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ISOLATION AND CHARACTERIZATION OF A NEW FLAVONE DIGLUCOSIDE FROM *Salix denticulata*

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*A new flavone diglucoside named 7,3′-dihydroxy-4′-methoxyflavone-5-*O*-β-D-glucopyranosyl (6″→1‴)-β-D-glucopyranoside (**1**), along with four known flavonoids, luteolin (**2**), isoquercetin (**3**), catechin (**4**), and diosmetin (**5**), has been isolated and characterized from* Salix denticulata*. The structure of the new flavone diglucoside was characterized by means of high field 1D and 2D NMR and MS spectral analysis.*

Keywords: flavone diglucoside, *Salix denticulata,* Salicaceae.

Salix (Salicaceae) is a genus of deciduous shrubs or trees with 300 species all over the world, out of which 35–40 species are found in India [1]. The various species of *Salix* are medicinally used in the treatment of fever, jaundice, hepatitis, rheumatism, arthritis, and eczema [2, 3], and as a blood purifier. This species is occasionally used as a traditional medicine in India. The previous phytochemical research on *Salix* genus showed the presence of phenolics [4], condensed tannins [5], catechins [6], flavonols [7], and diterpenes [8]. As part of the research on medicinal plants of Garhwal Himalaya, we herein reported the isolation and characterization of a new flavonoid diglucoside along with four known flavonoids, luteolin [9], isoquercetin [10], catechin [11], and diosmetin [12], from the ethanolic extract of leaves of *Salix denticulata*.

Compound **1** showed molecular mass *m/z* 624 $[M]^+$ in its FAB-MS spectrum, corresponding to the molecular formula $C_{28}H_{32}O_{16}$. This was corroborated by the ^{13}C NMR spectrum, which showed signals for all 28 carbons of the molecule. The UV spectrum showed absorption maxima at 270, 304, and 340 nm, which was typical of a flavone with 5,7,3′,4′ tetraoxygenation [13]. The ^1H NMR spectrum showed two *meta* coupled doublet signals at δ 6.46 and δ 6.77 (J = 1.6 Hz each) assignable to H-6 and H-8, respectively, together with a singlet at δ 6.72 for H-3. The presence of two *meta* coupled protons of H-6 and H-8 along with the downfield shift of their carbon signals in the ^{13}C NMR spectrum to δ 99.6 and δ 94.81, respectively, indicated C-5 and C-7 oxygenated carbons. The flavone identity was supported by the carbon signal at δ 103.18 assigned to C-3 [14]. Ring B showed the pattern of three one-proton signals at δ 7.45 (J = 2.4), δ 6.93 (J = 8.4), and δ 7.43 (J = 2.4, 8.4 Hz), where the multiplicity indicated that one proton was coupled to the remaining two, which were not coupled to each other. A singlet at δ 3.61 indicated the methoxy group.

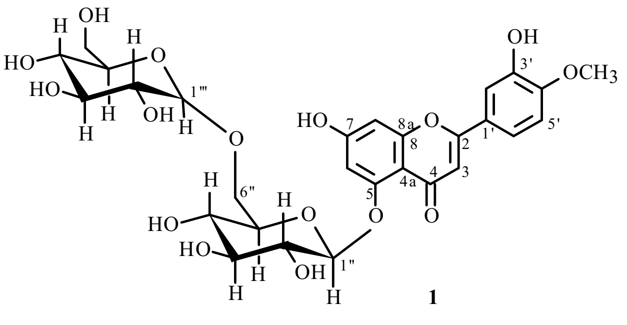

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TABLE 1. ^{1}H, ^{13}C and 2D NMR Data of Compound **1** (400, 125 MHz, DMSO-d$_6$, δ, ppm, J/Hz)

