

A new flavonoid diglycoside from *Biophytum reinwardtii* (Zucc.) Klotzsch. and evaluation of its antioxidant and anticancer activities

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A new flavonoid diglycoside namely apigenin-4',5-O-β-D-diglycoside **1** has been isolated from the whole plant of *Biophytum reinwardtii* (Zucc.) Klotzsch. and its structure has been elucidated by a combination of spectroscopic (¹H, ¹³C and 2D NMR spectroscopy), mass spectral data and chemical methods. The compound **1** has been evaluated for its antioxidant activities viz., DPPH free radical scavenging activity and modified ferric reducing/antioxidant power (FRAP) assay and anticancer activity viz., MTT assay. The compound **1** has been found to possess significant antioxidant and anticancer activities.

Keywords: *Biophytum reinwardtii*, oxalidaceae, flavonoid diglycoside, antioxidant activity, MTT assay

Biophytum reinwardtii (Zucc.) Klotzsch. (family: Oxalidaceae) is an annual herb seen during the rainy season throughout the warm parts of India. It is also distributed in Indo-Malaysia and China^{1,2}. The leaves and roots of *B. reinwardtii* are used to treat common fever. The plant is reported to possess tonic and stimulant properties, used for chest complaints, convulsions, cramps, inflammatory tumours and its ash for stomachache. The whole plant is dried, powdered and given internally to cattle to stop excess salivation. The leaves are diuretic and possess astringent and antiseptic properties. The pounded and bruised leaves or their juice is used in dressing burns and convulsions. Leaf paste is applied to wounds and cuts and leaf decoction given for asthma and phthisis. The mature leaves contain insulin like principle and are recommended for diabetes. Saline extract of the leaves showed hypoglycemic activity in rabbits¹. The whole plant is used as a sedative, for removing dandruff and as hair tonic. In Bihar state of India, the leaves and roots are given for insomnia, fever, gonorrhoea and lithiasis^{1,3}.

Amentoflavone (I3', II8-biapigenin) was isolated from the roots of *B. sensitivum*, a closely related species and proved to be a selective inhibitor of cyclooxygenase COX-1 catalyzed prostaglandin bio-synthesis when tested *in vitro* with an IC₅₀ value of 12.4 μM⁴. Two biflavones, cupressuflavone and amentoflavone, three flavonoids, luteolin 7-methyl ether, isoorientin and

3'-methoxyluteolin 7-O-glucoside, as well as two acids, 4-caffeoylquinic acid and 5-caffeoylquinic acid were isolated from the aerial parts of *B. sensitivum*⁵. To the best of our knowledge, no flavonoid compound has been reported from *B. reinwardtii*. In the present paper, we describe the isolation and structural elucidation of a new flavonoid diglycoside namely apigenin-4',5-O-β-D-diglycoside (compound **1**) from the whole plant of *B. reinwardtii*. The antioxidant activity of the compound **1** viz., DPPH free radical scavenging activity and modified ferric reducing/antioxidant power (FRAP) assay were investigated. The anticancer activity of compound **1** was also investigated by MTT assay.

Results and Discussion

A 80% aqueous methanol extract of *B. reinwardtii* was fractionated with petroleum ether, ethyl acetate and n-butanol in that order. The butanol extract was subjected to column chromatography over silica gel followed by preparative TLC to yield a pure flavonoid diglycoside, **1** (Figure 1). The compound **1** was checked for homogeneity by HPLC which afforded RT of 7.12 min.

The compound **1** was assigned the molecular formula C₂₇H₃₀O₁₅ by analysis of ESIMS (+ve and -ve modes). The compound **1** showed a quasi molecular ion peak at *m/z* 595.3274 [M+H]⁺ and 593.2058 [M-H]⁻ in the ESIMS. CHN analysis of **1** also agrees with the assigned molecular formula: (Found: C, 54.3%; H, 5.15%. C₂₇H₃₀O₁₅ requires: C, 54.55%; H,