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LIPIDS AND CARBOHYDRATES FROM *Althaea nudiflora* AND *A. armeniaca* ROOTS

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Lipids from roots of Althaea nudiflora *and* A. armeniaca *were studied. The carbohydrate and fatty-acid compositions were found. The lipids of* A. nudiflora *and* A. armeniaca *contain 22.3 and 12.6%, respectively, of cyclopropenoid fatty acids, the physical chemical properties of which are presented. The optimal degree of grinding, temperature, and raw-material-to-extractant ratio for aqueous extraction of water-soluble polysaccharides from althaea roots were chosen from a single statistically significant experiment.*

Key words: *Althaea nudiflora*, *Althaea armeniaca*, lipids, polysaccharides, pectinic substances.

Plants of the *Althaea* L. genus (Malvaceae) have a long history in folk medicine [1, 2]. The aerial part and roots were used by Avicenna for pneumonia and kidney ailments and as expectorants and antihemorrhagic agents [3]. The acidic polysaccharides and proteins of *A. officinalis* and *A. roseae* have been studied [4, 5].

The flora of Uzbekistan includes seven species of *Althaea* L. that are rich sources of water-soluble polysaccharides (WSPS) [6].

We investigated roots of *A. nudiflora* and *A. armeniaca* by grinding them, extracting the lipids, and successively extracting mono- and oligosaccharides (OS), WSPS, and pectinic substances (PS). Table 1 presents data for the content of the isolated substances.

The lipids were studied by TLC over silica gel in systems 1-3 and were identified by their chromatographic mobility, qualitative reactions, and comparison with authentic samples.

The qualitative compositions of the *A. nudiflora* and *A. armeniaca* lipids (L-1) were identical. They contained hydrocarbons and esters of sterols and triterpenes (17.8%); triacylglycerines (24.4%); free fatty acids (32.1%); triterpenols, diacylglycerines, and sterols (16.5%); and unidentified components (9.2%).

The majority of the lipids consists of triacylglycerines and fatty acids. The significant quantity of free fatty acids was confirmed by determining the acid number of the lipids, 77.2 mg KOH.

The fatty-acid composition of the lipids was determined by GLC.

Fatty acids	*A. nudiflora*	*A. armeniaca*
14:0	2.3	2.1
16:0	32.2	23.5
18:0	Tr.	1.8
18:1	33.1	46.5
18:2	26.6	23.6
18:3	4.8	2.5
Σ_{sat}	35.5	27.4
Σ_{unsat}	64.5	72.6

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TABLE 1. Carbohydrate and Lipid Content of *Althaea* Roots

Plant	Content, %				
	lipids	OS	PS	WSPS-1, 20°C	WSPS-2, 100°C
Althaea nudiflora	3.71	8.56	8.30	2.50	8.0
Althaea armeniaca L-1	4.05	6.10	6.20	4.50	7.30
Althaea armeniaca L-2	-	7.14	3.20	0.60	7.90

The lipids have the same qualitative composition of fatty acids. The total unsaturated acids in *A. armeniaca* (72.6%) and *A. nudiflora* (64.5%) are substantially different.

The quantitative content of fatty acids in *A. nudiflora* roots differs from that in the seeds [7], in which the 18:2 content exceeds 60% whereas that of 16:0 is less than half the content in roots.

It is known that many plants of the Malvaceae family contain cyclopropenoid fatty acids (CPA). A qualitative reaction for CPA [8] was positive. Under the GLC conditions used, CPA were not observed. Therefore, we determined their content by stepwise titration with HBr (0.1 N) in glacial acetic acid [9]. The CPA content in *A. armeniaca* lipids calculated based on malvic acid was 22.3%; in *A. nudiflora*, 12.6%.

Preparative paper chromatography (PC) was used to separate alcohol extracts of *A. nudiflora* and *A. armeniaca*. Glucose and maltose (system 4) were identified and quantified at 7.2:7.8 and 6.9:8.1% for the two plants, respectively. Photocolorimetric determination of sugars by the phenol—H_2SO_4 method confirmed their quantitative content.

