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FLAVONOIDS FROM *Pulicaria vulgaris* AND THEIR ANTIMICROBIAL ACTIVITY

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Pulicaria vulgaris (prostrata) is an annual plant with numerous branched reddish stems and small (6–12 mm) yellow flowerheads. It grows on moist and salty shores of rivers and lakes and valley meadows in almost all regions of Central Asia [1]. Previously, buddledin C was isolated from essential oil of this plant [2]. The goal of the present work was to study the constituent composition of the EtOH and $CHCl_3$ extracts of *P. vulgaris* and to determine the biological activity of the isolated compounds.

Raw material was collected in August 2019 in the vicinity of Stary Koluton village, Astrakhan District, Akmola Province. The dried aerial part (1 kg) was extracted (3×) with EtOH for 30 min at room temperature with ultrasound and then left overnight. The extract was filtered and concentrated under vacuum to produce a dry solid (76.6 g, 7.66%). The extract was separated by column chromatography over silica gel with elution by hexane–Me_2CO (gradient from 20:1 to 0:1) to produce 190 fractions and then by MeOH.

The six flavonoids quercetagetin 3,7,3′-trimethyl ether (**1**) [3]; quercetagetin 3,7,3′,4′-tetramethyl ether (**2**) [4]; quercetin 3,7,3′-trimethyl ether (**3**) [5]; sorbifolin (**4**) [6]; 6-hydroxyluteolin 7,3-dimethyl ether (**5**) [7]; and ladanein (**6**) [8] were isolated from various fractions.

5,6,4′-Trihydroxy-3,7,3′-trimethoxyflavone (1) (quercetagetin 3,7,3′-trimethyl ether), yellow powder (340 mg), mp 219–220°C. HR-ESI-MS, *m/z* 359.0743 $[M - H]^-$ (calcd for $C_{18}H_{15}O_8$, 359.0766). ^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 12.41 (1H, s, 5-OH), 7.69 (1H, s, H-2′), 7.66 (1H, d, J = 8.4, H-6′), 7.04 (1H, d, J = 8.4, H-5′), 6.54 (1H, s, H-8), 6.01 (1H, s, 4′-OH), 5.33 (1H, s, 6-OH), 4.01 (3H, s, 3′-OCH_3), 3.99 (3H, s, 7-OCH_3), 3.85 (3H, s, 3-OCH_3). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 156.31 (C-2), 138.77 (C-3), 178.84 (C-4), 145.36 (C-5), 129.27 (C-6), 152.96 (C-7), 90.26 (C-8), 149.84 (C-9), 106.45 (C-10), 122.72 (C-1′), 111.00 (C-2′), 146.44 (C-3′), 148.40 (C-4′), 114.67 (C-5′), 122,72 (C-6′), 60.28 (3-OCH_3), 56.22 (7-OCH_3), 56.59 (3′-OCH_3). COSY: H-5′→H-6′, H-6′→H-5′. HSQC: H-8→C-8, H-2′→C-2′, H-5′→C-5′, H-6′→C-6′, 3-OCH_3→3-OCH_3, 7-OCH_3→7-OCH_3, 3′-OCH_3→3′-OCH_3. HMBC: H-8→C-6, C-7, C-9, C-10; H-2′→C-4′, C-6′; H-5′→C-1′, C-3′; H-6′→C-2′, C-4′; 3-OCH_3→C-3; 7-OCH_3→C-7; 3′-OCH_3→C-3′; 5-OH→C-5, C-6, C-10; 6-OH→C-5, C-6, C-7; 4′-OH→C-4′, C-5′ [3].

5,6-Dihydroxy-3,7,3′,4′-tetramethoxyflavone (2) (quercetagetin 3,7,3′,4′-tetramethyl ether), brown crystals (9 mg), mp 204–205°C. HR-ESI-MS, *m/z* 373.0927 $[M - H]^-$ (calcd for $C_{19}H_{17}O_8$, 373.0923). ^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 12.41 (1H, s, 5-OH), 7.72 (1H, dd, J = 2.5, 8.6, H-6′), 7.67 (1H, d, J = 1.8, H-2′), 6.98 (1H, d, J = 8.6, H-5′), 6.55 (1H, s, H-8), 3.99 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 3.86 (3H, s, OCH_3). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 156.21 (C-2), 138.89 (C-3), 178.82 (C-4), 145.33 (C-5), 129.29 (C-6), 152.99 (C-7), 90.26 (C-8), 151.43 (C-9), 106.45 (C-10), 122.25 (C-1′), 110.91 (C-2′), 148.84 (C-3′), 149.82 (C-4′), 111.32 (C-5′), 123.1 (C-6′), 60.29 (3-OCH_3), 56.58 (OCH_3), 56.17 (OCH_3), 56.09 (OCH_3). NOE: H-8→OCH_3, H-5′→OCH_3, H-2′→OCH_3 [4].

