Chemistry of Natural Compounds, Vol. 45, No. 5, 2009

ANTIBACTERIAL ACTIVITY OF THE CHEMICAL CONSTITUENTS FROM *Ranunculus laetus*

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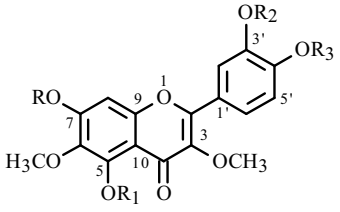
UDC 547.972

The genus *Ranunculus* belongs to the family Ranunculaceae, which comprises 50 genera and 2000 species, distributed throughout the northern hemisphere. It is also found in southern temperate regions in the tropics where they are usually confined to higher altitudes. In Pakistan, it is represented by 22 genera and about 114 species, of which several genera are of ornamental value while the others are toxic and used for medicinal purposes [1].

The plant *Ranunculus laetus* was collected from the Parachinar Kurram Agency, Pakistan, in April-May 2001, and was identified by Dr. Jahandar Shah (plant taxonomist) at the Department of Botany, University of Peshawar, Pakistan. The chloroform extract (86 g) was subjected to column chromatography using different solvent systems. The eluent obtained with *n*-hexane–$CHCl_3$ (8:2, v/v), was again subjected to silica gel column chromatography to obtain compounds **1**–**6**. These compounds were three known flavone jacein (**1**) [2], jacedin-5-*O*-*β*-D-glucoside (**2**) [3], and centaurein (**3**) [2], one known coumarin, 6,7-dimethoxycoumarin (**4**) [4], *β*-amyrin (**5**) [5], and *β*-sitosterol-3-*O*-*β*-D-glucoside (**6**) [6], which have been isolated from *Ranunculus laetus* for the first time. The structure elucidation of the isolated compounds were based primarily on 1D and 2D NMR analysis.

Compounds **1**–**6** were screened for bioactivity, and only **1**–**4** showed antibacterial activity (Table 1). Compounds **1**–**4** showed activity against *Bacillus subtilis*, *Escherichia coli*, *Shigella flexinari*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. It was observed that the area of the inhibition zone in compounds **1**–**3** was significant, while compound **4** showed weak activity. The results indicated that compounds **1**–**3** with hydroxyl groups are the most potent; compound **4** with two methoxyl showed weak inhibition.

Jacein (1): yellow powder, $[\alpha]_D^{25}$ –73.1° (*c* 1.48, MeOH), mp 205–207°C. UV (MeOH, λ_{max}, log ε, nm): 354 (4.32), 258 (4.30). IR (MeOH, ν_{max}, cm^{-1}): 3340, 3100, 1660, 1622, 1580–1460. PMR (300 MHz, CD_3OD, δ, ppm, J/Hz): 7.69 1H, d, J = 2.2, H-2′), 7.66 (1H, dd, J = 6.5, 2.2, H-5′), 6.92 (1H, d, J = 8.5, H-6′), 6.83 (1H, s, H-8), 3.94 (3H, s, OMe), 3.86 (3H, s, OMe), 3.77 (3H, s, OMe), 5.21(1H, d, J = 7.5, H-1″), 3.81 (1H, m, H-2″), 3.57 (1H, m, H-3″), 3.54 (1H, m, H-4″), 3.48 (1H, m, H-5″), 3.36–3.42 (2H, m, H-6″). EIMS *m/z* (rel. int %): 360 (100), 345 (64), 182 (11), 178 (6), 163 (22).