Extraction of the raw material residue with water at room temperature and at 100°C gave the WSPS (Table 1). It can be seen that their content depends on the species and habitat.

The isolated WSPS are light-cream-colored powders. Upon dissolving in water, they form viscous yellow solutions that give with I_2 solution (0.1 N) a bluish-violet color that is characteristic of starchy polysaccharides [10].

The relative viscosities of aqueous WSPS solutions (1%) are different:

A. armeniaca		*A. nudiflora*	
$\eta_{rel.\ WSPS-1}$	1.13	$\eta_{rel.\ WSPS-1}$	1.23
$\eta_{rel.\ WSPS-2}$	3.85	$\eta_{rel.\ WSPS-2}$	4.41

Total acid hydrolysis of all WSPS samples forms glucose, which was identified by PC (system 4).

Thus, the isolated polysaccharides contain a main chain of the single monosaccharide glucose.

Acetylation with acetic anhydride in pyridine gave WSPS peracetates that were oxidized with chromic anhydride in glacial acetic acid by the literature method [11]. The reaction products contained only glucose according to PC (system 4). This indicates that the glycoside bond between glucopyranoses has the α-configuration.

The IR spectra of WSPS from *A. nudiflora* and *A. armeniaca* contain bands at 930±4 cm^{-1} and 758±2 cm^{-1}, which are characteristic for an α-1→4 glycoside bond, and a band at 853 cm^{-1}, which belongs to an equatorial CH group [10-14]. A strong broad band with principal maximum at 3395 cm^{-1} and significantly narrower bands at 2935, 1655, 1459, 1420, 1371 cm^{-1}, a strong band near 1000-1200 cm^{-1} with clearly resolved maxima at 1006, 1083, and 1260 cm^{-1}, and sharp bands at 754 and 766 cm^{-1} were also observed. These are analogous to the IR spectrum of starch.

Thus, the WSPS from *A. nudiflora* and *A. armeniaca* are polysaccharides consisting of α-1→4 glucopyranoses and are typical starches.

Extraction of the remaining raw material with $H_2C_2O_4$ and $(NH_4)_2C_2O_4$ solution (0.5%) gave PS (Table 1).

The PS from *A. nudiflora* and *A. armeniaca* roots are light brown powders that dissolve in water to give viscous solutions.

The IR spectra of the PS contain absorption bands (cm^{-1}) at 3600 (OH^-), 1740 ($COOCH_3$ stretches), 1023 and 1100 (pyranose ring vibrations), 840 (indicative of the α-configuration of the glycoside bond), and 890 (β-bond between sugars), characteristic of pectins [15].

The degree of esterification of PS was 75 and 71% for *A. armeniaca* and *A. nudiflora*, respectively. The free carboxylic groups were 1.63 and 0.59 (K_f) and 1.98 and 1.94% (K_e).

Total acid hydrolysis gave rhamnose, glucose, galactose, and galacturonic acid according to PC (system 4).

Thus, PS of the plants are low-molecular-weight and highly esterified pectins.

The WSPS are the main active components in preparations of the roots [16]. Therefore, we investigated the effect of grinding, temperature, and ratio between extractant and raw material (hydromodulus) on the yield of extracted substances (ES) and process dynamics in order to select the optimal extraction conditions.

Roots ground to various sizes were extracted under static conditions. The yield of WSPS increased by 5.7% upon extraction of raw material of particle size 1.0-2.5 cm. Extremely finely ground raw material gave a cloudy extract that was difficult to clarify and filter. Therefore, further experiments were performed with the 1.0-2.5 cm fraction. We present data for the effect of grinding on the yield of ES and WSPS.

Fraction size, cm	Yield, % of content in raw material	
	ES	WSPS
Unground	34.3	34.6
>2.5	51.5	52.5
1.0-2.5	82.9	82.5
<1.0	88.8	88.2
Extraction temperature, °C		
45	58.2	55.4
65	75.4	73.4
90	86.3	86.0
100	88.0	87.5
Hydromodulus		
1:5	61.3	58.0
1:7	79.7	79.2
1:10	81.3	82.6
1:15	85.8	85.0
1:20	91.3	89.3

The optimal extraction temperature was chosen using the range 45-100°C. Its influence on the yield of ES and WSPS was determined.

