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LAMIACEAE CARBOHYDRATES. 1. PECTINIC SUBSTANCES AND HEMICELLULOSES FROM *Mentha x piperita*

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Pectinic substances from the aerial part of Mentha x piperita *were isolated and characterized and found to be a mixture of* β*-(1→4)-glucogalactan (MPG) and two* α*-(1→4)-rhamnopolygalacturonans (MPP′-1 and MPP′-2). It was shown that the pectin and its components exhibited membrane-stabilizing, antiatherogenic, and antioxidant activity. Hemicelluloses from* M. piperita *were a lignocarbohydrate complex.*

Key words: *Mentha x piperita*, Lamiaceae, pectinic substances, glucogalactan, rhamnopolygalacturonan, hemicelluloses, biological activity.

Mentha x piperita L. (Lamiaceae) is an official plant material that is widely used in medical practice. The main properties of pectinic substances (PS) from the variety Krasnodar 116 [1] and polysaccharides from tissue culture of domestic [2] and foreign [3, 4] varities of this species have been previously described.

Herein we report results from the isolation and study of the composition of PS and hemicellulose (HC) from the aerial part of *M. piperita* Krasnodar 2 variety.

We found 5.23-6.13% PS containing 97.02 ± 2.01% carbohydrates and <0.02% phenolic compounds with 0.85 ± 0.16% ash content, 0.52 ± 0.02% nitrogen, $[\alpha]_D^{20}$ +189° (*c* 0.1, water), and pH 5.5-5.8 (*c* 0.1, water) in the aerial part of *M. x piperita*.

The GalUA content was 54.5% with the ratio of neutral carbohydrates in the total complex PS being Glc:Gal:Ara:Rha: Xyl = 28:25:5:4:1 (Table 1). The quantitative characteristics were free carboxylic group content (K_C), 5.35 ± 0.11%; esterified carboxylic groups (K_E), 4.39 ± 0.14%; esterification degree (λ), 45-46%.

PS were separated using calcium pectinate in order to determine the component composition. This produced acidic and neutral components, MPP and MPG, respectively. Saponification by base of MPP gave demethoxylated pectinic acid MPP′, which was confirmed by analyzing the functional groups: K_C = 9.48 ± 0.28%; K_E = 0.34 ± 0.08%; λ = 3-4%.

Gel chromatography showed that MPP′ consisted of two components MPP′-1 and MPP′-2 in the ratio 1:2.3 with MW ~ $6.0 \cdot 10^4$ and $3.7 \cdot 10^4$ Da, respectively. The monosaccharide compositions of these were identical and differed only by the lack of Xyl in MPP′-2. Partial hydrolysis of MPP′-1 and MPP′-2 formed the same chromatographically homogeneous component MPP″, which contained 97.3% GalUA and 2.7% Rha. The molecular weight of MPP″ according to gel chromatography was $3.0 \cdot 10^4$ Da; the degree of polymerization, 185.

Enzymatic hydrolysis of MPP″ by pectinase produced GalUA. The positive specific rotation of MPP″ indicated that the glycoside bonds had the α-configuration. This was confirmed by the insignificant decrease of the specific rotation of the polyacetate (MPP″-Ac) compared with starting MPP″. The content of acetyls corresponded to substitution of two hydroxyls in anhydrogalacturonyl units. The IR spectrum of MPP″ contained the following absorption bands (cm^{-1}): 1725 (carboxylic C=O); 1610 and 1400 (ionized carboxylic); 1028, 1050, 1110 (pyran ring), and 832 (α-glycoside bond). The bands at 740 and 922 cm^{-1} shifted compared with pure GalUA (775 and 904 cm^{-1}). This is characteristic of polysaccharides with a 1→4 bond.

Methoxylated polygalacturonan was prepared and subjected to periodate oxidation in order to prove the type of bonding in MPP″. The product from oxidation of methoxylated polygalacturonan was levorotary and reduced a solution of silver in ammonia, i.e., acted like a polyaldehyde. The hydrolysate contained traces of GalUA, which eliminated a 1→3 bond from consideration. Oxidation by nitric acid of P_{al} produced oxalic and tartaric acids (1→4 bond). Oxidation by CrO_3 gave a hydrolysate in which GalUA and traces of Rha (α-bond) were detected.

