Further Flavonoids from *Polygonum viscosum* Buch-Ham. ex D. Don. (Polygonoceae)

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ABSTRACT: Three additional flavonoids such as 5,7,4′-trihydroxy-3,8,3′-trimethoxy flavonol (**1**), 5,7-dihydroxy-flavone (chrysin, **2**) and 5,6,7-trihydroxyflavone (baicalein, **3**) were obtained from the methanol extract of whole plant of *Polygonum viscosum*. The structure of the isolated compounds was established exclusively by ultraviolet (UV) spectroscopy, mass spectrometry (MS) and a series of Nuclear Magnetic Resonance (NMR) analysis.

Key words: *Polygonum viscosum*, Polygonoceae, flavonoid.

INTRODUCTION

Polygonum viscosum Buch-Ham. ex D. Don. (Fam. Polygonaceae), commonly known as Biskatale, is an annual herb (up to 1 meter) native to Nepal and also widely distributed in Bangladesh, northeast India, Japan and China.[1] The genus *Polygonum* is well known for many pharmacologically active compounds.[2] It is reputed for its application in the oriental systems for the treatment of various diseases such as fever, pain, infections, inflammation, cancer and tumor.[3] The methanol extract of the plant have yielded several novel flavonoids and a sesqueterpene.[1] This investigation reports the isolation and structure elucidation of three additional flavonoids such as 5,7,4′-trihydroxy-3,8,3′-trimethoxy flavonol (**1**), 5,7-dihydroxyflavone (**2**) and 5,6,7-trihydroxyflavone (**3**). This is the first report of their isolation from the genus *Polygonum*.

MATERIALS AND METHODS

General experimental procedure. UV (methanol) and IR (KBr) were obtained on Spectronic, Shimadzu UV and Perkin Elmer FTR spectrometer, respectively. ^{1}H (400 MHz, C_5D_5N) and ^{13}C (100 MHz, C_5D_5N) NMR data were acquired on a Varian Inova 400 instrument and HR-EIMS were recorded with a VG Micromass 2AB mass spectrometer. Vacuum liquid chromatography (VLC) column was packed with Kiselgel 60H (100-200 mesh). Analytical- and preparative-thin layer chromatography (TLC and PTLC) were performed on glass plates coated with silica gel (Kiselgel 60 PF_{254}). Solvent systems ranging from non-polar to polar were used for VLC. UV light (254 and 365 nm) and vanillin/H_2SO_4 reagents[4] were used to visualize the spots on the developed TLC and PTLC plates. All other chemicals and reagents were of analytical grade.

Plant material. Whole plant parts of *Polygonum viscosum* was collected from Panchori, Chittagong and authenticated by Prof. Dr. Abul Hassan (Department of Botany, University of Dhaka) where a voucher (DUB number 764) has been maintained.

Extraction and isolation. Air dried and coarsely powdered dried whole plant parts (2 kg) were soaked in methanol (5 L) for 10 days at room temperature with occasional shaking and then filtered using Whatmann filter paper no. 1. The extract was concentrated with a rotary evaporator at 40 ^oC to yield 10.6 gm of dried extract. Vacuum liquid chromatography of an aliquot (2.8 gm)of the dried

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extract followed by the repeated PTLC using toluene-EtOAc (96:4) provided 6 mg of compound **1** (R_f 0.530), 4.5 mg of compound **2** (R_f 0.410) and 5 mg of compound **3** (R_f 0.390).

Properties of isolated compounds

5,7,4′-Trihydroxy-3,8,3′-trimethoxyflavonol (**1**): Yellow needles from EtOAc- pet. ether, m.p. 216°C[7]; Found $[M]^+$ 360.0856, $C_{18}H_{16}O_8$, requires 360.0845; UV λ_{max} (MeOH) nm: 254, 273, 334, 359; IR ν_{max} (KBr) cm^{-1}: 3450, 1660, 1570, 1470, 1370, 1220, 1165, 1020, 800, 785; ^1H NMR (Table 1); EIMS, *m/z* (rel. int.): 360 $[M]^+$ (68), 359 (9), 346 (20), 345 (100), 331 (3), 317 (5), 295 (7), 166 (4), 151 (4), 95 (2).

