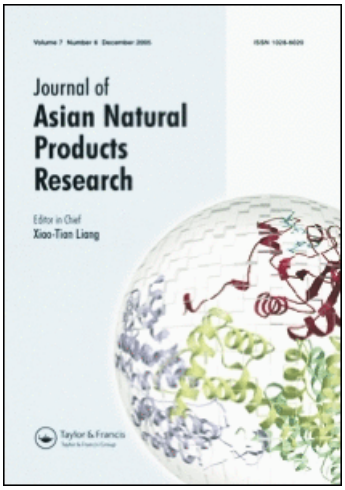

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Verticilliside, a new flavone *C*-glucoside from *Enicostemma verticillatum*

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Verticilliside, a new flavone *C*-glucoside from *Enicostemma verticillatum*

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Verticilliside (**1**), a new flavone *C*-glucoside, has been isolated from the ethyl acetate soluble fraction of *Enicostemma verticillatum* and its structure is assigned on the basis of the spectroscopic data. 5,7,4′-Trihydroxyflavone 8-*C*-β-D-glucopyranoside (**2**) and isoorientin 3′-*O*-methyl ether (**3**) have also been isolated for the first time from this species.

Keywords: *Enicostemma verticillatum*; Gentianaceae; flavone *C*-glucoside; Verticilliside

1. Introduction

The genus *Enicostemma* belonging to the family Gentianaceae comprises four species [1,2]. One of these is *Enicostemma verticillatum,* which is widely distributed in South America, Africa, and Asia [1]. In Pakistan, it is mainly found in Thatta, Badin, Hyderabad, Mirpur, Gharo, and Manghopir [1]. *E. verticillatum* is a bitter tonic and is used as a substitute for chirayita as a blood purifier. The literature survey revealed that no phytochemical or pharmacological studies have so far been carried out on *E. verticillatum*. This prompted us to carry out phytochemical investigation on this species resulting in the isolation and structural elucidation of a new flavone *C*-glucoside named as Verticilliside (**1**), along with 5,7,4′-trihydroxyflavone 8-*C*-β-D-glucopyranoside (**2**) [3] and isoorientin 3′-*O*-methyl ether (**3**) [4], which are isolated for the first time from this species.

2. Results and discussion

The ethyl acetate soluble fraction of *E. verticillatum* was subjected to column chromatography over silica gel eluting with different mobile phases. Compounds **1**–**3** were finally obtained and their structures were established by IR, mass and NMR spectroscopy including 2D-NMR techniques. Verticilliside (**1**) was obtained as a yellow amorphous powder, $[\alpha]_D^{25}$ − 11.5 ($c = 0.12$, CH_3OH). The HR-FAB-MS of **1** in negative ion mode gave $[M-H]^-$ peak at *m*/*z* 491.1181 corresponding to the molecular formula $C_{23}H_{23}O_{12}$. It further showed prominent fragment at *m*/*z* 329 due to the loss of a hexose unit. The IR spectrum showed the presence of hydroxyl groups (3455–3300 cm^{-1}), conjugated carbonyl (1665 cm^{-1}), olefinic bond (1650 cm^{-1}), and aromatic moiety (1503 and 1460 cm^{-1}). The UV spectrum exhibited characteristic absorption bands for the flavones at 275, 290, and 325 nm. The ^1H NMR spectrum provided signals of functional groups including a chelated hydroxyl group at δ 12.50 (1H, s), two aromatic methoxy protons at δ 3.85 and 3.79 (6H, s, 3′-OMe, 6-OMe), four aromatic protons at δ 6.91 (1H, s, H-3), 6.94 (1H, d, $J = 8.2$ Hz, H-5′), 7.63 (1H, dd, $J = 1.9$, 8.2 Hz, H-6′), and 7.58 (1H, d, $J = 1.9$ Hz, H-2′). The signal at δ 4.92 (1H, d, $J = 9.7$ Hz,

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H-1″) demonstrated the β-configuration of the hexose residue. The signals of oxymethine protons of sugar unit appeared between δ 3.51 and 4.31, while oxymethylene protons resonated at δ 3.81 (1H, dd, $J = 7.4$, 10.8 Hz, H-6″a) and δ 3.92 (1H, dd, $J = 4.4$, 10.8 Hz, H-6″b).

The ^{13}C NMR spectra (BB and DEPT) showed 23 signals comprising of two methyl, one methylene, nine methane, and 11 quaternary carbons. The signals at δ 166.5, 104.3, 183.6, 154.7, and 105.3 were typical of C-2, C-3, C-4, C-5, and C-10 of a flavone moiety. Apart from further peaks of the aromatic carbons, it showed the signals of hexose moiety at δ 73.4 C-1″, oxymethine carbons ranging between δ 70.5 and 81.7 and oxymethylene carbon at δ 61.5 C-6″. The hexose was identified as glucose through comparison of NMR chemical shifts of the hexose moiety with those reported for closely related compounds including vitexin [5] and precatorin [6]. The chemical shift of the glucose moiety in ^{1}H and ^{13}C NMR, particularly those of H-1″ at δ 4.92 (d, $J = 9.7$ Hz) and C-1″ (δ 73.4) corresponded to *C*-glucoside. The resistance to hydrolysis further indicated that **1** is a flavonoidal *C*-glucoside. The position of the sugar moiety at C-8 was confirmed by HMBC experiments in which H-1″ (δ 4.92) showed ^{2}J correlation with C-8 (δ 106.4) and ^{3}J correlations with C-9 (δ 154.2) and C-7 (δ 160.1), respectively. These data revealed the structure of Verticilliside (**1**) as 5,7,4′-trihydroxy-3′,6-dimethoxy flavone 8-*C*-β-D-glucopyranoside (Figure 1). 5,7,4′-Trihydroxyflavone 8-*C*-β-D-glucopyranoside (**2**) and isoorientin 3′-*O*-methyl ether (**3**) were isolated for the first time from the genus *Enicostemma* and their structures were established by mass and NMR spectrometry and by comparison with the previously reported data.

