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POLYSACCHARIDES FROM *Arctostaphyllos uva-ursi*

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Bearberry [*Arctostaphyllos uva-ursi* (L.) Spreng., Ericaceae] is an official medicinal plant used in medical practice to treat a broad spectrum of diseases of the urogenital tract [1]. The active compounds of *A. uva-ursi* are phenolic glycosides, tannins, and flavonoids [2]; other classes of compounds are insufficiently studied. Considering the fact that the forms of *A. uva-ursi* used most often are aqueous extracts (decoctions, tinctures, and dry extracts obtained from aqueous extraction), we analyzed water-soluble polysaccharide components and pectinic substances in addition to hemicellulose of this type of plant raw material.

Water-soluble polysaccharides (WSPS) were isolated from ground leaves of *A. uva-ursi* (200 g) by successive extraction in a Soxhlet apparatus using $CHCl_3$, EtOAc, and $(CH_3)_2CO$, after which the raw material was worked up exhaustively with water at 20 and 100°C. The extracts obtained at the different temperatures were concentrated separately and dialyzed through a cellulose-acetate membrane (Filtrak) against distilled water. The undialyzed remainder was precipitated by acetone (1:5). The resulting precipitates were centrifuged, demineralized by cation-exchanger KU-2-8 (H^+-form), and deproteinized using pronase from *Streptomyces griseus* (Sigma) [3] to produce two fractions of water-soluble polysaccharides, WSPSc and WSPSh in yields of 0.708 and 0.512 g.

WSPSc was a heterogeneous fraction that contained at least three components according to gel chromatography. The reaction with iodine was negative. The IR spectrum exhibited strong absorption bands for an α-bond (762.7 and 852.1 cm^{-1}), pyranose ring (810.7, 1037.1, 1081.2, 1146.8), and carboxylic group (1419.4, 1618.4) (Table 1). The presence of a weak band for ester (1737.1) suggested that acetyls were present, the content of which (K_{Ac}) was 2.44–2.54%. The WSPSc contained mannose, glucose, rhamnose, and arabinose in the ratio 7.9:3.6:1.3:1.0 in addition to galacturonic acid (13.3% of WSPSc mass) (Table 2).

Fraction WSPSh had physicochemical properties similar to those of WSPSc except for the content of galacturonic acid (23.0%) and acetyls (8.21–8.62%). The component composition of the fraction was identical to that of WSPSc with a greater amount of compound with MW 83 kDa. Thus, WSPS of *A. uva-ursi* contained probably glucomannans with an impurity of uronide-containing components.

Pectinic substances (PS) were isolated from raw material after removal of WSPS using a mixture of oxalic acid (0.5%) and ammonium oxalate (0.5%) (1:1) at a 1:50 ratio (5×) and 100°C. The extract was dialyzed. The dialysate was precipitated by acetone (1:4). The resulting precipitate was centrifuged and dried by solvent exchange. Yield of PS, 6.39 g.

PS of *A. uva-ursi* did not react with iodine, $[\alpha]_D^{20}$ +144° (Table 1). The IR spectrum contained absorption bands for pyranose ring (773.7, 832.2, 1097.4, 1337.1 cm^{-1}), α-bond (853.3), and carboxylic group (1599.3) [4]. The PS included galacturonic acid (59.2%) and neutral monosaccharides galactose, arabinose, glucose, xylose, and rhamnose in the ratio 9.6:8.1:2.5:1.4:1 (Table 2). The content of free (K_f) and esterified (K_e) carboxylic groups was 10.41 and 3.85%, respectively, according to potentiometric analysis. This indicated that it was a low-esterified pectin (degree of esterification λ, 27%). The IR spectrum contained bands for ester (1236.8, 1736.6) that disappeared after basic work up. This fact suggests the presence of acetyls, the content of which was 9.88-9.94% (IR spectroscopy, hydroxylamine method). Gel chromatography over Sephadex G-200 showed that the PS from *A. uva-ursi* were heterogeneous. The range of MW was 15–80 kDa.

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TABLE 1. Physicochemical Properties of *A. uva-ursi* Polysaccharide Fractions

