 40 mL. After that, the solution was dialyzed through an OrDial D-Clean cellophane film (Belgium) with a pore size of 5 kDa for 72 h in 3000 mL of purified water at room temperature; the water was changed after 12 h. Then, the solution was evaporated in vacuum, frozen and dried on a SP Scientific Advantage EL-85 lyophilized drier (United States).

The raw materials cake, remaining in the retort after extraction of water-soluble polysaccharides with water was used to extract of 0.7% PSs with ammonium oxalate solution in the same way as for extracting of WSPs.

For the quantitative determination of the polysaccharide complexes, the gravimetric method was used [19].

The method of DEAE-cellulose ion exchange column chromatography ($Cl^-$-form, sorbent capacity was ranged from 0.9 to 1.0 meq/g, particle size was ranged from 100 to 200 μm) was used for separation of the WSPs from *C. Scabiosa*.

The quantity of WSPs from *C. scabiosa* (0.300 g) was dissolved in 0.01 mol/L sodium chloride solution and centrifuged for 5 min, 1500 rpm. The solution was separated from the precipitate. After that, the solution was applied to a column (24 × 2.5 cm) with DEAE-cellulose (Whatman, Germany). The column was successively washed with 500 mL sodium chloride solutions with an increasing concentration of 0.01, 0.1, 0.2, 0.3, 0.4, and 0.5 mol/L. The eluent speed was 46 mL/h. Fractions of 25 mL were taken. Smith's method was used for the control of the yield of polysaccharides from the column [21]. The fractions obtained were concentrated and purified using a Vivaflow 200 machine (United States) with a 5000 MWCO cassette. After that, the fractions were frozen and lyophilized.

## RESULTS AND DISCUSSION

A sequential scheme that allows the more complex use of raw materials was proposed for the isolation of water-soluble polysaccharides (WSPs) and pectin substances (PSs) from the aerial parts of studied objects. Purified water was used as an extractant for the separation of WSPs with subsequent precipitation in the aqueous extraction of polysaccharide complexes

**Table 1.** Characterization of polysaccharide complexes from the aerial parts of *C. scabiosa* and *C. pseudomaculosa*

| Name of fractions | Yield*, % | Uronic acids content, % | Protein content, % |
|---|---|---|---|
| WSPs from *C. scabiosa* | 2.7 ± 0.3 | 63.7 ± 3.9 | 6.8 ± 0.9 |
| WSPs from *C. pseudomaculosa* | 0.97 ± 0.50 | 46.1 ± 1.8 | 8.3 ± 1.1 |
| PSs from *C. scabiosa* | 7.6 ± 0.4 | 61.6 ± 4.6 | 5.9 ± 1.2 |
| PSs from *C. pseudomaculosa* | 3.9 ± 0.3 | 45.8 ± 2.2 | 7.8 ± 1.2 |

* Yields of the fractions were estimated as a percentage of the air-dried plant raw material mass.

with ethanol. Pectin substances were extracted with 0.7% ammonium oxalate solution with subsequent precipitation with ethanol from the cake left after the separation of the WSPs. The following conditions were selected: double extraction on a boiling water bath, ratio of the extractant to the raw material 1 : 20 (first extraction) and 1 : 10 (second extraction), extraction time was 2 h and 1 h, respectively. Deproteinization was carried out by reprecipitation with ethanol and centrifugation of the precipitate of denatured proteins. The method of dialysis through a semipermeable membrane was used for purification from phenolic compounds, saponins, low-molecular compounds. The change in pH (pH < 7) of used extractants did not lead to an increase in yields of WSPs and PSs. In addition, in such conditions, the undesirable hydrolysis of native polysaccharides is available. The completeness of extraction was checked by reaction with Fehling's reagent after prehydrolysis of PSs in the extraction with 20% sulfuric acid solution. Experimentally, it was determined that the completeness of extraction is achieved by double extraction.

It was determined that the content of WSPs in the aerial parts of *C. scabiosa* was 2.8 times greater (2.7 ± 0.3%, $n = 3$) than in *C. pseudomaculos* (0.97 ± 0.50%, $n = 3$); the content of PSs in the aerial parts of *C. scabiosa* was 2 times greater (7.6 ± 0.4%, $n = 3$) than in *C. pseudomaculos* (3.9 ± 0.3%, $n = 3$) (Table 1).