Chrysin (**2**)**:** Yellow powder, m.p. 283-284°C[8]; Found $[M]^+$ 254.2443, $C_{15}H_{10}O_4$, requires 254.2443; UV λ_{max} (EtOH) nm: 286, 303, 314; IR ν_{max} (KBr) cm^{-1}: 3200, 2900, 1660, 1540, 1470, 1350, 1240, 1170, 1030, 840, 680; ^1H NMR (Table 1); ^{13}C NMR (Table 1); EIMS, *m/z* (rel. int.): 254 $[M]^+$ (100), 226 (33), 197 (3.3), 152 (41), 124 (33), 105 (6), 102 (10), 96 (14), 69 (28).

Baicalein (**3**)**:** Brown crystals, m.p. 265-266°C; Found $[M]^+$ 270.2438, $C_{15}H_{10}O_5$, requires 270.2440; UV λ_{max} (EtOH) nm: 292, 309, 320; IR ν_{max} (KBr) cm^{-1}: 3400, 3100, 2320, 1650, 1580, 1480, 1380, 1160, 1020, 900, 860, 780, 690, 570; ^1H NMR (Table 2); EIMS, *m/z* (rel. int.): 270 $[M]^+$ (100), 242(3.1), 168(44), 140 (15), 135 (6.3), 121 (4.5), 105 (5), 77 (16), 51 (11), 32 (19).

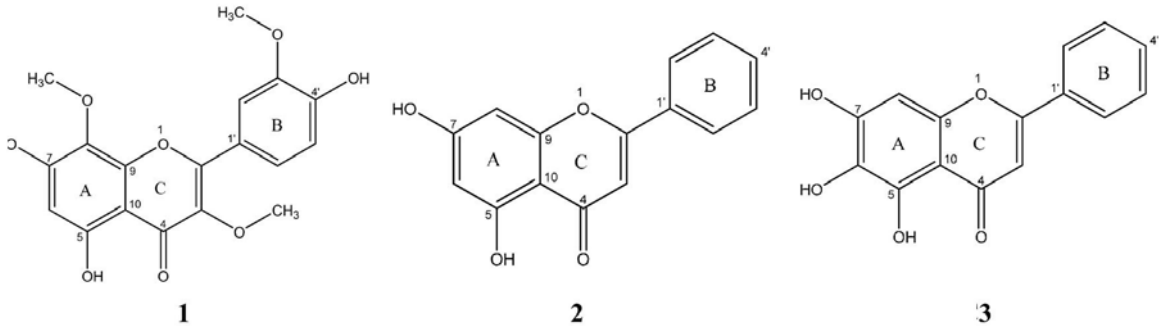
RESULTS AND DISCUSSION

Repeated chromatographic fractionation and purification of a methanol extract of aerial parts of *P. viscosum* provided three compounds (**1-3**). The structure of the isolated compounds was solved by extensive analyses of their spectroscopic data as well as by comparison with published values.

Compound **1** was crystallized as yellow needles from EtOAc-pet. ether that melted at 216°C[7]. The HR-EIMS revealed the molecular ion peak $[M]^+$ at *m/z* 360.0845 establishing the molecular formula as $C_{18}H_{16}O_8$. Addition of sodium acetate to the methanolic solution of compound **1** revealed an average shift of 9 nm in band II (254 to 263 nm) which suggested a free hydroxyl group at C-7.[5,6] Addition of aluminum chloride/ HCl did not cause any appreciable shift in the band 1 indicating the absence of a 3-OH group.

The ^1H NMR spectrum (Table 1) showed aromatic A-ring proton signals at δ 6.83 for H-6 and 13.14 for the chelated hydroxyl proton at C-5 of the flavonoid skeleton. The B-ring protons appeared at δ 8.01 (d, *J*=2.1 Hz), 7.39 (d, *J*=8.0 Hz) and 8.06 (dd, *J*=8.0, 2.1 Hz) appropriate for H-2′, H-5′ and H-6′. Three singlets at δ 3.90, 4.02 and 4.04, each of three proton intensity, were attributed to the methoxy group at C-3′, C-8 and C-3, respectively. These ^1H NMR data were in close agreement to 5,7,4′-Hrihydroxy-3,8,3′-trimethoxyflavonol (**1**), a flavonoid previously reported from *Cyanostegia angustifoli.*[7] It has also been synthesized by Fukui *et al*.[8]