Fraction	Yield, % of raw matl. mass	$[\alpha]_D^{20}$, ° (c 1.0, H_2O)	MW, kDa (% of fraction mass)	K_{Ac}, %*	ν, cm^{-1}
$WSPS_C$	0.35	+24.9	83 (15.4), 47 (73.4), 22 (11.1)	2.54/2.44	685.9, 762.7, 810.7, 825.8, 852.1, 875.4, 995.9, 1037.1, 1081.2, 1146.8, 1231.9, 1322.4, 1369.0, 1419.4, 1508.9, 1618.4, 1737.1, 2864.1, 3452.6
$WSPS_h$	0.26	+34.2	83 (37.2), 47 (61.5), 22 (1.2)	8.62/8.21	763.7, 825.6, 874.7, 915.3, 959.1, 1028.4, 1094.0, 1151.2, 1229.4, 1261.6, 1373.4, 1443.9, 1617.7, 1738.4, 2164.7, 2874.2, 3418.1
PS	3.20	+144.0	15–80	9.94/9.88	773.7, 832.2, 853.3, 892.9, 931.4, 953.1, 1019.4, 1097.4, 1144.1, 1236.8, 1337.1, 1421.2, 1599.3, 1736.6, 2145.5, 2893.5, 3400.3
HC_A	1.03	–	10 (7.7), 24 (32.1), 32 (38.1), 45 (22.0)	–	767.0, 841.2, 884.0, 950.3, 1015.1, 1056.9, 1102.1, 1143.2, 1392.1, 1651.0, 2246.2, 3313.3
HC_B	0.56	–	10 (5.4), 24 (74.8), 45 (19.7)	–	765.7, 842.0, 887.9, 954.4, 1010.4, 1052.8, 1098.4, 1147.4, 1391.7, 1652.0, 2238.4, 3322.7

*Chemical analysis (hydroxylamine method, IR spectroscopy).

TABLE 2. Monosaccharide Composition of *A. uva-ursi* Polysaccharide Fractions

Fraction	Monosaccharide composition, mol%							
	Ara	Gal	Glc	Man	Rha	Xyl	GalUA	GlcUA
$WSPS_C$	6.3	–	22.4	49.7	8.2	–	13.3	–
$WSPS_h$	9.7	–	21.7	42.6	1.1	1.8	23.0	–
PS	14.5	17.3	4.5	–	1.8	2.6	59.2	–
HC_A	3.2	0.5	26.4	21.2	3.3	22.1	19.0	4.2
HC_B	12.7	0.7	46.2	10.9	6.0	14.0	1.5	7.9

Hemicellulose components (HC) were isolated from raw material after extraction by the oxalate mixture using water and then NaOH solution (5%, 1:20, twice for 6 h each). The combined extract was neutralized by AcOH. The resulting precipitate was centrifuged and washed with AcOH (10%), water and EtOH (95%) to afford fraction HC_A (2.06 g). The supernatant after removal of HC_A was dialyzed, concentrated, and precipitated with acetone. The solid was centrifuged and dried by solvent exchange to afford fraction HC_B (1.12 g).

The principal properties of the HC fractions were:

HC_A: carbohydrates 34.15%; ash 12.20%; N < 1%; lignin 44.7%, uronic acids 23.2% including GalUA and GlcUA 4.5:1, Glc:Xyl:Man:Rha:Ara:Gal 8.3:6.9:6.6:1:1:tr.

HC_B: carbohydrates 47.11%, ash 5.15%, N < 1%, lignin 37.0%, uronic acids 9.4% including GalUA and GlcUA 1:5.3, Glc:Xyl:Ara:Man:Rha:Gal 7.7:2.3:2.1:1.8:1:tr.

IR spectra of HC_A and HC_B were similar and contained bands for pyranose ring (767.0, 1015.1, 1102.1, 1143.2/ 765.7, 1010.4, 1098.4, 1147.4 cm^{-1}), 2*a* α-bond (841.2/842.0), 2*b* β-bond (884.0/887.9), carboxylic group (1651.0/1652.0), and a band characteristic of xylose-containing HC (950.3/954.4) (Table 1) [5]. Both HC fractions were heterogeneous and contained polymeric components with MW 10–45 kDa according to gel chromatography.

The studies showed that HC from *A. uva-ursi* were heterogeneous lignin-hydrocarbon complexes, the polysaccharide components of which were xyloglucans.

The monosaccharide composition of the polysaccharides was determined after hydrolysis with TFA (2 M, 100°C, 4 h for WSPS; 2 M, 120°C, 6 H for PS; 3 M, 100°C, 6 h for HC) after removal of acid in vacuo in the presence of MeOH using HPTLC on Sorbfil PTSKh-AF-V plates (Sorbpolimer) [6]. Homogeneity was found using gel chromatography over Sephadex G-200 (Pharmacia, 2 × 90 cm column) with elution by NaCl (0.3%, WSPS, PS) and NaOH (0.01 M, HC) with detection by phenol:H_2SO_4 [7]. Optical rotation was measured on a SM-3 polarimeter (Zagorsk Optico-Mechanical Plant). Potentiometric

studies were performed using a pH-410 pH-meter (Akvilon). IR spectra were recorded in films on KRS-5 plates on a Spectrum 100 (Perkin–Elmer) IR-Fourier spectrometer in the range 4000–650 cm^{-1}. Carbohydrate content was determined by the anthrone method [8]; protein, by the literature method [9]; ash content, gravimetrically after ashing; galacturonic-acid content (K_{GalUA}), by reaction with 3,5-dimethylphenol [10]; free (K_f) and esterified (K_e) carboxylic groups, by potentiometry [11]; and acetyl content (K_{Ac}), by the hydroxylamine method [12] and IR spectroscopy [13]. The lignin content in HC was determined by the Komarov method [14].

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