The polysaccharide complexes found, WSP and PS, were characterized by the following parameters: qualitative monomeric composition (uronic acid content, protein content) and molecular weight distribution.

The monomeric composition of all isolated polysaccharide complexes was studied using GLC-MS method after acid hydrolysis. Water-soluble polysaccharides and pectin substances from the studied species have a similar qualitative monomeric composition. According to the results of the analysis, the monomeric units of WSPs and PSs are D-galacturonic acid, L-rhamnose, D-xylose, D-mannose, D-glucose and D-galactose.

The determination of the molecular weight distribution revealed that the WSPs of *C. scabiosa* contains two basic components with MW 1490 kDa (62.5%) and 15 kDa (33.6%) and two in lesser amounts, 1060 kDa (1.7%) and 400 kDa (1.7%) with a total content of uronic acids 63.7 ± 3.9% ($n = 5$). WSPs from *C. pseudomaculosa* also contain two main components with MW 770 kDa (40.8%) and 14 kDa (57.6%), and one in small amounts, 440 kDa (1.6%) with a total content of uronic acids 46.1 ± 1.8% ($n = 5$). PSs from *C. scabiosa* contain two main components with MW 399 kDa (55.8%) and 103 kDa (37.9%), and one in small amounts 32 kDa (6.3%) with a total content of uronic acids 61.6 ± 4.6% ($n = 5$). PSs from *C. pseudomaculosa* contain one main component with MW 354 kDa (84.4%) and two in smaller amounts 92 kDa (7.2%) and 15 kDa (8.4%) with a total content of uronic acids 45.8 ± 2.2% ($n = 5$). Based on these results, it was established that the content of uronic acid in the polysaccharide complex from *C. scabiosa* was 1.3 times higher than in the polysaccharide complexes from *C. pseudomaculosa* (Table 1).

The protein content in WSPs from *C. scabiosa* was 6.8 ± 0.9% ($n = 5$), and in WSPs from *C. pseudomaculosa*, 8.3 ± 1.1%. The protein content in PSs from *C. scabiosa* was 5.9 ± 1.2%, and in PSs from *C. pseudomaculosa*, 7.8 ± 1.2% (Table 1).

On the basis of the chemical and pharmacological researches of *C. scabiosa* and taking into account higher yields of WSPs and PSs from *C. scabiosa* than from *C. pseudomaculos*, the next step was isolation of polysaccharide fractions from the WPSs of *C. scabiosa* and the investigation of their chemical structure for a deeper study of the chemical structure of the WSPs. The acidulous polysaccharides, which were eluted from the column after using 0.1 M (CS1), 0.1 M (CS2) and 0.2 M (CS3) solutions of sodium chloride were obtained as a result of ion-exchange chromatography on DEAE-cellulose of the WSPs from *C. scabiosa* using an aqueous solution of sodium chloride with increasing concentration.

It was found that all three fractions contain less than 0.5% protein. The fractions contain a large amount of uronic acids (CS1, CS2 and CS3: 28.2 ± 6.4; 40.6 ± 5.2 and 41.2 ± 5.4%, respectively, $n = 3$). That indicates the acid nature of the PSs.

The fractions were analyzed by the SE-HPLC method to determine the homogeneity and purity. Chromatographic analysis revealed that all investigated fractions have different molecular weights: CS1 was 667 kDa, CS2 was 722 kDa, CS3 was 1027 kDa, the main monomeric units of which were D-galactose,

**Table 2.** Characteristics of the fractions from WSPs of *C. scabiosa*

| Name of fractions | Yield*, % | Molecular weight, kDa | Uronic acids content, % | Protein content, % | Monomer composition |
|---|---|---|---|---|---|
| CS1 | 11.3 | 667 | 28.2 ± 6.4 | Less than 0.5 | Gal A, Rha, Gal, Xyl, Glu |
| CS2 | 12.1 | 722 | 40.6 ± 5.2 | Less than 0.5 | Gal A, Rha, Gal, Xyl, Glu |
| CS3 | 9.5 | 1027 | 41.2 ± 5.4 | Less than 0.5 | Gal A, Rha, Gal, Xyl, Glu |

* Yields of the fractions were estimated as a percentage of the weight of the applied to the column WSPs. Gal A is D-galacturonic acid, Rha is L-rhamnose, Gal is D-galactose, Xyl is D-xylose, Glu is D-glucose.

