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POLYPRENOLS FROM *Althaea armeniaca* LEAVES

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The accumulation dynamics of polyprenols in leaves of 1-, 2-, and 3-year-old Althaea armeniaca *growing in Tashkent were studied according to vegetative phase. Optimal conditions for isolating the polyprenols were determined. It has been shown that the content of polyprenols was highest during fruiting in the second year of growth.*

Key words: polyprenols, *Althaea armeniaca*, accumulation dynamics, HPTLC.

Plants of the genus *Althaea* (Malvaceae) are especially interesting because of the wide use of preparations based on them in medicine [1]. Several species of *Althaea* are indigenous to Uzbekistan: *A. armeniaca*, *A. officinalis*, and *A. cannabina* L. [2].

A. armeniaca is a perennial plant that flowers in June-August and bears fruit in July-September. Roots of *Althaea* are included in the pharmacopoeiae of many countries and are used in medicine as an expectorant, coating, emollient, and anti-inflammatory agent for diseases of the respiratory tract, GI tract, and many other diseases [1].

Our goal was to study polyprenols (PP) of *A. armeniaca* according to vegetative phase because it is known that PP are biologically active and have low toxicity [3–5].

We have previously studied PP from leaves of 1-year-old *A. armeniaca* growing in Tashkent in ontogenesis [6] and found that the PP content increased as the plant developed. The maximum PP content occurred during fruiting and seed ripening and decreased during leaf shedding.

Considering that the maximum PP accumulation occurred during fruiting and in order to find possible changes in PP content during the first, second, and third year of growth, we continued research on PP during fruiting of 2- and 3-year-old *A. armeniaca*. Leaves were extracted with alcohol (3×) and then benzene. As it turned out, benzene extraction produced a small amount (<0.1%) of total extracted compounds. Therefore, we decided not to use it further as an extractant. The resulting extracts were separated over a column of silica gel (KSK, 160/250 mesh, 1:40 extract:adsorbent) with elution by hexane:$CHCl_3$ mixtures of gradually increasing polarity. This produced PP fractions with 10–12 isoprene units and PP fractions with other biologically active compounds.

Next extracted compounds underwent alkaline hydrolysis. The unsaponified fraction was isolated. The quantitative content of PP homologs in the unsaponified fraction was determined by high performance thin-layer chromatography (HPTLC). Table 1 lists the resulting comparative amounts of PP according to age.

Table 1 shows that the accumulation of PP fractions was highest during the second year of growth whereas their content decreased during the third year. The ratio of polymeric homologs also changed. Thus, the ratio of PP during the first, second, and third years was 1:2.48:2.43 (n = 10), 1.59:1.40:1 (n = 11), and 1:1.53:1.16 (n = 12). This showed that not only the total PP content but also the fractions of one homolog or another changed during ontogenesis.

The PP were identified chemically by comparing results of physicochemical analysis (IR and PMR spectra) with the literature [7, 8] and TLC (benzene:ethylacetate 24:1) with iodine vapor as the detector.

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TABLE 1. Accumulation Dynamics of Polyprenols as a Function of Age of *Althaea armeniaca*

Growth period, year	PP yield from air-dried mass, %	PP content, % of total unsaponified substances			
		n = 10	n = 11	n = 12	Total
1	2.35	1.32	64.29	13.61	79.22
2	2.66	3.28	56.57	20.83	80.68
3	2.16	3.21	40.46	15.77	59.44

TABLE 2. Yield of Extracted Compounds as a Function of Particle Size of *Althaea armeniaca* Leaves, %

Particle size, mm	1st extract	2nd extract	3rd extract	Total yield
0.5–1.0	3.4	3.19	2.5	9.1
2.0–3.0	3.9	3.70	3.2	10.8
4.0–5.0	3.6	2.97	2.2	8.7
6.0–7.0	3.4	2.91	0.8	7.1

TABLE 3. Yield of Polyprenols from Leaves of 2-Year-Old *Althaea armeniaca* as a Function of Extraction Time, %