C atom	δ_C	δ_H	HSQC	HMBC
2	161.24	–	C	–
3	103.18	6.72 s	CH	1′,2,4,4a
4	181.95	–	C	–
4a	105.43	–	C	–
5	164.61	–	C	–
6	99.60	6.46 (d, J = 1.6)	CH	4,5,7,8,4a
7	162.94	–	C	–
8	94.81	6.77 (d, J = 1.6)	CH	6,7,8a
8a	157.00	–	C	–
1′	121.40	–	C	–
2′	113.70	7.45 (d, J = 2.4)	CH	1′,3′,6′
3′	145.84	–	C	–
4′	150.04	–	C	–
5′	116.14	6.93 (d, J = 8.4)	CH	1′,2′,4′
6′	119.25	7.43 (dd, J = 2.4, 8.4)	CH	1′,4′
1″	104.07	5.05 (d, J = 6.8)	CH	5
2″	76.56	3.08 m	CH	1″
3″	73.37	2.92 (t, J = 8.4)	CH	4″
4″	75.61	3.51 (t, J = 8.4)	CH	3″
5″	69.34	3.25 m	CH	3″,6″
6″	68.30	3.91 (dd, J = 1.8, 13.2) 3.32 (dd, J = 1.8, 13.2)	CH$_2$	4″,5″
1‴	99.93	4.16 (d, J = 7.2)	CH	6″
2‴	76.27	3.25 m	CH	1‴
3‴	73.11	3.25 m	CH	4‴
4‴	75.61	3.41 m	CH	3‴,5‴
5‴	69.54	3.25 m	CH	6‴
6‴	65.67	3.67 (dd, J = 1.6,12.8) 2.98 (dd, J = 1.8,12.8)	CH$_2$	5‴
OCH$_3$	58.7	3.61 s	CH$_3$	4′

In the ^{1}H NMR, two anomeric proton signals at δ 5.05 (J = 6.8 Hz) and δ 4.16 (J = 7.2 Hz) indicated two sugars with *β*-configuration. The individual sugar chemical shifts were assigned using 1D (^{1}H, ^{13}C, DEPT) and 2D NMR (COSY, HSQC, HMBC) data, which showed that both sugar were *β*-D-glucose and which was further confirmed on TLC after acid hydrolysis.

The exact positions of the methoxy group and sugar rings were subsequently confirmed by the observation of HMBC correlations between Glc H-1‴ with Glc C-6″ and Glc H-1″ with C-5 (164.61), suggesting that the chain structure was Glc (1‴→6″) Glc, and this chain was attached to C-5 of the aglycone through an *O*-glycosidic linkage. The correlation between methoxy protons (δ 3.61) and δ 150.04 showed its position at C-4′. So, based on the above observation, the structure of **1** was established as 7,3′-dihydroxy,4′-methoxyflavone-5-*O*-*β*-D-glucopyranosyl (6″→1‴)-*β*-D-glucopyranoside for the first time.

The remaining isolated compounds **2–5** were identified based on comparison of their spectral data with the literature those reported for the first time from *S. denticulata*.

EXPERIMENTAL

UV: Perkin–Elmer Lambda 15 UV/VIS spectrophotometer; IR: Perkin–Elmer Infrared 15; ^{1}H NMR spectra: Bruker AVANCE 400 (400 MHz); ^{13}C NMR spectra: Bruker AVANCE 400 (125 MHz), TMS as an internal reference; 2D NMR spectra: COSY, HMQC (optimized for J_{HC} = 145 Hz), and HMBC (optimized for J_{HC} = 7 Hz); FAB-MS: Joel SX 102; CC: silica gel (60–120 mesh (Merck).

Plant Material. The plant material was collected from Tungnath, Chamoli, Uttarakhand, India at altitude of 3000 meters in July 2007 and identified by the taxonomist of the Botany Department of HNB Garhwal University, Uttarakhand, India. A voucher specimen is deposited in the Botany Department, HNB Garhwal University, Uttarakhand, India.

Isolation and Identification. The shredded dried leaves of *Salix denticulata* (6 kg) were extracted with 95% ethanol, and concentrated extract was defatted with *n*-hexane. The polar extract was subjected to silica gel chromatography and eluted with a binary solution of $CHCl_3$–MeOH with increasing polarity to afford six fractions. The fraction collected at 85:15 ($CHCl_3$–MeOH) polarity was again subjected to repeated column chromatography to yield compounds **1–5**.

7,3′-Dihydroxy-4′-methoxyflavone-5-*O*-*β*-D-glucopyranosyl (6″→1‴)-*β*-D-glucopyranoside (1), $C_{28}H_{32}O_{16}$, light yellow solid, mp 198–201°C. UV (λ_{max}, nm): 270, 304, 340. FAB-MS: *m/z* 624 $[M]^+$, 603, 576, 391, 287. ^1H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), see Table 1.

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