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PHENYLPROPANOIDS AND POLYSACCHARIDES FROM *Plantago depressa* AND *P. media* GROWING IN BURYATIA

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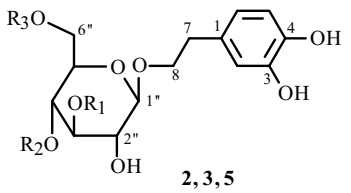
The composition of phenylpropanoids and polysaccharides from leaves of Plantago depressa *and* P. media *(Plantaginaceae) growing in Buryatia was studied. Caffeic and chlorogenic acids and acteoside were found in the studied plants; isoacteoside and plantamajoside, in* P. media*. The dominant compound in* P. depressa *was acteoside (8.72 mg/g); in* P. media*, plantamajoside (7.69 mg/g). These were responsible for the pronounced antiradical activity. Polysaccharide components that were extracted by water and an oxalate mixture were highly esterified pectinic substances with anti-atherogenic activity.*

Keywords: *Plantago depressa*, *P. media*, Plantaginaceae, phenylpropanoids, water-soluble polysaccharides, pectinic substances, biological activity, DPPH.

Six species of *Plantago* are indigenous to the flora of Buryatia. Of these, *P. depressa* and *P. media* are widely distributed over the whole territory of the Republic and are used in traditional medicine under the common name *tha ram* for diseases of the gastrointestinal tract (diarrhea, enteritis, colitis) and for tuberculosis and pneumonia [1, 2]. Studies of the chemical composition of *P. depressa* and *P. media* showed that iridoids [3, 4] and phenylpropanoids [5, 6] were present. Information on the polysaccharide components of *P. depressa* and *P. media* has not been reported. The pharmacologically active components of *Plantago* extracts are phenylpropanoids, which are responsible for the antioxidant activity [7, 8], and polysaccharides, which exhibit immunostimulating activity [9]. The goal of our work was to study the chemical composition of phenylpropanoids, water-soluble polysaccharides, and pectinic substances from leaves of *P. depressa* and *P. media* growing in Buryatia.

Preliminary experiments established that the total alcohol extracts of *P. depressa* and *P. media* exhibited high antiradical activity with IC_{50} values (DPPH method) of 34.72 and 28.85 μg/mL, respectively (Table 1). Fractionation of the extracts of both species produced series of fractions, among which the EtOAc extracts with the maximum amount of phenylpropanoids showed the highest activities. Chromatographic separation of these fractions with monitoring by HPTLC and the HPTLC/DPPH method (HPTLC autography) succeeded in isolating the most active compounds and afforded five compounds. These were **1–4** from *P. depressa* and **1**, **2**, **4**, and **5** from *P. media*. The compounds were identified using physicochemical data as the phenylpropanoids caffeic acid (**1**), acteoside (**2**), isoacteoside (**3**), chlorogenic acid (**4**), and plantamajoside (**5**). Leaves of *P. depressa* were found previously to contain **2**, β-hydroxyacteoside, cistanoside F, campenoside I, and orobanchoside [5]. Compounds **1**, **3**, and **4** were observed in this species for the first time. The presence of only **2** was observed in *P. media* so that **1**, **4**, and **5** were found in it for the first time.



2, 3, 5

2: R_1 = Rha, R_2 = caffeyl, R_3 = H
3: R_1 = Rha, R_2 = H, R_3 = caffeyl
5: R_1 = Glc, R_2 = caffeyl, R_3 = H

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TABLE 1. Antiradical Activity of Fractions and Pure Compounds from *Plantago depressa* and *P. media*, IC_{50}^{DPPH}, μg/mL[a]

Fraction	*P. depressa*		*P. media*		Compound	*P. depressa*
	FPr, mg/g[a]	IC_{50}^{DPPH}, μg/mL[b]	FPr, mg/g[a]	IC_{50}^{DPPH}, μg/mL[b]		FPr, mg/g[a]
EtOH-extract	36.34 ± 0.97	34.72 ± 0.69	24.71 ± 0.53	28.85 ± 0.58	**1**	11.62 ± 0.24
Hexane	–	> 2000	–	> 2000	**2**	3.43 ± 0.10
$CHCl_3$	–	437.62 ± 9.91	–	529.03 ± 11.10	**4**	4.75 ± 0.15
EtOAc	158.64 ± 4.10	8.57 ± 0.26	182.03 ± 6.11	8.23 ± 0.20	**5**	3.62 ± 0.12
H_2O	22.73 ± 0.62	51.76 ± 0.69	11.84 ± 0.43	34.82 ± 1.05	GA[c]	4.42 ± 0.15

[a]Total phenylpropanoid content; [b]index for 50% binding of DPPH radicals; [c]GA (gallic acid) as a reference compound.