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TABLE 1. Quantitative Monosaccharide Composition of PS and Its Components and Periodate Oxidation Results, mol %

Fraction	Ara	Gal	Glc	Rha	Xyl	GalUA	$NaIO_4$*	HCOOH isolation*
PS	3.8	17.8	19.7	3.1	0.7	54.5	-	-
MPP	1.7	14.8	10.2	1.7	0.6	71.0	-	-
MPP′	1.9	10.7	10.0	1.9	1.7	73.7	-	-
MPP′-1	2.2	13.7	11.9	1.4	8.8	53.4	0.92	0.324
MPP′-2	1.9	11.8	5.8	2.1	-	78.3	0.94	0.250
MPP″	-	-	-	2.7	-	97.3	1.02	0.016
MPG	9.8	48.8	34.2	6.5	0.8	-	0.97	0.305
MPG′	-	100	-	-	-	-	1.01	0.030

*mol/anhydrous unit.

Oxidation of MPP″ by $NaIO_4$ consumed 1.02 mol/anhydrous unit (1→4 hexapyranose). The amount of HCOOH was 0.016 mol/anhydrous unit, which indicated a linear or slightly branched structure for the macromolecule with the given degree of MPP″ polymerization. The position of Rha in the MPP″ structure was established by methylation with diazomethane and reduction with $NaBH_4$ followed by Hakomori methylation of the reduced product until the hydroxyl absorption band in the IR spectrum disappeared. TLC of the hydrolysate from the permethylate after formolysis detected 2,3,6-tri-*O*-Me-Gal; 2,3,4,6-tetra-*O*-Me-Gal; and 3,4,6-tri-*O*-Me-Rha by comparison with authentic samples. 2,3,6-Tri-*O*-Me-Gal was the main hydrolysis product of the permethylate and proved that the GalUA units were 1→4 bonded. The absence of dimethyl Gal derivatives indicated that the MPP″ structure was unbranched and that Rha was located in the main chain. Based on the results, it was concluded that MPP″ was α-(1→4)-polygalacturonan with a (1→2)-bonded Rha unit.

The results from periodate oxidation and molecular weight data for MPP′-1 and MPP′-2 suggested that MPP′-1 had one branching per three units of the main chain whereas MPP′-2 had four. In general the results indicated that the acidic components of *M. piperita* pectin were two branched polymers, the main chain of which was a rhamnopolygalacturonan.

The neutral component MPG was chromatographically homogeneous with molecular weight $3.25 \cdot 10^4$ Da. It did not contain GalUA. The principal monosaccharides were Gal and Glc in a 1.4:1 ratio. Periodate oxidation of MPG produced 0.305 mol HCOOH. This corresponded to two branching sites per seven units of the main chain.

Hydrolysis with cation exchanger gave MPG′, the only hydrolysis product of which was Gal. The molecular weight was $1.6 \cdot 10^4$ Da; degree of polymerization, 98. The absence of hexose in the hydrolysate after CrO_3 oxidation indicated that the Gal units were β-bonded. This was confirmed by an absorption band at 890 cm^{-1} in the IR spectrum. The MPG′ structure is linear or slightly branched according to the amount of HCOOH released by periodate oxidation, 0.03 mol per single anhydrous unit. The presence of erythritol in the hydrolysate from the reduced polyol after Smith degradation indicated that the Gal units were 1→4 bonded.

The main hydrolysis product of MPG′ permethylate was 2,3,6-tri-*O*-Me-Gal with 2,3,4,6-tetra-*O*-Me-Gal present in trace quantities (TLC). This confirmed the conclusion that the main chain of MPG′ was not branched and that this polymer was the galactan core of MPG.

The bonding of the second dominant monosaccharide Glc to the polygalactan chain was established by studying the composition of the products from partial hydrolysis of MPG. Ara, Rha, Xyl, Gal, Glc, maltose, maltotriose, and substance G were detected. Preparative isolation (paper chromatography, PC) and quantitative hydrolysis found that G consisted of Gal and Glc in a 1:1 ratio. Therefore, Glc was located in side chains. Thus, MPG was a branched β-(1→4)-bonded galactan containing Glc units in side chains.

