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CARBOHYDRATE AND PROTEIN COMPONENTS OF *Helianthus tuberosus* AND THEIR BIOLOGICAL ACTIVITY

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Carbohydrates from leaves, stems, and tubers of cultivated Helianthus tuberosus *were studied. The quantitative contents of mono- and oligosaccharides, water-soluble polysaccharides, pectinic substances, and hemicellulose were determined. The qualitative monosaccharide composition and physical chemical properties were found. The amino-acid composition was determined. It was shown that syrup from the tubers exhibited hypoglycemic activity.*

Keywords: *Helianthus tuberosus*, polysaccharide, pectin, hemicellulose, proteins, inulin syrup, fructose.

Jerusalem artichoke (*Helianthus tuberosus* L., Asteraceae) is a perennial herbaceous plant that is cultivated in many countries [1], including Uzbekistan.

Tubers of J. artichoke are used in modern medicine to reduce blood sugar. Therefore, it is prescribed for diabetes mellitus, weight reduction, and for gastritis, lethargy, reduced work capacity [2], and dysbacteriosis [3]. Tubers of J. artichoke have been used for a long time to produce a fructose syrup and typically have a high content of polyfructans, in particular, inulin, which facilitates elimination of heavy-metal salts and assimulation of Ca and Fe. There is evidence that L-inulin has immunomodulating properties [4–6].

In continuation of our research we studied carbohydrates from the new variety *H. tuberosus* (Faiz Baraka) that was elaborated at the Uzbekistan Scientific Institute of Plant Culture by individual and mass selection from voucher samples [7, 8]. The starting material was air-dried ground raw material (leaves, stems, and roots, separately) that was treated with a mixture of $CHCl_3$:MeOH (2:1) in order to remove lipophilic compounds. The remaining raw material was extracted successively with EtOH (82°) to isolate sugars soluble in alcohol (SSA); with H_2O at room temperature, water-soluble polysaccharides (WSPS); a mixture of oxalic acid and ammonium oxalate, pectinic substances (PS); and NaOH solution, hemicellulose (HMC).

Polysaccharides were precipitated from the extracts by alcohol. Table 1 presents the carbohydrate content in the various organs of cultivated *H. tuberosus*.

SSA from tubers consisted of fructose, glucose, and fructooligosaccharides (paper chromatography, PC). Samples of WSPS were amorphous powders that were soluble in H_2O and did not contain starch according to a negative reaction with iodine.

TABLE 1. Polysaccharide Content in Various Organs of Jerusalem Artichoke

Plant organ (air-dried raw matl., %)	SSA	WSPS	PS	HMC
Leaves	11.4	3.3	9.1	14.7
Stems	20.0	1.4	4.0	8.0
Tubers	30.0	7.7	3.0	2.7

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Table 1 shows that the polysaccharides were distributed unevenly in the various plant organs. In a quantitative sense, WSPS dominated in tubers (7.7%) and PS and HMC, in leaves (9.1 and 14.7%, respectively). Tubers contained 0.86% nitrogen per dry mass. The protein content as determined by the Kjeldahl method [9] was 5.45%. The amino-acid composition is presented below without tryptophan (Trp) determination.

Amino acid	*Content, wt%*	*Amino acid*	*Content, wt%*
Asp	1.57	Ile*	0.2
Thr*	0.7	Leu*	1.5
Ser	1.0	Tyr	0.8
Glu	3.6	Phe*	0.8
Pro	1.0	His*	0.8
Gly	1.1	Lys*	0.6
Ala	1.0	Arg*	1.4
Val*	0.7	∑	16.77.

According to the composition, J. artichoke contains essential amino acids* with the nonessential amino acids well balanced. This is responsible for its high nutritional value.

An expansion of the variety of pectins is often connected with the search for new raw material sources, as which J. artichoke is proposed. Cell walls of J. artichoke contain structural polysaccharides such as PS (4.0–9.1%) and HMC (2.7–14.7%).

Pectins were amorphous white and grayish-brown powders without an aroma. They were sour to the taste, soluble in H_2O, and practically insoluble in organic solvents. Pectin was isolated from the solutions as a colloidal precipitate upon adding alcohol, acetone, and polyvalent metals. The products of pectin acid hydrolysis contained mainly galacturonic acid and neutral sugars. The MW was determined by the literature method [10]. The calculations were made using the equation $[h] = K \cdot M \cdot 1.1 \cdot 10^{-5}$ MW/22. Table 2 presents the PS properties obtained using the titration method [11].

The PS had low esterification indices of 50.1% for leaves; 34.5, stems; and 36.3, tubers. This enabled them to be assigned as low-esterified pectins.

Hemicellulose was a light-brown amorphous powder. Its aqueous solutions did not give a reaction with starch. Galactose, glucose, xylose, arabinose, and rhamnose were detected in the hydrolysate.