Particle size, mm	Extract No.	Extraction time, h									
		1	2	3	4	5	6	7	8	9	10
2.0–3.0	1	0.04	0.06	0.07	0.082	0.093	0.100	0.103	0.106	0.106	0.106
	2	0.03	0.048	0.058	0.07	0.089	0.093	0.095	0.095	0.095	
	3	0.024	0.037	0.046	0.053	0.063	0.065	0.065	0.065		

Ground leaves of various particle size (0.5–1.0, 2.0–3.0, 4.0–5.0, and 6.0–7.0 mm) were extracted in order to determine the optimum conditions for extracting compounds from *A. armeniaca* leaves as a function of extraction time and particle size of raw material. The extraction was carried out three times with alcohol at raw material:solvent ratios 1:4, 1:3, and 1:3 and durations 10, 9, and 8 h, respectively. Yields were measured each hour. Table 2 shows yields of total extracted compounds as functions of particle size.

Table 2 shows that the maximum (10.8%) yield of total extracted compounds occurred for particle size 2.0–3.0 mm. The filters became clogged by very small particles (0.5–1.0 mm). This interfered with the extraction.

Next we studied the completeness of PP extraction from the plants as a function of extraction time using leaves of particle size 2.0–3.0 mm. Table 3 lists the results.

The maximum yield of PP was found for extraction by ethanol of leaves from 2-year-old plants by standing for 8 h the first time and 7 and 6 h the second and third times, respectively. Increasing the extraction time further did not increase the amount of extracted compounds. The extraction of PP was not complete for the first and second extractions so we carried out the extraction three times for completeness.

Thus, the yield of PP was greatest for ethanol extraction (3×) of leaves of particle size 2.0–3.0 mm from 2-year-old *A. armeniaca* and extraction times 8, 7, and 6 h, respectively.

Next we used a different method, emulsion extraction [9], which is based on treatment of ground plant material with aqueous base solutions with stirring and with or without added small quantities of organic solvents, in order to isolate PP fractions. This method has recently been widely used to extract PP from conifer trees and gave good results. We used aqueous NaOH solution (5%) and small quantities of ethanol or petroleum ether (10:3 ratio). Table 4 gives the yields of neutral compounds (NC).

TABLE 4. Yield of Neutral Compounds Obtained by Emulsion Extraction from *Althaea armeniaca* Leaves

Extraction type	Extraction conditions			NC yield	
	method	number of extractions	extraction time, h	g	%
5% aqueous NaOH + petr. ether	Stirring	2	4–4	0.0149	0.15
5% aqueous NaOH + petr. ether	Stirring	3	12–8–8	0.095	0.95
5% aqueous NaOH + alcohol + petr. ether	Stirring	3	12–5–4	0.096	0.96
5% aqueous NaOH + alcohol + petr. ether	Standing	3	12–8–8	0.087	0.87

TABLE 5. Polyprenol Content in Total Neutral Compounds Isolated by Emulsion Extraction

Extraction type	Quantative composition of polyprenols, %			
	n = 10	n = 11	n = 12	Total
Althaea armeniaca + 5% aqueous NaOH	8.4	27.86	20.84	57.1
Althaea armeniaca + 5% aqueous NaOH + petr. ether	8.9	31.02	20.80	60.72
Althaea armeniaca + 5% aqueous NaOH + alcohol + petr. ether	11.94	39.19	21.07	72.2
Standard	17.62	57.32	17.36	92.32

Table 4 shows that the yield of extracted compounds from the emulsion method was greatest if aqueous NaOH solution (5%) and extraction (3×) with stirring for 12, 8, and 8 h, respectively, in the presence of small amounts of petroleum ether were used. This method produced fractions with 57.1% PP (Table 5). The extraction was performed using aqueous NaOH solution (5%) in the presence of small amounts of petroleum ether (10:3 ratio) for comparison. In this instance, the PP content increased to 72.2%. Table 5 lists the quantitative content of the PP fractions.

The degree of extraction and ratio of PP homologs varied for the emulsion extraction method.

EXPERIMENTAL

The quantitative PP content was determined using HPTLC on a Camag instrument (Switzerland). Sorbfil HPTLC-AF-UV plates (10 × 10) were developed using $CHCl_3$. Compound (1.0 mg) was dissolved in solvent (1 mL) and sampled (5 μ L). The solvent system was toluene:EtOAc (19:1); distance from origin to solvent front, 70.0 mm; air drying, 20°C, 15 min; distance between tracks 7.7 mm; and wavelength 200 nm.