A high yield of WSPS was achieved at 90°C and above. Therefore, the optimal temperature was set at 90-100°C.

During the study of the relationship between the amount of extractant and raw material, the mass fraction of extractant was chosen so that the extraction of the desired product would be greatest without unjustified consumption of extractant or increased processing time and energy expenditure.

The extraction at 90-100°C with 1.0-2.5 cm raw material was performed with hydromodulus 1:5, 1:7, 1:10, 1:15, and 1:20 taking into account moisture absorption.

For a single extraction, increasing the hydromodulus increases the extraction of ES and WSPS, as expected. The maximum yield in the matrix we used was 91.3% (ES) and 89.3% (WSPS) at hydromodulus 1:20. However, considering that multiple extractions with a smaller amount of extractant may deplete more completely the raw material [17], we performed a triple extraction at ratios 1:5, 1:3, and 1:2 (total hydromodulus 1:10) and obtained yields of 88.9% (ES) and 87% (WSPS).

The dynamics of WSPS extraction was studied to determine the duration of the extraction and the attainment of phase equilibrium. The times needed for the most complete depletion of raw material using three phase contacts and different ratios of raw material and water and for the attainment of phase equilibrium were measured.

The extraction curves are typical isotherms approaching equilibrium. Equilibrium concentrations of ES and WSPS during the first phase contact are attained after 100 min. Phase equilibrium during the second contact is attained after 70 min; during the third, after 40 min (Fig. 1).

The results indicate that triple extraction of the roots ground to 1.0-2.5 cm with water at an overall hydromodulus 1:10 and 90-100°C extracts 88.9% of the ES and 87% of the WSPS.

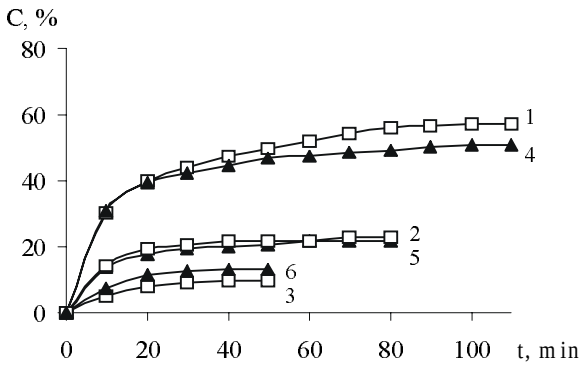

Fig. 1. Change of concentration of ES and WSPS with time (1-3 washes): ES (1-3), WSPS (4-6).

EXPERIMENTAL

IR spectra were obtained on a Perkin—Elmer 2000 Fourier spectrometer.

GLC of fatty acid methyl esters was performed on a Chrom-4 chromatograph with a flame-ionization detector and a column packed with Chromaton-N-RW-DMCS with 15% Reoplex-400.

Solvent systems were $(C_2H_5)_2O$—C_6H_{14} (3:7, 1; 4:6, 2; 2:8, 3).

Lipids were extracted with $CHCl_3$. Nonlipid components were removed from the $CHCl_3$ extract using aqueous $CaCl_2$ (0.04%). Lipids were saponified and fatty acids were isolated using the literature method [18].

PC was performed on Filtrak FN-11 paper using butanol—pyridine—water (6:4:3, system 4). Quantitative measurements were made on chromatograms using areas of the corresponding spots. Spots were visualized using acidic anilinium phthalate. Monosaccharides were identified using standards. The melting point of maltose was 107.8°C.

Roots of *A. nudiflora* L. (Tashkent), *A. armeniaca* Tep.-1 (Tashkent), and *A. armeniaca* Tep.-2 (Syrdar′ya region) were used. OS, WSPS, and PS were successively extracted from air-dried raw material (100 g) using the literature method [19].