TABLE 2. Phenylpropanoid Content in **1**–**5** from *Plantago depressa* and *P. media* Leaves, mg/g of Air-dried Raw Material

Species	FPr, mg/g[a]	**1**	**2**	**3**	**4**	**5**
P. depressa	28.11 ± 0.84	5.94 ± 0.17	8.72 ± 0.26	0.75 ± 0.02	0.93 ± 0.03	Tr.
P. media	16.89 ± 0.51	4.42 ± 0.12	0.64 ± 0.02	–	1.22 ± 0.04	7.69 ± 0.23
P. major[b]	10.42 ± 0.31	0.83 ± 0.02	2.92 ± 0.08	–	0.42 ± 0.01	0.74 ± 0.02

[a]Total phenylpropanoid content; [b]commercial raw material sample; Tr.: traces.

The quantitative contents of **1**–**5** in *P. depressa* and *P. media* and in a commercial sample of *P. major* pharmacopoeial raw material were determined using HPLC (Table 2). The dominant compounds for *P. depressa* were **2** (8.72 mg/g) and **1** (5.94 mg/g); for *P. media*, **5** (7.69) and **1** (4.42); for *P. major*, **2** (2.92). The total contents of phenylpropanoids were determined by the Arnow method [7] and were 112.43 and 67.57 mg/g for *P. depressa* and *P. media*, respectively, which are greater than the analogous value for *P. major*.

Considering reports in the literature about the pronounced antioxidant properties of phenylpropanoids, we determined the antiradical activities of the isolated compounds and found that acteoside (**2**), plantamajoside (**5**), and chlorogenic acid (**4**) typically had the highest activities (Table 1). The results together with the HPTLC-autography mobilities indicated that these components had the greatest influence on the antiradical activity.

Raw material was extracted to remove alcohol-soluble components. Polysaccharides were isolated by extraction with water and an oxalate mixture. This produced fractions of water-soluble polysaccharides (WSPS) and pectinic substances (PS) in yields of 6.7% (WPSP) and 5.5% (PS) from *P. depressa*; 8.0 (WSPS) and 3.5 (PS), from *P. media*. The total WSPS contents in the studied samples of *P. depressa* and *P. media* were according to the anthrone method 6.98 ± 0.20% and 8.35 ± 0.25, respectively; PS, 6.01 ± 0.18 and 3.87 ± 0.11, respectively.

The studies showed that the carbohydrate fraction in the polysaccharides was 95–97%; ash, protein, and phenols, <0.5%. The high indices of specific rotation ($[\alpha]_D^{20}$) and galacturonic acid contents (41.9–61%) indicated that the isolated polysaccharide fractions were PS. The P_d-WSPS and P_m-WSPS contained 19.2 and 24.4 mol% glucose, respectively (Table 3). The reaction of these fractions with iodine solution suggested that they contained glucan-type starch. Gel chromatography with detection by iodine solution established that the content of iodine-positive components was 17–28% of the fraction masses and that their molecular weights (MWs) were in the range 2.5–4 kDa. Therefore, membrane dialysis (5 kDa pores) was used for purification. This produced purified fractions P_d-WSPS′ (75% yield of P_d-WSPS mass) and P_m-WSPS′ (67% yield of P_m-WSPS mass).

A study of the monosaccharide composition of P_d-WSPS′, P_d-PS, P_m-WSPS′, and P_m-PS showed that the dominant neutral monosaccharides in all fractions were galactose, arabinose, xylose, mannose, and glucose in ratios 1.4:4.4:1.4:0.7:1, 10.3:7.7:4.9:2.7:1, 4.6:1.9:1.4:1.6:1, and 4.9:3.4:2.6:1.9:1, respectively. Rhamnose and fucose were detected in trace quantities (Table 3). Gel chromatography established that all polysaccharide fractions were heterogeneous and did not have a common characteristic MW distribution. They contained three groups of polymers with MWs >150 (I), 110-50 (II), and 20–8 kDa (III). The highest content of group I components was found in P_d-WSPS′ (27%) and P_m-WSPS′ (20%); for PS, group II characteristically dominated P_m-PS (82%) and P_d-PS (79%).