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4′,5-Dihydroxy-3,7,3′-trimethoxyflavone (3) (quercetin 3,7,3′-trimethyl ether), yellow powder (7.5 mg, mp 199–201°C. HR-ESI-MS, *m/z* 345.0969 $[M + H]^+$, 343.0817 $[M - H]^-$ (calcd for $C_{18}H_{17}O_7$, 345.0974). ^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 12.59 (1H, s, 5-OH), 7.68 (1H, dd, J = 2.5, 8.6, H-6′), 7.55 (1H, d, J = 2.5, H-2′), 7.09 (1H, d, J = 8.6, H-5′), 6.95 (1H, s, H-8), 6.92 (1H, s, H-6), 3.89 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.82 (3H, s, OCH_3). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 156.79 (C-2), 138.91 (C-3), 178.83 (C-4), 162.06 (C-5), 97.94 (C-6), 165.51 (C-7), 92.26 (C-8), 156.06 (C-9), 106.09 (C-10), 122.51 (C-1′), 110.91 (C-2′), 146.41 (C-3′), 148.39 (C-4′), 114.66 (C-5′), 122.75 (C-6′), 60.29 (3-OCH_3), 55.92 (OCH_3), 56.18 (OCH_3), 60.27 (OCH_3). HSQC: H-6→C-6, H-8→C-8, H-2′→C-2′, H-5′→C-5′, H-6′ →C-6′, 3-OCH_3→3-OCH_3, 7-OCH_3→7-OCH_3, 3′-OCH_3→3′-OCH_3. HMBC: H-6→C-7, C-9, C-10; H-8→C-6, C-7, C-9, C-10; H-2′→C-6′, C-4′, C-2; H-5′→C-1′, C-3′; H-6′→C-2′, C-4′; 3-OCH_3→C-3; 7-OCH_3→C-7; 3′-OCH_3→C-3′; 5-OH→ C-5, C-6, C-10. NOE: H-6→OCH_3, H-2′→OCH_3 [5].

5,6,4′-Trihydroxy-7-methoxyflavone (4) (sorbifolin), crystalline yellow compound (8.1 mg), mp 286–289°C. HR-ESI-MS, *m/z* 299.0558 $[M - H]^-$, 323.0531 $[M + Na]^+$, (calcd for $C_{16}H_{11}O_6$, 299.0555). ^{1}H NMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.66 (1H, s, 5-OH), 10.36 (1H, s, 4′-OH), 8.72 (1H, s, 6-OH), 7.96 (2H, d, J = 8.6, H-2′, 6′), 6.94 (2H, d, J = 7.3, H-3′, 5′), 6.91 (1H, s, H-8), 6.82 (1H, s, H-3), 3.92 (3H, s, 7-OCH_3). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm): 164.27 (C-2), 103.01 (C-3), 182.72 (C-4), 146.72 (C-5), 130.45 (C-6), 154.86 (C-7), 91.66 (C-8), 150.13 (C-9), 105.55 (C-10), 121.88 (C-1′), 128.92 (C-2′, 6′), 116.46 (C-3′, 5′), 161.1 (C-4′), 56.81 (7-OCH_3). HSQC: H-3→C-3, H-8→C-8, H-2′→C-2′, H-3′→C-3′, H-5′→C-5′, H-6′→C-6′, 7-OCH_3→7-OCH_3. HMBC: H-3→C-10, C-2, H-8→ C-10, C-6, C-9, H-2′, 6′→C-2′, C-6′, C-4′, C-2, H-3′, 5′→C-1′), 7-OCH_3→C-7, 5-OH→C-5, C-10, C-6. COSY: H-2′, 6′)→H-3′, 5′. NOE: H-8→7-OCH_3 [6].

5,6,4′-Trihydroxy-7,3′-dimethoxyflavone (5) (6-hydroxyluteolin 7,3-dimethyl ether), yellow crystalline compound (14.9 mg), mp 254–256°C. HR-ESI-MS, *m/z*: 329.0658 $[M - H]^-$, 353.0637 $[M + Na]^+$, 353.0638 (calcd for $C_{17}H_{13}O_7$, 329.0661). ^{1}H NMR spectrum (500 MHz, acetone-d_6, δ, ppm, J/Hz): 12.71 (1H, s, 5-OH), 7.62 (1H, d, J = 1.8, H-2′), 7.60 (1H, dd, J = 1.8, 8.0, H-6′), 6.99 (1H, d, J = 8.0, H-5′), 6.84 (1H, s, H-8), 6.69 (1H, s, H-3), 3.97 (3H, s, OCH_3), 3.96 (3H, s, OCH_3). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm): 163.69 (C-2), 102.26 (C-3), 181.94 (C-4), 146.10 (C-5), 129.73 (C-6), 154.05 (C-7), 90.95 (C-8), 149.44 (C-9), 104.84 (C-10), 120.33 (C-1′), 109.85 (C-2′), 148.10 (C-3′), 149.44 (C-4′), 115.79 (C-5′), 120.33 (C-6′), 56.12 (7-OCH_3), 55.75 (4′-OCH_3). NOE: H-8→OCH_3, H-2′→OCH_3 [7].