Hemicellulose (HC) components were isolated from raw material using basic extraction after removal of PS. This produced two HC fractions, groups A and B (HC_A and HC_B, respectively), the contents of which were 6.98-7.90 (HC_A) and 3.02-3.94% (HC_B) of the raw material mass.

The principal properties were as follows. HC_A: carbohydrates, 17.13 ± 0.51%; ash content, 10.86 ± 0.27%; nitrogen, 24.02 ± 1.12%; $[\alpha]_D^{20}$ -36° (*c* 0.1, 0.5% KOH); Glc—Xyl—Rha—Ara, 52.3:35.4:7.3:4.9; HC_B: carbohydrates, 22.04 ± 0.88%; ash content, 16.44 ± 0.39%; nitrogen, 13.31 ± 0.89%; $[\alpha]_D^{20}$ -52° (*c* 0.1, 0.5% KOH); Xyl—Man—Glc—Gal—Ara, 54.3:25.5:14.9:2.8:2.4.

TABLE 2. Physical Chemical Properties of HC_A and HC_B

Component	C_{EtOH}, %	Yield, %	$[\alpha]_D^{20}$,° (c 0.1, 0.5% KOH)	Carbohydrate content, %	Ara	Gal	Glc	Man	Rha	Xyl	Content of MW components·10^4Da, % of mass fraction
				HC_A							
HCA-1	30.0	8.5	-41	32.45±4.47	1.4	-	50.5	-	2.1	45.9	5.9 (100)
HCA-2	72.0	17.5	-47	14.95±0.77	5.1	-	67.4	-	7.5	19.9	5.9 (8), 3.1 (92)
HCA-3	80.0	11.8	-38	10.75±0.57	7.5	-	50.3	-	9.5	32.6	3.1 (17), 1.9 (83)
HCA-4	-	6.2	-32	3.30±0.07	7.7	-	63.4	-	15.3	13.5	1.25 (100)
				HC_B							
HCB-1	33.0	22.0	-67	33.28±0.41	8.1	-	14.4	44.1	-	33.4	7.0 (100)
HCB-2	40.0	38.1	-58	36.89±0.82	-	4.7	15.2	14.8	-	65.3	4.6 9100)

TABLE 3. Membrane-stabilizing and Antiatherogenic Activity of PS Components

Component	IC_{50}, mg/mL		Osmotic resistance of erythrocyte membrane		Binding of atherogenic lipids, %
	Peroxide hemolysis	Osmotic hemolysis	C_{NaCl}, %	% of control	
PS	0.0546	0.1691	0.32	+22	24.33±0.11
MPP	0.0368	0.1607	0.29	+29	26.80±0.21
MPP″	0.0290	0.1452	0.27	+34	50.22±0.41
MPG	0.0752	2.0724	0.39	+5	6.53±0.24
GalUA	0.0000	0.0000	0.41	0	5.31±0.09
Quercetin	-	-	0.33	+20	-
Control	-	-	0.41	-	-
Heparin	-	-	-	-	100

It is well known that HC and lignin must be present together [5]. It was found that the lignin contents were 42 and 39% for HC_A and HC_B, respectively. The carbohydrate contents in the isolated lignin samples were less than 0.5%.

Fractionation of HC_A using sequential precipitation by ethanol produced four components (HCA-1, HCA-2, HCA-3, and HCA-4) that were colored and insoluble in water (Table 2). Gel chromatography indicated that HC_A was heterogeneous. The molecular weights of the components fell in a broad range. The negative rotation of $[\alpha]_D^{20}$ and a strong absorption band in the IR spectrum at 889 cm^{-1} indicated that β-bonds were present.

Fractionation of HC_B using gradient percipitation by ethanol isolated two homogeneous (gel chromatography) components HCB-1 and HCB-2 (Table 2). The principal monosaccharides of both components were Glc, Man, and Xyl in 1:3:2 and 1:1:4 ratios. The negative specific rotation suggested that β-bonds were present.