Polysaccharides were hydrolyzed using H_2SO_4 (2 N); PS for 24 h; WSPS, 5 h. Neutralized and purified hydrolysates were analyzed by PC.

Acetylation and Chromic Oxidation of WSPS. Dry WSPS (0.4 g) were dissolved in formamide (5 mL) at 35°C, cooled, treated with pyridine (4 mL) and acetic anhydride (5 mL), and stirred for 48 h.

Dry peracetate (0.2 g) was oxidized by CrO_3 (0.6 g) as usual [11]. Glucose was detected in the peracetate oxidation products by PC (system 4).

The viscosity of aqueous solutions of PS of *A. nudiflora* and *A. armeniaca* was measured using an Ostwald viscometer at 20°C:

A. nudiflora		*A. armeniaca*	
$\eta_{rel.\ PS}$	3.27	$\eta_{rel.\ PS}$	2.98
MW_{PS}	27930	MW_{PS}	21970

Molecular weights were calculated from the dependence of molecular weight on viscosity using the literature formula [20].

The ES content was determined by refractometry; WSPS, by gravimetry after precipitation of the resulting extract using alcohol.

The extraction was performed in 20-L extractors heated under static conditions at 45-100°C at hydromodulus 1:5-1:20.

REFERENCES

1. A. A. Grossheim, *Flora of Kazakhstan* [in Russian], Acad. Sci. USSR, Moscow-Leningrad (1962), p. 6.
2. B. G. Bolynskii, K. N. Bender, and S. L. Freidman, *Medicinal Plants in Scientific and Folk Medicine* [in Russian],

Saratov (1967), p. 77.
3. Kh. Kh. Kholmatov and A. I. Kosimov, *Medicinal Plants* [in Russian], IBN SINA, Tashkent (1994).
4. M. Tomoda, K. Shimada, N. Shimizu, and T. Takasu, *Chem. Pharm. Bull.*, **29**, 2277 (1981).
5. M. Tomoda, K. Shimada, and N. Shimizu, *Chem. Pharm. Bull.*, **31**, 2677 (1983).
6. *Flora of Uzbekistan* [in Russian], Acad. Sci. Uzbek SSR, Tashkent (1959), Vol. 4, p. 166.
7. A. U. Umarov, Doctoral Dissertation in Chemical Sciences, Inst. Chem. Plant Sub., (1976).
8. D. Gol′de, *Zhir. Masl.*, **2**, 97 (1933).
9. F. C. Magne, J. A. Harris, R. A. Pittam, and E. L. Skau, *J. Am. Oil Chem. Soc.*, 519 (1966).
10. *State Pharmacopeia* [in Russian], Moscow (1968).
11. J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, **26**, 661 (1972).
12. S. A. Barker, E. J. Bourne, M. Stasey, and D. H. Whiffen, *J. Chem. Soc.*, 171 (1954).
13. S. A. Barker, E. J. Bourne, M. Stasey, and D. H. Whiffen, *J. Chem. Soc.*, 34 (1968).
14. W. J. Black, A. Sakko, and R. H. Marchessanuet, *J. Mol. Biol.*, **42**, 379 (1969).
15. M. P. Filipov, *Infrared Spectra of Pectinic Substances* [in Russian], Khimiya, Kishinev (1978).
16. M. D. Mashkovskii, *Medicinal Preparations* [in Russian], Meditsina, Moscow (1987), Vol. 1.
17. I. A. Murav′ev, *Medical Technology* [in Russian], Meditsina, Moscow (1980).
18. *Handbook of Study Methods, Mechanochemical Control, and Production Accounting in the Oil-Fat Industry* [in Russian], VNIIZh, Leningrad (1967).
19. M. A. Khodzhaeva, I. Talibaev, Kh. R. Mukhamedova, and M. T. Turakhozhaev, *Khim. Prir. Soedin.*, 362 (1995).
20. S. M. Kovalenko and O. D. Kurilenko, *Izv. Vyssh. Uchebn. Zaved., Pishch. Tekhnol.*, 175 (1972).