The presence of a significant quantity of HMC enables stems and leaves of J. artichoke to be recommended for paper production. This facilitates grinding of the cellulose mass and improves its properties [12].

The chemical composition of *H. tuberosus* differs from that of potato by the lack in the composition of starch and solanine. This enables them to be used raw [13]. Because J. artichoke stores poorly, syrup available for use at any time of the year should be prepared from it.

Syrup is prepared from J. artichoke tubers by taking the juice. Then, the pressings are extracted with hot H_2O. The extract is mixed with the juice. Protein impurities are removed. The syrup characteristics are presented below:

Parameter	*Content, %*
Dry solid	At least 71%
pH	3.0–4.5
Density	1.350–1.370
Quantitative protein content	5.45
Quantitative inulin content	26.0

Biological tests of J. artichoke syrup found hypoglycemic activity for it. Thus, the hypoglycemic effect after just a single administration to rats at a dose of 0.4 mL/100 g was 21.2% after 3 h ($p < 0.01$). The syrup was more active than arfazetin and adebit, the effects of which were 11.0 and 14.2% ($p \geq 0.05$). The blood sugar level continued to remain at a reduced level over the while period of multiple administrations of J. artichoke syrup (two weeks). They dropped by 23.3 and 27.8% with respect to the initial value ($p < 0.05$) after 7 and 14 d. The reference drugs showed that blood glucose dropped by 14.1 and 16.5%, respectively, under the influence of arfazetin during these same periods; of adebit, by 18.8 and 19.9% ($p < 0.05$).

The effect of J. artichoke syrup was even more evident during disrupted carbohydrate exchange when the initial blood glucose level was rather high. Thus, the blood glucose after 15, 30, 45, and 60 min was greater than the initial level by 42.4, 63.4, 34.4, and 12.2%, respectively, for exogenous injection of glucose to control rats. For rats of the test group, which were administered J. artichoke syrup 2.5 h before the start of the experiment, blood glucose rose during these periods by only 27.6, 38.1, and 8.3%. The blood glucose level at the last observation point was even below the initial value by 3.5%. The difference from the control was statistically significant ($p < 0.05$).

TABLE 2. Physical Chemical Characteristics of Pectins from Various Organs of Cultivated *H. tuberosus* (Faiz Baraka)

Plant organ	Viscosity η_{rel}, *c* 0.5%, H_2O	MW	Titration data			
			K_f	K_e	K_t	l
Leaves	1.7	15.000	10.8	10.8	21.6	50.1
Stems	6.8	86.000	12.6	6.6	19.2	34.5
Tubers	1.36	9.000	6.3	3.6	9.9	36.3

η, relative viscosity; K_f, free carboxylic groups; K_e, esterified carboxylic groups; K_t, total carboxylic groups; l, degree of esterification.

TABLE 3. Effect of Jerusalem Artichoke Syrup on Glucose Level in Blood with Alloxan Hyperglycemia (M ± m, n = 6)

Experimental conditions	Blood glucose level, mg%						
	initial level	after 1 h		after 2 h		after 3 h	
		mg%	% w.r.t. initial	mg%	% w.r.t. initial	mg%	% w.r.t. initial
Control	210.6 ± 9.4	206.0 ± 8.2	–2.2	205.0 ± 8.4	–2.7	201.3 ± 8.0	–4.4
J. artichoke syrup	228.0 ± 9.6	183.3 ± 9.5	–19.6	153. 3 ± 11.4	–32.8	141.0 ± 11.5	–38.2
Arfazetin	224.0 ± 10.5	205.0 ± 8.8	–8.5	187.7 ± 10.1	–16.2	172.0 ± 8.6	–23.2
Adebit	227.0 ± 10.4	191.6 ± 4.0	–15.6	160.0 ± 5.2	–29.5	141.6 ± 4.8	–37.6

A pronounced hypoglycemic effect of J. artichoke syrup was also determined for alloxan hyperglycemia and alloxan diabetes. In the first instance, syrup was given starting 72 h after alloxan injection, i.e., at the peak of secondary hyperglycemia (rats with an initial blood glucose level in the range 200–250 mg% were used). Table 3 presents the results. In the second instance, J. artichoke syrup was administered to rats with developed alloxan diabetes (i.e., three weeks after alloxan administration). Animals with a stable high blood glucose level in the range 220–250 mg% (moderately serious diabetes) were also used for the test. Administration of J. artichoke syrup for a week lowered the blood glucose level by 31.7% with $p < 0.01$ (initial level 233.3 ± 16.0; after 7 d, 159.3 ± 12.5). The glycemia level in control animals did not change significantly. Arfazetin exhibited a hypoglycemic effect of 10.4% ($p < 0.25$); adebit, 25.4% ($p < 0.05$).