The membrane-stabilizing, antiatherogenic, and antioxidant activities of the total PS complex, MPP, MPP″, MPG, and GalUA were determined experimentally.

PS, MPP, and MPG had positive effects on membrane strength in the peroxide hemolysis model (Table 3). The effect of PS on osmotic hemolysis was due to the presence of acidic fractions. The MPG activity was over 12 times less than that of MPP. The last two had a stabilizing effect in the determination of the osmotic resistance of erythrocyte membranes after incubation with polysaccharide solutions. Acidic components increased the osmotic limit to 0.27% NaCl, which is higher than the limiting physiological osmotic loading of erythrocytes of 0.32-0.34%. GalUA did not seem capable of stabilizing membranes up to 0.15 mg/mL. Higher concentrations had a hemolytic effect.

Judging from the antiatherogenic activity of the prepared compounds, PS and its acidic components are more active than MPG, reaching 24-50% of heparin activity (Table 3).

TABLE 4. Antioxidant Activity of PS Components

Component	A_{OX}, %, component concentration, μg/mL							
	Carotene-H_2O_2-DMSO				Fe^{2+}-ascorbate-SMO			
	300	150	30	3	400	200	100	20
PS	84.84	62.66	23.85	0.00	39.41	23.53	17.65	0.00
MPP	46.21	36.78	9.24	0.00	21.76	19.41	8.82	0.00
MPP″	2.03	0.00	0.00	0.00	5.88	1.18	0.00	0.00
MPG	0.00	0.00	0.00	0.00	17.65	8.82	4.12	0.00
GalUA	14.97	13.86	13.31	0.00	8.82	2.94	0.00	0.00
Quercetin	100	100	71.35	30.68	100	100	100	54.71

Total PS complex had the highest antioxidant activity for β-carotene destruction in DMSO—H_2O_2. Its activity was 33% that of quercetin at a concentration of 30 μg/mL (Table 4). The situation was analogous for Fe^{2+}-ascorbate-induced oxidation of sorbitan mooleate. The decrease of A_{OX} for the components was probably due to a decrease in the overall degree of branching in the complex, which in turn affected that ability to trap free radicals.

EXPERIMENTAL

The aerial part of *M. x piperita* L. was obtained through a drugstore chain (Travy Bashkirii, batch No. 070604). The variety was Krasnodar 2.

Isolation of polysaccharides was carried out after removing lipophilic ($CHCl_3$:EtOH, 9:1) and alcohol- (80% ethanol) and water-soluble components using a mixture of oxalic-acid and ammonium-oxalate solutions (0.5%, 1:1) with subsequent concentration of the extracts and precipitation by ethanol (PS). HC were isolated at room temperature using extraction with KOH (5%). Fraction HC_A was obtained after neutralization of the extract with HCl (10%); HC_B, after concentration and precipitation by ethanol.

The contents of carbohydrate components were determined by the anthrone method [6]; of phenols, by the literature method [7] calculated as gallic acid; of protein, by the ninhydrin method after hydrolysis and calculated as alanine [8]; ash content, by gravimetry after ashing; acetyl content, by reaction with hydroxylamine [9]; optical rotation, on a Coers polarimeter with l = 10 cm at 20°C; and pH, using a Checker™1 pH-meter (Hanna Instruments). Spectrophotometric studies were performed on a Cecil CE 2011 spectrophotometer in 10-mm quartz cuvettes. IR spectra were recorded on a Vector 22 spectrometer in KBr disks (6 mg compound per 600 mg KBr).

PC was carried out on Filtrak FN-2 (carbohydrates) and FN-16 (organic acids) paper; TLC, on Silufol (Kavalier) plates; HPTLC, on Armsorb (Reakhrom) plates impregnated with phosphate buffer (0.07 M Na_2HPO_4 and 0.07 M KH_2PO_4, 96.7:3.3, pH 8.0).