TABLE 3. Monosaccharide Composition of Polysaccharide Fractions from *Plantago depressa* and *P. media*, mol%

Fraction	Ara	Fuc	Gal	Glc	Man	Rha	Xyl	GalUA
P_d-WSP	16.2	0.5	5.4	19.2	2.6	0.7	5.1	50.2
P_d-WSP′	19.6	0.6	6.4	4.5	3.1	0.9	6.1	58.7
P_d-PS	11.5	Tr.	15.4	1.5	4.0	0.6	7.3	59.6
P_m-WSP	6.7	0.2	15.6	24.4	5.4	0.8	4.9	41.9
P_m-WSP′	8.6	0.4	19.7	4.3	6.9	1.1	6.2	52.7
P_m-PS	9.4	Tr.	13.6	2.8	5.4	0.4	7.3	61.0

Tr.: traces.

TABLE 4. Antiradical (IC_{50}^{DPPH}) and Anti-atherogenic Activity (K_B) of Polysaccharide Fractions from *Plantago depressa* and *P. media*

Fraction	IC_{50}^{DPPH}, μg/mL[a]	K_B, %[b]	Fraction	IC_{50}^{DPPH}, μg/mL[a]	K_B, %[b]
P_d-WSPS′	1.95 ± 0.05	39.87 ± 1.19	P_m-PS	2.58 ± 0.08	42.77 ± 1.28
P_d-PS	1.42 ± 0.04	39.15 ± 1.14	Quercetin[c]	0.011 ± 0.001	–
P_m-WSPS′	2.29 ± 0.07	35.20 ± 1.04	Heparin[c]	–	100

[a]Index for 50% binding of DPPH radicals; [b]percent binding of ALP relative to the control (heparin) at concentration 20 mg/mL; [c]reference compound.

A study of the functional group composition showed that the content of acetyl groups was 1.82% (P_m-WSPS′) to 4.73% (P_d-PS); free carboxylic acids, 1.14 (P_m-WSPS′) to 4.65 (P_d-WSPS′ and P_m-PS); methoxylated carboxylic acids, 10.35 (P_d-WSPS′) to 12.33 (P_mWSPS′); methoxyls, 7.13 (P_d-WSPS′) to 8.49 (P_m-WSPS′); degree of esterification, in the ranges 66.4-69.0 (P_d-WSPS′) up to 87.4-91.5% (P_m-WSPS′). Therefore, all polysaccharide fractions were highly esterified pectins.

A study of the biological activity of the polysaccharides from *P. depressa* and *P. media* showed that they typically had low antiradical activity (IC_{50} 1.42-2.58 mg/mL) and high anti-atherogenic activity (Table 4). The degree of binding blood-serum atherogenic lipoproteins was 39.15% (P_d-PS) to 42.77% (P_m-PS) of that of heparin.

EXPERIMENTAL

General Comments. Plant raw material (*P. depressa* and *P. media* leaves) were collected near Goryachinsk village (Pribaikal region, Buryatia Republic; 52°98′53″ N, 108°98′27″ E; June 25, 2008). The species were determined by Candidate of Biological Sciences D. V. Sandanov (IGEB, SB, RAS). Raw material was stored in the herbarium of the Department of Biologically Active Compounds, IGEB, SB, RAS (No. P1-16/4-14/0708 and No. P1-16/13-15/0708). The commercial sample of *P. major* raw material was acquired through a pharmacy network (Krasnogorskleksredstva).