1 - 3

1: R = Glc, $R_1 = R_3 = H$, $R_2 = CH_3$
2: R = R_3 = H, R_1 = Glc, $R_2 = CH_3$
3: R = Glc, $R_1 = R_2 = H$, $R_3 = CH_3$

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 0009-3130/09/4505-0720

TABLE 1. Antibacterial Activity of Compounds **1–4**

Bacteria	**1**	**2**	**3**	**4**
Bacillus subtilis	+ + +	+ + +	+ + +	+
Escherichia coli	+ +	+ +	+ +	+
Shigella flexinari	+ +	+ +	+ + +	+
Staphylococcus aureus	+ + +	+ + +	+ + +	+
Pseudomonas aeruginosa	+ +	+ +	+ + +	+ +

+ + + +: significant, + + +: high, + +: high active; +: moderate. Standard drug imipenem**: + + + +.**
Incubation period 8 h, 37°C; colony forming unit = 10^4–10^6; size of well = 5 mm radius.

Jacedin-5-*O*-*β*-D-glucoside (2): yellow powder, mp 192–196°C, $[\alpha]_D^{25}$ –73.1° (*c* 1.48, MeOH). UV (MeOH, λ_{max}, log ε, nm): 353 (3.76), 277 (3.60). IR (MeOH, ν_{max}, cm^{-1}): 3340, 3100, 1660, 1622, 1580–1460. PMR (300 MHz, CD_3OD, δ, ppm, J/Hz): 7.91 (1H, dd, J = 8.2, 2.0, H-5′), 7.30 (1H, d, J = 2.0. H-3′), 6.98 (1H, d, J = 8.2, H-6′), 6.89 (1H, s, H-8), 3.90 (3H, s, OMe), 3.86 (3H, s OMe), 3.74 (3H, s, OMe), 5.23 (1H, d, J = 7.2, H-1″), 3.76 (1H, dd, J = 7.6, 8.1, H-2″), 3.61 (1H, s, m, H-3″), 3.55 (1H, m, H-4″), 3.51 (1H, m, H-5″), 3.35–3.46 (2H, m, H-6″). HRFAB MS (neg) *m/z* : 521.4480 $[M–H]^+$ (calcd for $C_{24}H_{25}O_{13}$, 521.4475).

Centaurein (3): yellow powder, mp 190–200°C, $[\alpha]_D^{25}$ –76.6° (*c* 1.4, MeOH). UV (MeOH, λ_{max}, log ε, nm): 349 (4.31), 258 (4.30). IR (KBr, ν_{max}, cm^{-1}): 3400, 3100, 1660, and 1620, 1582–1464. PMR (300 MHz, CD_3OD, δ, ppm, J/Hz): 7.79 (1H, d, J = 2.2, H-3′), 7.58 (1H, dd, J = 8.5, 2.2, H-5′), 6.87 (1H, d, J = 8.5, H-6′), 6.86 (1H, s, H-8), 3.89 (3H, s, OMe), 3.78 (3H, s, OMe), 3.75(3H, s, OMe), 5.33 (1H, d, J = 7.2 Hz, H-1″), 3.91(1H, m, H-2″), 3.67 (1H, m, H-3″), 3.62 (1H, m, H-4″), 3.57 (1H, m, H-5″), 3.37–3.45 (2H, m, H-6″). EIMS *m/z* (rel.int. %): 360 (100), 359 (38), 345 (7.6), 317 (15.6), 178 (4), 163 (3.1).

6,7-Dimethoxycoumarin (4): mp 143–145 °C. UV (MeOH, λ_{max}, log ε, nm): 395 (2.03), 345 (3.41), and 265 (3.11). IR ($CHCl_3$, ν_{max}, cm^{-1}): 1682 and 1615. PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.66 (1H, d, J = 9.2, H-4), 6.83 (1H, s, H-5), 6.82 (1H, s, H-8), 6.29 (1H, d, J = 9.2, H-3), 3.93 (3H, s, OMe-6) and 3.90 (3H, s, OMe-7). EIMS *m/z* (rel. int.): 206 (100), 191 (31), 178 (10), 163 (33), 135 (14), 107 (12), 79 (16). HR-EIMS *m/z*: 206.0568 (calcd for $C_{11}H_{10}O_4$, 206.0579).

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