SUPPLEMENTARY MATERIAL

Chemical constituents from the flowering buds of *Bauhinia tomentosa* Linn (FBBT)

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Abstract

Isolation and characterization of compounds, 1-(2'-hydroxy-4'-methoxyphenyl)-3-(4''-methoxyphenyl)-2-hydroxypropane-1,3-dione (**1**), 5-hydroxyflavone (**2**), 3,5,7,3',4'-pentahydroxyflavone (**3**), 3,5,7,2',4'-pentahydroxyflavone (**4**) and 5,7,3',4'-tetrahydroxyflavone-3-O-rhamnoside (**5**) are reported from the air dried flowering buds of *Bauhinia tomentosa* Linn. Their structures are determined on the basis of extensive chemical and spectral evidences. Compound (**1**) is reported for the first time from the plant source. While compounds (**2**) and (**4**) are reported for the first time from this genus, compound (**2**) is reported for the second time from the natural source.

Keywords: *Bauhinia tomentosa*; Fabaceae; Propanedione; Flavonoids; Hydroxy flavones

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Experimental

1 General Methods:

UV-Visible spectra were recorded in a JASCO- spectra manager (V-550) double beam spectrophotometer and IR spectra were recorded on FT-IR JASCO spectrophotometer using KBr pellets. NMR spectra (^1H -NMR, ^{13}C -NMR, DEPT-135, HH-COSY, CH-COSY and HMBC) were recorded in CDCl_3 and $\text{DMSO}-d_6$ on Bruker DRX-300 (300 MHz) Ultrashield TM spectrometer using TMS as an internal standard. Chemical shifts (δ) are expressed in ppm, coupling constants (J) are expressed in Hz, and splitting patterns are described as follows: s = singlet; d = doublet; dd = doublet of doublet. Verification of the product and its purity analysis were carried out on a Shimadzu System (Shimadzu, Japan) equipped with a Shimadzu RP-18 column. The purity analysis was performed using the following spectroscopic gradient: from 0 to 1 min in methanol, then from 1 to 35 min 90% methanol and 10 % H_2O . Injection Volume is 5 μl , flow rate is 0.30 mL/min and the oven temperature is 30 $^\circ\text{C}$. The compound was analyzed by a PDA-UV-Vis detector at 254 nm.

Electrospray ionization mass spectrometry (ESI-MS) analysis was performed in both the positive ion mode and negative ion mode on a liquid chromatography-ion trap mass spectrometer (LCQ Fleet, Thermo Fisher Instruments Limited, US). The collision voltage and ionization voltage were 33 V and 4.98 kV, respectively, using nitrogen as atomization and desolvation gas. Helium was used as the nebulizer at 40 psi. The desolvation temperature was set at 300 $^\circ\text{C}$. The scan range of mass spectrum was 150–2000 m/z. For TLC, silica gel-G and Whatman No. 1 filter paper was used and the plates were visualized using iodine/UV lamp in long and short wavelength.

2 Plant material:

The fresh flowering buds of *B. tomentosa* were collected from the vicinity of our resident, Madurai, Tamilnadu, India during December 2012 and were identified by Dr. G. V. S. Moorthy at the Botanical survey of India, Coimbatore, Tamilnadu. A botanically identified voucher specimen (voucher No: 34556) was deposited at the herbarium of Botanical survey of India, Coimbatore, Tamilnadu.

3 Extraction and isolation:

The air dried flowering buds of *B. tomentosa* (1.5 Kg) were successively extracted with continuous soxhlet extraction method. The extraction was done with different solvents in their

increasing order of polarity such as hexane, chloroform, ethyl acetate and ethanol in succession. All the extracts were concentrated *in vacuo*. The CHCl_3 and alcohol extracts on concentration gave a dark yellow and dark brown colored semi-solid respectively, which was subjected to chromatography over a silica gel column employing a stepwise gradient solvent system of hexane-benzene-methanol mixture in varying proportions to yield five solid compounds characterized as 1-(2'-hydroxy-4'-methoxyphenyl)-3-(4''-methoxyphenyl)-2-hydroxypropane-1,3-dione (**1**) (Yield: 18mg), 5-hydroxyflavone (**2**) (Yield: 22mg), 3,5,7,3',4'-pentahydroxyflavone (**3**) (Yield: 40mg), 3,5,7,2',4'-pentahydroxyflavone (**4**) (Yield: 24mg) and 5,7,3',4'-tetrahydroxyflavone-3-O-rhamnoside (**5**) (Yield: 28g).