Column chromatography was performed over silica gel L 100/400 (Chemapol) and Sephadex LH-20 (Pharmacia); gel chromatography, over Sephadex G-200 (Pharmacia). Silica-gel plates PTSKh-AF-V (160 μm, Imid Ltd.) were used for preparative TLC; Sorbfil PTSKh-AF-V (Imid Ltd.), for HPTLC. The solvent systems were BuOH:AcOH:H_2O (1, 4:1:1) and EtOAc:AcOH:H_2O (2, 10:2:3). Spectrophotometric studies were carried out on a UV-Vis-mini spectrophotometer (Shimadzu). Optical rotation was measured on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant). Potentiometric studies used a pH-410 pH-meter (Akvilon). IR spectra were recorded on KRS-5 plates in the range 4000–650 cm^{-1} on a Spectrum 100 IR-Fourier spectrometer (Perkin–Elmer). GC/MS was performed on a 5973N GC/MS (Agilent Technologies) with a 6890N mass-selective detector (Agilent Technologies) with a diffusion pump using a PH-Innowax capillary column (30 m/250 μm/0.50 μm). ^{13}C NMR spectra were recorded from DMSO-d_6 solutions (1%) on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz. HPLC was performed in a Summit liquid chromatograph (Dionex) using a Prodigy ODS 3 column (Phenomenex, 250 × 4.6 mm, 5 μm), mobile phase H_2O:MeOH:AcOH (14:6:1), flow rate 1 mL/min, 20°C, and a UVD 170S UV-detector at 330 nm. Dialysis was carried out in dialysis tubes with exclusion limit 1 and 5 kDa (Sigma). Quantitative analysis of gel chromatograms was made using the program Leonardo 1.01 (Nauka Plyus). We used 1,1-diphenyl-2-picrylhydrazyl (DPPH, MP Biomedicals Inc.); gallic and galacturonic acids; quercetin, acteoside (Sigma), heparin (5000 U/mL, Ferein). The total content of phenylpropanoids in the raw material was determined by the Arnow method calculated as acteoside [7]; polysaccharides, by the anthrone method [10] calculated as galacturonic acid.

Extraction and Isolation of Phenylpropanoids. Ground raw material of *P. depressa* (250 g) was extracted by EtOH (70%, 1:20, 80°C, 5×). The alcohol extract was concentrated to an aqueous residue that was worked up with hexane, $CHCl_3$, and EtOAc. The EtOAc fraction (8.52 g) was separated by column chromatography over SiO_2 (2 × 40 cm) using a $CHCl_3$:EtOH gradient (100:0→10:90) with subsequent rechromatography of subfractions over Sephadex LH-20 (1.5 × 40 cm) using $CHCl_3$:EtOH (100:0→80:20) and EtOH:H_2O (95:0→70:30) and preparative TLC (solvent system 1). Fractions were monitored using HPTLC (solvent system 2, 5% KOH detection) and HPTLC-autography.

HPTLC-autography. The studied solutions (2 μL) were placed in bands (8 mm) on chromatographic plates. The chromatograms were developed vertically to a height of 60 mm in an unsaturated chamber using solvent system 2. The plates were dried in a stream of cold air. Detection used immersion in DPPH solution (0.2%) in $CHCl_3$:MeOH (1:1). Densitograms were obtained after 15 s using a Sorbfil scanner (Imid Ltd.) and processed using the program set Sorbfil TLC Videodensitometer 2.0 (Imid Ltd.).

Chromatographic separation of the EtOAc fraction of *P. depressa* isolated **1** (18 mg), **2** (76 mg), **3** (4 mg), **4** (8 mg); of the EtOAc fraction (6.40 g) of *P. media* (195 g raw material), **1** (11 mg), **2** (14 mg), **4** (10 mg), and **5** (37 mg).

Caffeic acid (1), mp 220°C. UV spectrum (MeOH, λ_{max}, nm): 248, 300, 329. ^{13}C NMR spectrum (δ, ppm, 125.7 MHz, DMSO-d_6): 125.5 (C-1), 115.0 (C-2), 145.3 (C-3), 148.4 (C-4), 115.9 (C-5), 121.2 (C-6), 144.2 (C-7), 114.8 (C-8), 168.9 (C-9) [11].

Acteoside (2), mp 146°C, $[\alpha]_D^{20}$ –70.8° (*c* 0.65, MeOH). UV spectrum (MeOH, λ_{max}, nm): 247, 290, 331. MS (*m*/*z*): 625 $[M + H]^+$. ^{13}C NMR spectrum (δ, ppm, 125.7 MHz, DMSO-d_6): 3,4-dihydroxyphenethyl: 131.5 (C-1), 116.5 (C-2), 144.6 (C-3), 146.1 (C-4), 117.1 (C-5), 121.2 (C-6), 72.5 (C-7), 36.4 (C-8); caffeyl: 127.7 (C-1′), 115.0 (C-2′), 146.7 (C-3′), 149.7 (C-4′), 116.6 (C-5′), 123.2 (C-6′), 148.1 (C-7′), 115.2 (C-8′), 168.3 (C-9′); glucose: 104.2 (C-1″), 76.0 (C-2″), 81.7 (C-3″), 70.4 (C-4″), 76.0 (C-5″), 62.4 (C-6″); rhamnose: 103.0 (C-1‴), 70.4 (C-2‴), 75.5 (C-3‴), 71.2 (C-4‴), 70.7 (C-5‴), 18.9 (C-6‴). Acetate of **2**: amorphous. UV spectrum (MeOH, λ_{max}, nm): 218sh, 281 [12].