The constituent composition of the $CHCl_3$ extract of *P. vulgaris* was also studied. The aerial part was extracted with refluxing $CHCl_3$ for 1 h at 65°C. The dried extract (105 g) was worked up with aqueous EtOH (1:2) to remove ballast compounds and produce an extract (35 g) that was separated by column chromatography over silica gel with elution by petroleum ether–EtOAc (20:1 to 0:1) to give 217 fractions.

5,6-Dihydroxy-7,4′-dimethoxyflavone (6) (ladanein, scutellarein 4′,7-dimethyl ether), greenish-yellow compound (20 mg), mp 218–220°C. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 12.59 (1H, s, 5-OH), 7.84 (2H, d, J = 8.7, H-2′, 6′), 7.01 (2H, d, J = 8.6, H-3′, 5′), 6.59 (2H, s, H-3, 8), 5.35 (1H, s, 6-OH), 4.00 (3H, s, 7-OCH_3), 3.89 (3H, s, 4′-OCH_3). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm): 164.26 (C-2), 104.08 (C-3), 182.68 (C-4), 145.75 (C-5), 129.59 (C-6), 152.78 (C-7), 90.49 (C-8), 150.71 (C-9), 105.97 (C-10), 123.79 (C-1′), 128.09 (C-2′, 6′), 114.58 (C-3′, 5′), 162.64 (C-4′), 55.64 (7-OCH_3), 56.55 (4′-OCH_3). HSQC: H-3→C-3, H-8→C-8, H-2′, 6′→C-2′, 6′, H-3′, 5′→C-3′, 5′, 7-OCH_3→7-OCH_3, 4′-OCH_3→4′-OCH_3. HMBC: H-3, 8→C-10, C-6, C-4, C-9, C-7, C-2, C-1′, H-3′, 5′→C-1′, C-3′, C-4′, C-5′, H-2′, 6′→C-2, C-4′, 7-OCH_3→C-7, 4′-OCH_3→C-4′, 6-OH→C-5, C-6, C-7, 5-OH→C-5, C-6, C-10. COSY: H-2′, 6′→H-3′, 5′) [8].

All isolated compounds were tested for antimicrobial activity against five strains (*S. aureus*, *B. cereus*, *S. enteritidis*, *E. coli*, and *C. albicans*). The strains for determining the antimicrobial activity were obtained from the Central Museum RGP Republican Collection of Microorganisms. Antimicrobial tests were conducted by culturing growth medium using a published protocol [9]. The results for antimicrobial activity (Table 1) showed that **4** and **5** were active against Gram-positive bacteria *B. cereus* at all tested concentrations.

In several instances, the percent inhibition showed negative values, meaning that the compound enhanced (did not inhibit) growth of the bacteria. This fact could not be denied; however, the negative values were most probably due to a combination of experimental changes and corrections for colored compounds in control samples without bacteria. This could occur during metabolism by the bacteria of several of the colored constituents [9].

Thus, six flavonoids (**1**–**6**), the structures of which were proven unambiguously using spectral data and the antimicrobial activities of which were determined, were isolated for the first time from the aerial part of *P. vulgaris*.

TABLE 1. Antimicrobial Activity of Compounds Isolated from *P. vulgaris*, % Inhibition as Compared to Solvent DMSO

Compound	*S. aureus*	*B. cereus*	*E. coli*	*S. enteritidis*	*C. albicans*
			100 μg mL^{-1}/50 μg mL^{-1}		
1	47.2	46.4	38.8	21.3	3.5
2	44.1	39.4	34	39	–6.3
3	–7.4	39.5	27.9	41.4	–4.05
4	15.8	105	23.6	46.3	–0.1
5	46.6	130.6	10.01	106.3	–10.2
Ampicillin (250 μg mL^{-1})	95	90	95	78	–
			50 μg mL^{-1}/25 μg mL^{-1}		
1	–6	39.7	6.2	–7	–2.05
2	38.7	27.8	13	18.6	–5.5
3	28.9	43.7	15	5.7	–3.6
4	21	99.3	–2.2	14	–7.5
5	12.6	90.8	5.2	–0.5	–11.8
Nystatin (62.5 μg mL^{-1})	–	–	–	–	98
			25 μg mL^{-1}/12.5 μg mL^{-1}		
1	–15.4	48.2	29.3	18.3	–1.8
2	–15	–13.2	18	2.9	–4.7
3	33	5.1	29.3	7.1	1.7
4	19.8	80.4	–23.7	–38.3	–7.5
5	–2.6	66.9	–26.1	–5	–9.4

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