Solvent systems were isopropanol:water (80:20, 1), *n*-propanol:$CHCl_3$:DMSO:phosphate buffer (51:29:11:9, 2), $CHCl_3$:CH_3OH (9:1, 3), $CH_3CO_2C_2H_5$:HCOOH:H_2O (3:1:1, 4), $CH_3CO_2C_2H_5$:CH_3CO_2H:HCOOH:H_2O (18:3:1:4, 5).

Developers were *p*-hydroxydiphenyl:phosphate (1), diphenylamine:phosphate (2), resorcinol:*p*-hydroxydiphenyl: aniline:phthalate:phosphate (3), bromphenol blue (4), $KMnO_4$:$NaIO_4$:benzidine (5).

Total hydrolysis of PS and MPP components was carried out as before [10] using compound (100 mg) dissolved in H_2SO_4 (10 mL, 80%) for 17 h at 4-5°C, treated with water (70 mL), heated on a boiling-water bath for 4 h, neutralized with $CaCO_3$, and centrifuged. The aqueous phase was concentrated to the minimal volume in vacuo at 40°C and analyzed by HPTLC (system 2, developer 3).

The quantitative monosaccharide composition was determined by HPTLC and densitometry using a Mustek 2000 planchette scanner and the TLC-Manager 3.1.1 scanning densitometer program (@PinSoft 2005). Glucose (Roquette), arabinose, galactose, mannose (Acros Organics) galacturonic acid (Fluka BioChemica), rhamnose (Dia M), and xylose (Reakhim) were used as standards.

Properties of PS were determined as before [11].

Gel Chromatography. The studied compound (10 mg) was dissolved in NaCl solution (1 mL, 0.3%) and transferred to a Sephadex G-100 column (Pharmacia, Uppsala, 1.5 × 60 cm) with elution by NaCl solution (0.3%) at 0.2 mL/min flow rate and 0.5 mL eluate volumes. The column was calibrated beforehand using Dextran 100000, 70000, 50000, 20000, and 10000 (BioChemica for GPC, Fluka). The internal volume of the column was determined using "blue" dextran (2000000 Da, Pharmacia, Uppsala). Polysaccharide yields were determined using anthrone sulfate at 625 nm.

Separation of PS Using the Ca Salt. PS (10 g) were dissolved in water (400 mL). The pH was adjusted to 7.5-7.8 using NH_3 solution (20%). $CaCl_2$ solution (50 mL, 2.5%) was added. The resulting precipitate was centrifuged, washed with water, treated with $(COOH)_2$ solution (400 mL, 2%), and heated at 80°C for 30 min. The precipitate of calcium oxalate was filtered off. The solution was concentrated and precipitated by ethanol (MPP). Yield 3.91 g (39%), $[\alpha]_D^{20}$ +228° (*c* 1.00, 0.1 M NaOH), ash content <0.2%. The solution was concentrated after precipitation of the Ca salt and precipitated by ethanol (MPG). Yield 1.31 g (13%).

Saponification of MPP. MPP (2 g) was dissolved in water (150 mL), treated with NaOH solution (4 mL, 8 M), stirred continuously at 18-20°C for 30 min, and treated with conc. HCl (4 mL). The resulting precipitate (MPP′) was centrifuged for 10 min. Yield 1.84 g (92%), $[\alpha]_D^{20}$ +232° (*c* 1.00, 0.1 M NaOH).

Isolation of MPP″. MPP′ (1.5 g) was dissolved in water (100 mL), treated with H_2SO_4 (70 mL, 3 M), and heated at 100°C for 4 h. The resulting precipitate (MPP″) was centrifuged and washed with H_2SO_4 (1%) and then acetone until the rinsings were neutral. Yield 1.02 g (68%), $[\alpha]_D^{20}$ +239° (*c* 1.00, 0.1 M NaOH).

Enzymatic hydrolysis of MPP″ was carried out using α-pectinase (5000 PSU/g, Fluka) by the literature method [12]. Hydrolysis products were analyzed by PC (system 5, developer 1).