Isoacteoside (3), mp 137°C. UV spectrum (MeOH, λ_{max}, nm): 247, 290, 330. Mass spectrum (*m*/*z*): 625 $[M + H]^+$. ^{13}C NMR spectrum (δ, ppm, 125.7 MHz, DMSO-d_6): 3,4-dihydroxyphenethyl: 131.5 (C-1), 116.7 (C-2), 144.7 (C-3), 146.4 (C-4), 117.2 (C-5), 121.3 (C-6), 72.5 (C-7), 37.2 (C-8); caffeyl: 127.8 (C-1′), 115.2 (C-2′), 146.5 (C-3′), 149.7 (C-4′), 116.6 (C-5′), 123.2 (C-6′), 147.6 (C-7′), 115.1 (C-8′), 169.2 (C-9′); glucose: 104.6 (C-1″), 75.6 (C-2″), 84.0 (C-3″), 70.0 (C-4″), 75.4 (C-5″), 64.9 (C-6″); rhamnose: 102.7 (C-1‴), 70.4 (C-2‴), 75.9 (C-3‴), 73.1 (C-4‴), 70.7 (C-5‴), 18.3 (C-6‴) [13].

Chlorogenic acid (4), mp 201°C. UV spectrum (MeOH, λ_{max}, nm): 242, 300sh, 331. ^{13}C NMR spectrum (δ, ppm, 125.7 MHz, DMSO-d_6): caffeyl: 125.4 (C-1), 115.6 (C-2), 145.5 (C-3), 148.2 (C-4), 114.6 (C-5), 121.3 (C-6), 145.0 (C-7), 114.4 (C-8), 165.7 (C-9); quinyl: 73.2 (C-1′), 36.3 (C-2′), 71.8 (C-3′), 68.2 (C-4′), 70.4 (C-5′), 37.0 (C-6′), 175.4 (C-7′) [11].

Plantamajoside (5), mp 147°C, $[\alpha]_D^{20}$ –44.6° (*c* 0.91, MeOH). UV spectrum (MeOH, λ_{max}, nm): 232sh, 243sh, 290, 300sh, 331. Mass spectrum (*m*/*z*): 641 $[M + H]^+$. ^{13}C NMR spectrum (δ, ppm, 125.7 MHz, DMSO-d_6): 3,4-dihydroxyphenethyl: 131.5 (C-1), 116.6 (C-2), 144.7 (C-3), 146.5 (C-4), 117.3 (C-5), 121.2 (C-6), 72.8 (C-7), 36.4 (C-8); caffeyl: 127.7 (C-1′), 115.1 (C-2′), 146.7 (C-3′), 149.8 (C-4′), 116.6 (C-5′), 123.4 (C-6′), 147.7 (C-7′), 116.0 (C-8′), 168.6 (C-9′); glucose 1: 103.9 (C-1″), 76.0 (C-2″), 84.0 (C-3″), 70.9 (C-4″), 76.1 (C-5″), 62.4 (C-6″); glucose 2: 105.6 (C-1‴), 75.8 (C-2‴), 77.6 (C-3‴), 71.2 (C-4‴), 77.9 (C-5‴), 62.0 (C-6‴). Acetate of **5**: mp 95°C. UV spectrum (MeOH, λ_{max}, nm): 281 [12].

Extraction and Isolation of Polysaccharides. The raw material of *P. depressa* remaining after removal of alcohol-soluble components was extracted with water (1:25, 3×, extract 1) and then with an equal volume of a mixture of oxalic acid and ammonium oxalate solutions (0.5%, 1:20, 2×, extract 2) on a boiling-water bath. Extracts 1 and 2 were concentrated separately in vacuo at 40°C to 200–250 mL and dialyzed (1 kDa cut-off). The undialyzed residue was precipitated by HCl (1%) in EtOH (95%) (1:5). The resulting precipitates were centrifuged (3000 g, 20 min), washed with EtOH, and dried to afford fractions of P_d-WSPS (16.75 g) and P_d-PS (13.75 g).