We used Silufol AL SIL G/UV plates (Germany) for TLC (20 × 20 cm), solvent system benzene:EtOAc (24:1), $KMnO_4$ in H_2SO_4 or iodine vapor detector. The solvent systems for column chromatography were $CHCl_3$ and $CHCl_3$:hexane of gradually increasing polarity.

Isolation of Total PP from *A. armeniaca* Leaves. Leaves from 1-, 2-, and 3-year-old *A. armeniaca* (10 g each) were ground to 0.5–1.0, 2.0–3.0, 4.0–5.0, and 6.0–7.0 mm; extracted with ethanol (96%, 3 × 100 mL) and benzene (1 × 100 mL). The extracts were combined and concentrated in vacuo. The resulting total extracted compounds were separated into fractions by column chromatography (18 × 98 cm column, KSK 160/250 mesh silica gel adsorbent, adsorbent:extract 40:1) with elution by hexane:$CHCl_3$ of gradually increasing polarity. A total of 120 fractions (30–40 mL) was collected. Fractions 23–25 contained PP. Yields of PP in mixed fractions were calculated by a semi-quantitative method [10]. Table 2 lists the yields of PP.

Isolation of Unsaponified Fraction. Total extracted compounds (10 g) were treated with aqueous KOH (39 mL, 50%), ethanol (96%, 300 mL), water (18 mL), and petroleum ether (100 mL) and stirred on a magnetic stirrer at 120 rpm for 3 h. The extraction was carried out three times. The petroleum-ether extracts were combined and washed with water until the pH was 7. Solvent was distilled in a rotary evaporator. The yield of unsaponified fraction was 4.83% of the air-dried mass.

Emulsion Extraction Method. We used the literature method [9] with some changes. Leaves (10 g) were ground to 2.0–3.0 mm, treated with aqueous NaOH (5%, 100 mL) and petroleum ether (30 mL), and stirred on a magnetic stirrer at 120 rpm for 4 h. The extraction was carried out twice. Then the extracts were combined, transferred to a separatory funnel, and washed with water until the pH was 7. Solvent was distilled in a rotary evaporator. For comparison, the same experiment was performed under different conditions by adding alcohol (70%), standing, and varying the time (4 to 12 h) and the number of extractions.

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REFERENCES

1. M. D. Mashkovskii, *Drugs* [in Russian], Vol. 1, Abu Ali Ibn Sino, Tashkent, 1998, p. 344.
2. *Plant Resources* [in Russian], **2**, Nauka, Leningrad, 1986.
3. N. K. Khidyrova and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 87 (2002).
4. T. Chojnacki and T. Vogtman, *Acta Biochim. Pol.*, **31**, 115 (1984).
5. V. G. Kasradze, E. V. Salimova, O. S. Kukovinets, F. Z. Galin, N. S. Makara, L. T. Karachurina, A. V. Kuchin, and A. A. Koroleva, *Khim. Prir. Soedin.*, 242 (2003).
6. N. K. Khidyrova, E. V. Van, R. Kh. Shahidoyatov, N. M. Mamatkulova, and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 387 (2007).
7. A. M. Rashkes, N. K. Khidyrova, Ya. V. Rashkes, U. Z. Mirkhodzhaev, U. K. Nadzhimov, and Kh. M. Shakhidoyatov, Rep. Uzb. Pat. No. 1543 (1993); *Byull.* No. 1 (1995).
8. A. M. Rashkes, U. Kh. Saitmuratova, N. K. Khidyrova, Kh. M. Shakhidoyatov, and V. B. Leont′ev, *Khim. Prir. Soedin.*, 65 (1998).
9. A. A. Koroleva, L. P. Karmanova, and A. V. Kuchin, *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.*, **48**, 3, 97 (2005).
10. A. A. Akhrem and A. I. Kuznetsov, *Thin-Layer Chromatography* [in Russian], Nauka, Moscow, 1966, pp. 52–53.