Isolation of GalUA after enzymatic hydrolysis of MPP″ was performed as before [12], mp 153°C (dec.), $[\alpha]_D^{20}$ +55.4° (*c* 1.00, H_2O) {lit. [12] mp 155-159°C (dec.), $[\alpha]_D^{20}$ +55.4-55.6° (*c* 1.00, H_2O)}. Oxidation by conc. HNO_3 [13] produced a compound with mp 222°C, mucic acid.

Acetylation of MPP″. MPP″ (100 mg) was dissolved in pyridine (10 mL), treated with acetic anhydride (30 mL), left at 40°C for 48 h, and treated with HCl (50 mL, 3%). The resulting precipitate was centrifuged, washed with ethanol, and dissolved in acetone (10 mL). The insoluble part was discarded. The solution was poured into dry diethylether (50 mL). The acetate precipitate was washed with ethanol and dried, $[\alpha]_D^{20}$ +245° (*c* 1.0, acetone), K_E 31.56%.

Methoxylation, periodate—nitric-acid oxidation of MPP″, and hydrolysis of MPP″ acetate were carried out as before [14, 15] to produce samples of methoxylated polygalacturonan {$[\alpha]_D^{20}$ +202° (*c* 0.5, H_2O), K_M 7.15%} and polyaldehyde {$[\alpha]_D^{20}$ -91° (*c* 3.0, H_2O)}.

Products of polyaldehyde oxidation were analyzed by PC (system 4, developer 4). Tartaric acid was isolated by preparative PC [16].

Tartaric acid, mp 170.8-171.0°C, anilide mp 181.0-182.5°C, $[\alpha]_D^{20}$ +12° (*c* 1.5, H_2O).

Periodate oxidation and Smith degradation were carried out as before [17]. Periodate consumption was calculated from the absorption decrease at 223 nm [18]; quantitative determination of HCOOH, by titration with NaOH solution (0.01 M). The hydrolysate after $NaBH_4$ reduction was analyzed by HPTLC (system 2, developer 5). Oxidation by CrO_3 was performed by the literature method [19].

Methylation and Reduction of MPP″. A suspension of MPP″ (0.7 g) in methanol (10 mL) was esterified by diazomethane at +2°C for 24 h. The esterified product was dissolved in water (50 mL) and reduced with $NaBH_4$ (1 g). After 10 h the solution was passed over KU-2-8 cation exchanger (H^+-form). The effluent was concentrated in vacuo in the presence of methanol. The esterification and reduction were carried out 10 times to afford reduced MPP″ (0.2 g) containing GalUA (10.4%). Reduced MPP″ and MPG′ were methylated by the Hakomori method [20] and demethylated by the Ciucanu-Kerek method [21]. Reduced MPP″ (0.2 g) afforded the permethylate (52 mg).

Formolysis and Hydrolysis. The permethylate (50 mg) was heated at 80°C in formic acid (5 mL, 90%) for 1 h. Formic acid was removed in vacuo in the presence of methanol. The solid was treated with H_2SO_4 (3 mL, 1 M) and heated at 100°C for 24 h. The hydrolysate was investigated by TLC (system 3, developer 2).

Hydrolysis of MPG by Cation Exchanger. MPG (1 g) was dissolved in water (50 mL) and treated with KU-2-8 (H^+-form) to the level of the solution. The mixture was heated on a boiling-water bath for 2 h, cooled, and filtered. The cation exchanger was washed. The solution was concentrated in vacuo and precipitated by acetone. The resulting precipitate was dried with acetone (MPG′, 450 mg).

Partial Hydrolysis of MPG. MPG (100 mg) was dissolved in H_2SO_4 (15 mL, 0.25 M), heated at 100°C for 5 min, and neutralized with $CaCO_3$ and KU-2-8 cation exchanger. The solution was concentrated in vacuo and analyzed by PC (system 1, developer 1).

Preparative PC was performed on Filtrak FN-8 paper using system 1.

Fractionation of HC by Ethanol. Fraction HC_A (or HC_B) (1 g) was dissolved in KOH solution (100 mL, 5%) and gradually treated with ethanol (5 mL each, 95%) until the concentration was 80%. The resulting precipitates were centrifuged and dried by changing solvent. The solution was acidified by H_2SO_4 (5%). The resulting precipitate was freed of mineral salts by reprecipitation from water and treatment with KU-2-8 (H^+-form).