D-xylose, D-galacturonic acid, L-rhamnose, and D-glucose (Table 2).

The $^1$H NMR-spectroscopy was used for identification the nature of glycosidic bonds in carbohydrate chains of polysaccharide fractions CS1, CS2 and CS3. Spectra data of standard samples of mono-, di- and polysaccharides were used in the identification of the spectra.

The signals of protons in the region from 3.5 to 4.3 ppm indicate the presence of the residues of α-D-glucopyranose bound by the α(1→4)-glycoside bond in the structures of all tested compounds. The intensive signal in the region of 3.7 ppm corresponds to protons of the (CH3-O) methoxy group. The signal in the region of 3.88 ppm refers to the signals of protons at the 3rd position of the carbon atom in the residues of α-D-galactopyranose and α-D-glucopyranose. Moreover, 1.16 and 3.4 ppm signals, which indicate the presence of α-L-rhamnopyranose residues in these compounds, are observed in the spectra of CS2 and CS3 compounds.

The method of sequential isolation of water-soluble polysaccharides and pectin substances from the aerial parts of the studied objects was proposed. It was established that the aerial parts of *C. pseudomaculosa* have a considerable quantity of water-soluble polysaccharides and pectin substances. The content of WSPs and PSs in the *C. scabiosa* were 2–3 times higher than in *C. pseudomaculosa*. Furthermore, the content of uronic acids in the polysaccharide complexes from *C. scabiosa* was 1.3 times higher than in the polysaccharide complexes from *C. pseudomaculosa*. The monomer units of the polysaccharide complexes from *C. scabiosa* and *C. pseudomaculosa* are the residues of D-galacturonic acid, L-rhamnose, D-xylose, D-mannose, D-glucose, and D-galactose.

Three polysaccharide fractions (with molecular weights of 667, 722 and 1027 kDa) in the structure of which the residues of α-D-galactopyranose, α-L-rhamnopyranose, α-D-glucopyranose bound by a α(1→4)-glycoside bond, D-galacturonic acid and D-xylose are present were isolated by ion-exchange chromatography from water-soluble polysaccharides of *C. scabiosa*, which is a more promising object.

## REFERENCES


1. Fedorova, A.A., *Rastitel'nye resursy SSSR: tsvetkovye rasteniya, ikh khimicheskii sostav, ispol'zovanie; semeistva Asteraceae* (Plant Resources of the USSR: Flowering Plants, Their Chemical Composition, and Use. Family Asteraceae), Leningrad, 1987.
2. Khammar, A. and Djeddi, S., Pharmacological and biological properties of some *Centaurea* species, *Eur. J. Sci. Res.*, 2012, vol. 84, no. 3, pp. 398–416.
3. Vele, T., Dejan, D., and Vlatka, V., Constituents of the roots of plants species *Centaurea scabiosa, J. Serb. Chem. Soc.*, 1994, vol. 59, no. 12, pp. 979–981.
4. Kadyrova, T.V., Ermilova, E.V., Krasnov, E.A., Kaminskii, I.P., Lar'kina, M.S., and Dudko, V.V., The chemical composition of promising plants, their antioxidant activity, and pharmacological activity, in *Novye dostizheniya v khimii i khimicheskoi tekhnologii rastitel'nogo syr'ya: tezisy dokladov Vseross. nauch. konf.* (New Advances in Chemistry and Chemical Technology of Plant Raw Materials: Abstracts of All-Russia Sci. Conf.), Barnaul, 2007, p. 86.
5. Lar'kina, M.S., Saprykina, E.V., Kadyrova, T.V., Ermilova, E.V., and Peshkina, R.A., Antioxidant activity of *Centaurea scabiosa* extract in toxic damage of rat liver, *Vopr. Biol. Med. Farmatsevt. Khim.*, 2011, no. 8, pp. 25–28.
6. Kadyrova, T.V., Lar'kina, M.S., Ermilova, E.V., Krasnov, E.A., and Avramchik, O.A., Antioxidant activity of extracts from the above-ground part of *Centaurea scabiosa* L. (Asteraceae), *Rastit. Resur.*, 2010, no. 1, pp. 102–106.
7. Kaminskii, I.P., Krasnov, E.A., Kadyrova, T.V., Sazonov, A.E., Rakhimova, B.B., Ivasenko, S.A., and Adekenov, S.M., The antiepistoric properties of extracts from *Centaurea scabiosa* (Asteraceae), *Rastit. Resur.*, 2010, no. 1, pp. 106–112.
8. Lar'kina, M.S., Saprykina, E.V., Gereng, E.A., Kadyrova, T.V., Ermilova, E.V., and Peshkina, R.A., Hepatoprotective properties of *Centaurea scabiosa, Vopr. Biol. Med. Farm. Khim.*, 2011, no. 7, pp. 28–32.
9. Lar'kina, M.S., Saprykina, E.V., Kadyrova, T.V., and Ermilova, E.V., Effect of *Centaurea scabiosa* extract on the content of total lipids and their individual components in rats with hepatitis, in *Farmatsiya: sovremennoe sostoyanie i perspektivy: tezisy dokladov Mezhdunar. nauch.-prakt. konf.* (Pharmacy: Current State and Prospects, Abstracts of International Scientific-Practical Conference), Almaty, 2010, pp. 206–207.
10. Kadyrova, T.V., Krasnov, E.A., and Kornyakova, A.V., Anticonvulsant properties of extracts from *Cenaurea*

