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POLYSACCHARIDES from *Crotalaria alata*

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Water-soluble polysaccharides, pectinic substances, and hemicellulose were isolated from various organs of Crotalaria alata *cultivated in Uzbekistan. Water-soluble polysaccharides that were present in seeds were found to be galactomannans.*

Keywords: *Crotalaria alata*, water-soluble polysaccharides, pectinic substances, hemicellulose.

Crotalaria alata (Fabaceae) is cultivated at the Rusanov Botanical Garden of the Academy of Sciences of the Republic of Uzbekistan. It is an herbaceous annual plant, the aerial part of which is used as fodder for cattle; the seeds, as a source of polysaccharides such as galactomannans [1, 2].

We studied seeds, pods, and the aerial part of *C. alata* in order to find the distribution of galactomannans in the various plant parts. Plants were collected in October 2008 during seed ripening.

Carbohydrates from the various organs were isolated by the published method [3]. Ground raw material (separately) was defatted by $CHCl_3$:MeOH (1:1) and then extracted by EtOH (82°) to remove low-molecular-weight compounds. Water-soluble polysaccharides (WSPS) were extracted by cold (WSPS-C) and hot (WSPS-H) water. Pectinic substances (PS) were extracted by a mixture of oxalic acid and ammonium oxalate solutions (0.5%). Hemicellulose (HMC) was extracted by base solution (5%). The monosaccharide compositions of the isolated polysaccharides were studied by PC and GC after total acid hydrolysis. Table 1 presents the contents of polysaccharides and their monosaccharide compositions.

The polysaccharide contents in the various organs were different. The WSPS content was greatest in seeds (15%); PS, in pods. The isolated polysaccharides differed in both qualitative and quantitative monosaccharide composition.

WSPS isolated from *C. alata* seeds were a white amorphous powder that dissolved in water to form solutions with high relative viscosities (η_{rel}) of 49.8. The dominant monosaccharides in hydrolysates of WSPS-C and WSPS-H were mannose and galactose in 18:1 and 2.7:1 ratios, respectively. Treatment of the WSPS aqueous solutions with Fehling's solution produced a precipitate that was characteristic of mannose-containing polysaccharides. Therefore, the studied WSPS were galactomannans.

The dominant monosaccharides in WSPS-H isolated from the aerial part of the plant, in contrast with WSPS from seeds, were arabinose and xylose; from pods, xylose. Aqueous solutions of the WSPS had low viscosities of 1.5–2.0.

The PS were white powders with a cream tint. The PS content was elevated only in pods. Their content in seeds was 1.2%. They were characterized by high contents of arabinose and mannose. All hydrolysates of PS contained neutral monosaccharides in addition to galacturonic acid. The PS were highly esterified according to titrimetric analysis [4] (Table 2).

HMC were dark brown powders that were soluble in base and gave a negative reaction with I_2. The HMC content in the plant organs was insignificant. The qualitative monosaccharide compositions of the HMC did not differ greatly. However, there were differences in the amounts of individual monosaccharides, the principal ones of which were arabinose, xylose, and galactose (Table 1).

Thus, the study of the polysaccharide contents and their monosaccharide compositions in various organs of *C. alata* showed that they differed in quantitative content and qualitative monosaccharide composition. The greatest WSPS and PS contents were found in aerial organs and pods. The monosaccharide composition of the isolated polysaccharides indicated that they were heteropolysaccharides. The PS were highly esterified. Seeds of *C. alata* are a source of galactomannans.

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TABLE 1. Content and Monosaccharide Composition of *Crotalaria alata* Carbohydrates

Polysaccharide type	Yield, %	Ratio of monosaccharide units						
		Rha	Ara	Xyl	Man	Glc	Gal	UAc
				Seeds				
WSPS-C	15.0	Tr.	1.0	6.6	91.8	–	5.1	–
WSPS-H	7.1	–	1.0	–	13.4	–	4.96	–
PS	1.2	1.0	15.8	3.5	**24.5**	–	**4.5**	+
HMC	1.4	2.3	3.2	**7.2**	1.0	Tr .	3.7	+
				Pods				
WSPS-C	0.6	2.0	–	2.0	1.0	1.1	Tr .	+
WSPS-H	2.6	1.0	–	14.4	4.2	–	3.6	+
PS	2.6	1.0	Tr .	Tr .	5.0	1.6	2.0	+
HMC	0.09	–	1.0	2.4	1.7	1.8	2.4	–
				Aerial part				
WSPS-C	2.5	1.0	Tr .	–	Tr .	1.8	2.0	–
WSPS-H	0.6	1.0	3.2	–	Tr .	2.4	3.2	+
PS	0.7	1.7	Tr .	1.0	Tr .	10.0	1.2	+
HMC	0.7	1.3	1.8	**2.3**	1.9	2.0	1.0	–

Tr.: traces.

TABLE 2. Titrimetric Data for *C. alata* PS

Plant part	A_f, %	A_e, %	λ, %
Seeds	4.5	52.2	92.06
Pods	1.8	68.4	70.2
Aerial part	0.9	58.5	97.4

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 45–50°C. PC of samples was carried out on FN 1, 12, and 18 paper (Filtrak) using BuOH:Py:H_2O (6:4:3) with detection by anilinium biphthalate. GC used a Chrom-5 chromatograph with a flame-ionization detector, glass column (1.5 × 0.3 cm), Silicone XE-60 (5%), NAW (0.20–0.25 mm), 210°C, N_2 carrier gas, and 30 mL/min flow rate. Samples were analyzed as aldononitrile acetates [5]. Polysaccharides were hydrolyzed using H_2SO_4 (2 N) at 100°C. WSPS were hydrolyzed for 8 h; PS, 42; HMC, 72. Hydrolysates were neutralized by $BaCO_3$, deionized by cation-exchanger KU (H^+), and evaporated to 1 mL.

Isolation of WSPS. Raw material (seeds, 100 g; pods and aerial part, 50 g each) was separately defatted by $CHCl_3$:MeOH (1:1) for 1 h. Raw material was separated, dried, and extracted by EtOH (82°, 2×) for 1 h (1:5). The plant solids were extracted with cold water at room temperature (3×, 1:10 and 1:5 for seeds; 1:5 for pods and the aerial part). The extracts were combined, condensed, and precipitated by three times the volume of EtOH. The resulting precipitates were separated, washed, dehydrated by EtOH, and dried in vacuo over P_2O_5. Yields of WSPS-C: 15.01 g (seeds), 0.3 (pods), 1.28 (aerial part). Then, the plant solids were extracted separately with hot water (3×, 80°C) for 3 h. WSPS-H were worked up as described above. Yields of WSPS-H: 7.13 g (seeds), 1.2 (pods), 0.31 (aerial part).

Isolation of PS. The remaining raw material (separately) was treated with a mixture of oxalic acid and ammonium oxalate solutions (0.5%, 1:1) and extracted (2×, 1:2) at 80°C for 2 h. The extracts were combined, dialyzed against tapwater until neutral, condensed, and precipitated by alcohol. The resulting precipitates were separated, washed with alcohol, and dehydrated by acetone. Yields of PS: 1.2 g (seeds), 1.3 (pods), 0.35 (aerial part).

Isolation of HMC. HMC was extracted by base solution (2×, 5%) at room temperature. The extracts were dialyzed against tapwater until neutral, condensed, and precipitated by twice the volume of alcohol. The precipitates were worked up as described above. Yields of HMC: 1.47 g (seeds), 0.04 (pods), 0.35 (aerial part).

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