The lignin content in HC was determined by the Komarov method [22].

Peroxide Hemolysis. Suspensions (1 mL) of rinsed ram erythrocytes in physiological solution (1:150) were treated with a solution (1 mL) of the compound (0.001-10 mg/mL), H_2O_2 solution (1 mL, 0.8%), $FeSO_4$ solution (1 mL, $7.4 \cdot 10^{-3}$%); stirred; and left in the dark at room temperature for 24 h. All solutions were prepared using NaCl (0.9%). Optical density of the studied solution was determined at 420 nm. Water was used as the reference solution. Optical density of control solutions was determined in parallel. These were 100% hemolysis, the same composition without adding the component; 0% hemolysis, erythrocyte suspensions (1 mL); and NaCl (3 mL, 0.9%).

Osmotic Hemolysis. Suspensions (2 mL) of rinsed ram erythrocytes in physiological solution (1:200) were treated with an aqueous solution (1 mL) of compound (0.001-10 mg/mL) and water (1 mL), stirred, and left in the dark at room temperature for 24 h. Optical density of the studied solution was determined at 420 nm. Water was used as the reference solution. Optical density of controls was determined in parallel. These were 100% hemolysis, erythrocyte suspensions (2 mL); purified water (2 mL); 0% hemolysis, erythrocyte suspensions (2 mL); and NaCl (2 mL, 0.9%).

Membrane-stabilizing activity was estimated from the IC_{50} (mg/mL), which is the concentration causing 50% inhibition of hemolysis. The expressed activity was estimated using caffeic acid (MRTU 6-09-5985-69, Olainsk Chemical Reagent Plant).

Antiatherogenic Activity. Blood serum (0.2 mL) from a healthy donor was placed in a 10-mL tube, treated with $CaCl_2$ solution (2 mL, 0.025 M), and stirred. Optical density was determined at 410 nm. The solution was treated with the studied compound (40 μL, 1%) and stirred. Optical density was determined under the same conditions. Heparin (Heparin-Ferein, Ferein, ZAO Bryntsalov-A, 5000 units/mL) was used as the reference compound.

Osmotic Resistance of Erythrocyte Membranes. A suspension (5 mL) of rinsed erythrocytes from a healthy donor in NaCl solution (0.9%, 1:100) was treated with a solution of compound (0.5 mL; polysaccharides, 5-10 mg/mL water; flavonoids, 0.2-0.6 mg/mL in DMSO) and incubated at 37°C for 2 h. Erythrocytes were rinsed with NaCl (0.9%) until the rinsings were negative for carbohydrates (anthrone sulfate reagent) and flavonoids (Folin reagent). The precipitate of erythrocytes were placed in a flask with NaCl solution (10 mL, 0.9%) for titration and quickly titrated with water until completely hemolyzed (formation of blood lac). The NaCl concentration in the solution causing hemolysis was determined from the volume of added water. The control was a sample with added NaCl (0.5 mL, 0.9%).

Antioxidant Activity. A solution (10 mL) of β-carotene (water/DMSO, c = 0.012 mg/mL) was placed in a 20-mL tube; treated with H_2O_2 solution (5 mL, 0.15%), DMSO (1 mL), a solution of the compound (0.1-1.0), and water to give 20 mL; stirred; and stoppered. The mixture was thermostatted at 50°C for 2 h. Every 20 min starting at time = 0, samples (2 mL) were taken. Optical density was determined at 470 nm. The control sample did not contain the compound. Water was used as the reference solution. The antioxidant activity (%) was calculated using the formula

$$A_{OX} = [1 - (D_K^0 - D_K^{120})/(D_S^0 - D_S^{120})],$$

where D_K^0, D_K^{120}, D_S^0, and D_S^{120} are optical densities of the control and studied solutions after 0 and 120 min of incubation.

Fe^{2+}-ascorbate-induced oxidation of sorbitan mooleate (SMO) was carried out as before [23].

Quercetin (Acros organics) was used as the reference compound for determining the membrane-stabilizing and antioxidant activity.

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