*scabiosa* (Asteraceae), *Rastit. Resur.*, 2006, no. 4, pp. 70–75.

11. Lar'kina, M.S., Kadyrova, T.V., and Ermilova, E.V., Phenolic compounds of species of the genus *Centaurea* of the world flora (review), *Khim. Rastit. Syr'ya*, 2011, no. 4, pp. 7–14.
12. Lar'kina, M.S., Kadyrova, T.V., Ermilova, E.V., and Krasnov, E.A., Quantitative determination of flavonoids in the above-ground part of *Cenaurea scabiosa* L., *Khim.-Farm. Zh.*, 2009, no. 4, pp. 14–17.
13. Lar'kina, M.S., Kadyrova, T.V., Koval', V.V., Ermilova, E.V., and Yusubov, M.S., Flavonoids of the above-ground part of *Centaurea scabiosa* L., *Khim. Rastit. Syr'ya*, 2012, no. 4, pp. 175–180.
14. Krasnov, E.A., Kaminskii, I.P., and Kadyrova, T.V., Antimicrobial activity of extracts from the above-ground part of *Centaurea scabiosa* (Asteraceae), *Rastit. Resur.*, 2012, no. 2, pp. 262–266.
15. Ovodov, Yu.S., Polysaccharides of flowering plants: structure and physiological activity, *Bioorg. Khim.*, 1998, vol. 24, no. 7, pp. 483–501.
16. Krishtanova, N.A., Safonova, M.Yu., and Bolotova, V.Ts., Prospects for the use of plant polysaccharides as therapeutic and prophylactic agents, *Vestnik VGU*, 2005, no. 1, pp. 212–221.
17. Korzh, A.P., Gur'ev, A.M., Belousov, M.V., Yusubov, M.S., and Belyanin, M.L., The composition of water-soluble polysaccharides from *Calendula officinalis* L. flowers, *Khim.-Farm. Zh.*, 2012, no. 4, pp. 23–25.
18. Galambos, J.T., The reaction of carbazole with carbohydrates: I. Effect of borate and sulfamate on the carbazole color of sugars, *Anal. Biochem.*, 1967, vol. 19, pp. 119–132.
19. *Gosudarstvennaya farmakopeya SSSR* (State Pharmacopoeia of the USSR), vol. 2: *Obshchie metody analiza. Lekarstvennoe rastitel'noe syr'e* (General Methods of Analysis. Medicinal Plant Raw Materials), 11th ed., Moscow, 1990.
20. Neukom, H., Deuel, H., and Heri, W.J., Chromatographische Fraktionerung von Polysackariden an Cellulose-Anionenaustauschern, *Helv. Chim. Acta*, 1960, vol. 43, pp. 64–68.
21. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F., Colorimetric method for determination of sugars and related substances, *Anal. Chem.*, 1956, vol. 28, pp. 350–356.

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