Thus, J. artichoke syrup at the studied dose exhibited a pronounced hypoglycemic effect in normal animals and those with hyperglycemia induced by glucose dosing or alloxan (and with alloxan diabetes). The effect was more pronounced than the corresponding action of arfazetin. The hypoglycemic properties of J. artichoke syrup in tests on normal animals were also manifested to a greater extent than adebit. Their effects were equivalent overall in tests on animals with hyperglycemia.

EXPERIMENTAL

Solutions were evaporated in vacuo at 45 ± 5°C. Descending chromatography was performed in FN-1 and FN-14 papers using BuOH:Py:H_2O (6:4:3). Sugars were detected by anilinium acid phthalate and urea solutions. Gas chromatography (GC) was carried out on a Chrom-5 instrument with a flame-ionization detector and stainless-steel column (200 × 0.01 mm), 5% silicone XE-60 on Chromaton NAW-0.200–0.255 mesh, 210°C, N_2 carrier gas, and 60 mL/min for aldononitrile acetates. Aldononitrile acetates were prepared as before [14]. IR spectra were recorded in KBr pellets on a Perkin–Elmer Model 2000 Fourier-IR spectrometer (5 mg compound per 200 mg KBr).

Amino-acid composition of protein occurring in the syrup was determined after acid hydrolysis (5 N HCl, 24 h, 110°C) on an amino-acid analyzer.

Monosaccharide Composition of Polysaccharides. WSPS, PS, and HMC (0.1 g each) were hydrolyzed by H_2SO_4 (2 N) at 100°C for 8–16 h. The hydrolysates were worked up with $BaCO_3$ until neutral, filtered, evaporated, and analyzed by PC and GC. Monosaccharides (Rha, Xyl, Ara, Glc, Man, Fru, Gal) were used as standards.

WSPS, PS, and HMC were isolated successively after extraction of sugars soluble in alcohol using water at room temperature, a mixture of oxalic acid and ammonium oxalate (1:1) at 70°C, and base (10% aqueous solution). Three extractions

were performed. The first used a 1:10 ratio; the second, 1:5; and the third, 1:3. Polysaccharides were precipitated by 96° EtOH (1:3). The isolated polysaccharides were rinsed with alcohol and acetone and dried in vacuo to afford the corresponding WSPS, PS, and HMC. Table 1 presents the yields.

Preparation of J. Artichoke Syrup. Tubers (1 kg) were thoroughly rinsed and cleaned and passed through a fine (up to 1–2 mm) grater. The pulp was moistened with ascorbic acid solution during the mechanical grinding in order to avoid enzymatic darkening. The juice (0.5 L) was separated by manual pressing. The cake was extracted with H_2O (2 L) containing chalk (20 g) at 90°C for 30 min. The aqueous extract was drawn off and combined with the initially pressed juice. Then, the combined solutions were heated at 85–87°C for 30 min to precipitate the protein, which was separated by centrifugation for 10 min at 5,000 rpm. The resulting solution containing 14.25% solids was evaporated until their content was 70–71%.

Isolation of Inulin. Air-dried tubers (20 g) that were ground to 1 mm were extracted with H_2O (1:10, 1:5) at 85°C for 30 min. The extracts were centrifuged, purified by adding alcohol (1:1), and centrifuged after 20 min. Inulin was precipitated from the extracts by alcohol (1:3). The precipitate was rinsed with alcohol (80° then 96°) and dried. Yield 26.0%. White powder, $[\alpha]_D^{20}$ –36.5° (*c* 1.0, H_2O). The compound was chromatographically pure and lacked reducing properties. IR spectrum: 830, 890, 935 cm^{-1}.

Inulin and inuloids were identified by the literature method [15].

Isolation of Mono- and Oligosaccharides. The alcoholic mother liquor after inulin precipitation was evaporated to dryness. Yield 13.4%. PC detected fructose, glucose, saccharose, and a series of D-fructofuranosides that were a mixture of short fructose and glucose chains.

Determination of Hypoglycemic Activity of J. Artichoke Syrup. Male rats (140–150 g), both normal and with experimental hyperglycemia induced by i.p. injection of glucose at a dose of 3,000 mg/kg or s.c. injection of alloxan at a dose of 150 mg/kg, were used. The blood glucose level was determined by the *ortho*-toluidine method [16]. J. artichoke syrup was administered perorally at a dose of 0.4 mL/100 g (established as the most effective in preliminary experiments). The known hypoglycemic drugs arfazetin (herb mixture) and peroral adebit (*N*-butylbiguanide) were used for reference [17]. The first was also administered orally at a dose of 0.4 mL/100 g body mass (as a freshly prepared 2.5% tincture); the second, at a dose of 50 mg/kg. Results were processed statistically using the Student *t*-criterion.

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