Fractions P_m-WSPS (15.60 g) and P_m-PS (6.83 g) were isolated analogously from *P. media*.

The monosaccharide compositions were determined using HPTLC [14] and GC/MS (as methyl ethers) after hydrolysis in TFA (2 M, 100°C, 6 h) and removal of the acid in vacuo in the presence of MeOH.

Gel chromatography was performed over Sephadex G-200 (2 × 90 cm column) with NaCl (0.3%) eluent and detection by phenol:H_2SO_4 [15] and iodine solution (0.1 M).

Low-molecular-weight glucans were removed by dissolving P_d-WSPS and P_m-WSPS fractions (10 g each) in water (300 mL), transferring the resulting solutions to dialysis tubes with exclusion limit 5 kDa, and dialyzing against distilled water for 50 h. The undialyzed residues were precipitated by acetone. The precipitates were dried to afford fractions P_d-WSPS′ (8.12 g) and P_m-WSPS′ (7.84 g).

The carbohydrate contents were determined by the anthrone method [10]; galacturonic acid, by reaction with 3,5-dimethylphenol [16]; free and methoxylated carboxylic acids, by potentiometric titration [17]; acetyl groups, by the hydroxylamine method [18]. The degree of esterification was determined by potentiometric titration [17], IR spectroscopy [19].

P_d-WSPS′, $[\alpha]_D^{20}$ +109.2° (*c* 1.0, H_2O). Galacturonic acid content (K_{GalUA}), 58.7 mol%; acetyls (K_{Ac}), 2.15%; free carboxylic acids (K_{COOH}), 4.65; methoxylated carboxylic acids (K_M), 10.35; methoxyls (MeO), 7.13. The degree of esterification (DE) was determined by titration and IR spectroscopy as 69.0/66.4%. MW (content, %): >150 kDa, 27; 110–50 kDa, 63; 20–8 kDa, 9. IR spectrum (ν, cm^{-1}): 634, 751, 833, 892, 956, 1024, 1100, 1147, 1232, 1331, 1365, 1420, 1631, 1735, 2936, 3393.

P_d-PS, $[\alpha]_D^{20}$ +126.4° (*c* 1.0, H_2O). K_{GalUA}, 59.6 mol%; K_{Ac}, 4.73; K_{COOH}, 3.11; K_M, 12.14; MeO, 8.36; DE, 79.6/75.4%. MW (content, %): >150 kDa, 14; 110–50 kDa, 79; 20–8 kDa, 7. IR spectrum (ν, cm^{-1}): 634, 754, 832, 891, 956, 1024, 1077, 1100, 1147, 1236, 1331, 1372, 1418, 1616, 1740, 2935, 3412.

P_m-WSPS′, $[\alpha]_D^{20}$ +115.7° (*c* 1.0, H_2O). K_{GalUA}, 52.7 mol%; K_{Ac}, 1.82; K_{COOH}, 1.14; K_M, 12.33; MeO, 8.49; DE, 91.5/87.4%. MW (content, %): >150 kDa, 20; 110–50 kDa, 68; 20–8 kDa, 12. IR spectrum (ν, cm^{-1}): 602, 665, 832, 892, 957, 1021, 1050, 1103, 1143, 1231, 1331, 1370, 1440, 1623, 1737, 2935, 3408.

P_m-PS, $[\alpha]_D^{20}$ +137.8° (*c* 1.0, H_2O). K_{GalUA}, 61.0 mol%; K_{Ac}, 4.57; K_{COOH}, 4.65; K_M, 10.94; MeO, 7.54; DE, 70.2/69.2%. MW (content, %): >150 kDa, 7; 110–50 kDa, 82; 20–8 kDa, 11. IR spectrum (ν, cm^{-1}): 637, 758, 832, 850, 892, 921, 958, 1023, 1049, 1076, 1099, 1146, 1236, 1330, 1373, 1418, 1440, 1610, 1740, 2936, 3401.

Antiradical activity was determined by the DPPH method [20]; anti-atherogenic activity, by the literature method [21].

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