4 1-(2'-hydroxy-4'-methoxyphenyl)-3-(4''-methoxyphenyl)-2-hydroxypropane-1,3-dione (1)

Yellow color solid. M.p.166-168 °C. UV (CH_3OH) λ_{max} (nm) : 283, 376. IR (KBr) ν_{max} (cm^{-1}) : 1659 [$\nu_{\text{C=O}}$], 1605 [$\nu_{\text{C=O}}$], 3424 [$\nu_{\text{(OH)}}$]. ^1H , ^{13}C and HMBC Correlation Data, see Table S1. DEPT-135 (75 MHz, CDCl_3) (δ) 129.92 (CH, C-3'), 128.56 (CH, C-2'' & 6''), 114.03 (CH, C-3'' & 5''), 107.80 (CH, C-6'), 101.23 (CH, C-5'), 90.59 (CH, C-2), 55.51 (4''-O- CH_3), 55.46 (4'-O- CH_3). LC-MS [m/z] (rel. Int.) (+ve Mode) [339.06 ($[\text{M}+\text{Na}]^+$, 100.0%)] (calcd for $\text{C}_{17}\text{H}_{16}\text{O}_6$ 316.09).

HPLC chromatogram of Compound (1)

¹H-NMR spectrum of Compound (1)

¹³C-NMR spectrum of Compound (1)

DEPT 135 spectrum of Compound (1)

Comparison of ¹³C-NMR and DEPT 135 spectra of compound (1)

HH-COSY spectrum of Compound (1)

CH-COSY spectrum of Compound (1)

HMBC spectrum of Compound (1)

ESI-MS spectrum of compound (1) (Positive ion Mode)

^1H -NMR spectrum of Compound (2)

Comparison of ^{13}C -NMR and DEPT 135 spectra of compound (2)

^1H -NMR spectrum of Compound (3)

Comparison of ^{13}C -NMR and DEPT 135 spectra of compound (3)

¹H-NMR spectrum of Compound (4)

Comparison of ¹³C-NMR and DEPT 135 spectra of compound (4)

^1H -NMR spectrum of Compound (5)

Comparison of ^{13}C -NMR and DEPT 135 spectra of compound (5)

Position	^1H	^{13}C	HMBC
C-1 (-C=O)	-	193.87	-
H-C-2	6.63 (1H, s)	90.66	C-1, C-3, C-1''
C-3 (-C=O)	-	176.27	-
C-1'	-	112.68	-
C-2'	-	165.10	-
H-C-3'	7.67 (1H, d, $J = 8.4$ Hz)	129.89	C-1, C-4'
C-4'	-	165.66	-
H-C-5'	6.48 (1H, d, $J = 2.4$ Hz)	107.73	C-4', C-6'
H-C-6'	6.47 (1H, d, $J = 9.6$ Hz)	101.37	C-1'
C-1''	-	126.15	-
H-C-2''&6''	7.89 (2H, d, $J = 9.0$ Hz)	128.56	C-2, C-3', C-4''
H-C-3''&5''	6.97 (2H, d, $J = 9.0$ Hz)	114.08	C-1''
C-4''	-	162.96	-
2-OH	15.56 (1H, s)	-	-
2'-OH	12.64 (1H, s)	-	-
4'-OCH ₃	3.84 (3H, s)	55.42	C-4'
4''-OCH ₃	3.88 (3H, s)	55.45	C-4''

^a Measured in CDCl₃ at 300 MHz (^1H) and 75 MHz (^{13}C).

Table S1. ^1H (δ_{H}) and ^{13}C NMR (δ_{C}) Data^a of Compound (1)

Figure S1. Key HMBC connectivity for Compound (1)