3. Experimental

3.1 General experimental procedures

The melting points were recorded on a Buchi melting point apparatus and are uncorrected. Optical rotations were measured on a Jasco DIP-360 digital polarimeter using a 10-cm cell tube. The UV spectra were recorded on Hitachi-UV-3200 spectrometer. The IR spectra were recorded on Jasco-320-A spectrometer. The ^{1}H, ^{13}C NMR, and the 2D-NMR spectra were recorded on a Bruker AMX-400 spectrometer in methanol-d_4. Chemical shifts are in parts per million (δ), relative to tetramethylsilane as an internal standard, and scalar coupling constants are reported in Hertz. Mass spectra were measured on Finnigan MAT 12 and MAT 312 spectrometers and ions are given in *m/z*. Column chromatography was carried out using silica gel (70–220 mesh). Alumina sheets precoated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick; E-Merck, Darmstadt, Germany) were used for TLC to check the purity, and were visualized under UV light (254 and 366 nm) or spraying with ceric sulfate solution.

3.2 Plant material

The whole plant of *E. verticillatum* (Gentianaceae) was collected from Thatta region (Sindh, Pakistan) and identified by plant Taxonomist Prof. Dr Surraiya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, Karachi, Pakistan, where a voucher specimen (No. 15013) has been deposited.

3.3 Extraction and isolation

The shade-dried plant material (30 kg) was extracted (3 × 30 l) with methanol. The residue from the methanolic extract was partitioned between *n*-hexane and water. The water soluble fraction was further extracted with chloroform, ethyl acetate, and *n*-butanol. The ethyl acetate soluble fraction (40 g) was subjected to column chromatography over silica gel eluting with *n*-hexane–chloroform, chloroform, chloroform–methanol, and methanol in increasing order of polarity. The fraction, which was eluted with chloroform–methanol (9.5:0.5), showed two major spots on TLC. It was further subjected to column chromatography using chloroform–methanol (9:1) as eluent to afford compounds **2** (12 mg) and **3** (16 mg), from the top and tail

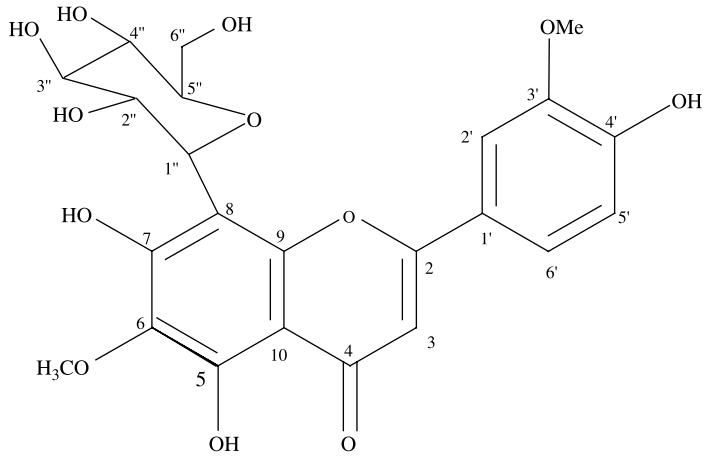

Figure 1. Structure of Verticilliside (**1**).

fractions, respectively. The fractions that were obtained from chloroform–methanol (9.2:0.8) were combined and further subjected to column chromatography using chloroform–methanol (8.8:1.2) as eluent to afford compound **1** (15 mg).

*3.3.1 Verticilliside (**1**)*

White amorphous powder; mp 210–211°C; $[\alpha]_D^{25} - 11.5$ ($c = 0.12$, CH_3OH). λ_{max} (MeOH) nm: 275, 290, and 325. IR (KBr) ν_{max} cm^{-1}: 3455–3300, 1665, 1650, 1503, and 1460. ^1H, ^{13}C NMR, and important

Table 1. ^1H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations for **1**.

H/C	^1H multiplicity J (Hz)	^{13}C (DEPT)	HMBC (H → C)
2	–	166.5	–
3	6.91, s	104.3	2, 4, 10
4	–	183.6	–
5	–	154.7	–
6	–	133.9	–
7	–	160.1	–
8	–	106.4	–
9	–	154.2	–
10	–	105.3	–
1′	–	121.8	–
2′	7.58, d, 1.9	111.2	1′, 3′, 4′
3′	–	150.7	–
4′	–	161.5	–
5′	6.94, d, 8.2	116.4	3′, 4′, 6′
6′	7.63, dd, 8.2, 1.9	121.2	1′, 2, 5′
1″	4.92, d, 9.7	73.4	7, 8, 9
2″	4.31, m	71.1	1″, 3″
3″	3.51, m	78.9	4″
4″	3.98, m	70.5	5″, 6″
5″	3.69, m	81.7	4″, 6″
6″	3.81, dd, 7.4, 10.8	61.5	5″
	3.92, dd, 4.4, 10.8	–	
OH-5	12.50, s	–	
OMe-3′	3.85, s	56.0	
OMe-6	3.79, s	60.5	

HMBC correlations are shown in Table 1. HR-FAB-MS *m/z*: 491.1181 $[M-H]^-$ (calcd for $C_{23}H_{23}O_{12}$, 491.1189).

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