Compound **2** was obtained as yellow powder that melted at 283-284°C.[9] The molecular formula was deduced as $C_{15}H_{10}O_4$ from the molecular ion peak $[M^+]$ at *m/z* 254.2443 in the HR-EIMS. The ^{13}C NMR revealed 15 carbon signals including eight methine and seven quaternary carbons (Table 2). The UV spectral absorption at 286, 303 and 314 nm in EtOH suggested a flavones skeleton. The ^1H NMR spectrum (Table 1) displayed a chelated hydroxyl proton at δ 12.80 suggesting a phenolic –OH at C-5 of the flavonoid nucleus. Two multiplets at δ 8.0 (2H) and 7.55 (3H) in the aromatic region indicated the presence of an unsubstituted phenyl group (ring B). The signals at δ 6.50 and 6.70 appeared as a pair of doublets (*J*=2.0 Hz), which demonstrated the *meta* coupled protons, H-8 and H-6, respectfully on ring A. The lone aromatic singlet at δ 6.95 was attributed to H-3. The above spectral features are almost identical to those published for 5,7-dihydroxyflavone (chrysin).[9] On this basis compound **2** was characterized as chrysin, a flavone previously known from many plants.



1 **2** **3**

Table 1. ^{1}H NMR (400MHz, C_5D_5N, δ in ppm, *J* in Hz) spectral data of flavonoids 1-3.

Protons	**1**	**2**	**3**
H-3	-	6.95, s	-
H-6	6.83, s	6.70, s	-
H-8	-	6.50, s	7.03, s
H-2′	8.01, d (2.1)	8.0, m	7.90, m
H-3′	-	7.55, m	7.44, m
H-4′	-	7.55, m	7.44, m
H-5′	7.39, d (8.0)	7.55, m	7.44, m
H-6′	8.06, dd (8.0,2.1)	8.00, m	7.90, m
OH-5	13.14, s	12.80, s	13.50, s
OMe-3	4.04, s	-	-
OMe-8	4.02, s	-	-
OMe-3′	3.90, s	-	-

The third compound (**3)** appeared as brown crystals that melted at 265-266^{o}C.[10] The HR-EIMS of compound (**3)** showed the molecular ion peak at *m/z* 270.2438 [M^{+}], which led to establish the molecular formula as $C_{15}H_{10}O_5$. The ^{13}C NMR spectrum (Table 2) exhibited 15 carbon signals, which were accounted for seven methine and eight quaternary carbons. The UV absorption bands at 292 and 320 nm suggested a flavonoid skeleton. The ^{1}H NMR spectrum (Table 1) was, in part, similar to that of 5,7-dihydoxyflavone (**2**), but ring A in compound (**3)** had only one unsubstituted aromatic carbon as indicated by the singlet at δ 7.03 (H-8). The upfield signal at δ 6.94 (s) was assigned to the proton H-3. Therefore, the remaining carbons, C-5, C-6 and C-7 of the A ring were substituted by three oxygenated groups. A singlet at δ 13.50 supported the presence of a chelated –OH proton at C-5. Two set of multiplets at δ 7.90 (H-2′, H-6′) and δ 7.44 (H-3′, H-4′ and H-5′)

Table 2. ^{13}C NMR (100MHz, C_5D_5N, δ in ppm) spectral data of compounds 2 and 3.

Carbon No.	**2**	**3**
2	163.2	163.7
3	105.2	105.5
4	181.9	183.2
5	161.5	149.4
6	99.1	131.5
7	164.5	155.9
8	94.1	95.3
9	157.5	151.2
10	104.0	105.6
1′	130.7	132.2
2′	126.4	126.7
3′	129.1	129.3
4′	132.0	131.8
5′	129.1	129.3
6′	126.4	126.7

in the aromatic region revealed that the ring B was an unsubstituted phenyl group. On the basis of these spectral data, compound **3** was identified as 5,6,7-trihydroxyflavone (baicalein), a compound previously isolated from *Oroxylum indicum.* These spectral data of **3** were consistent with those reported for baicalein (**3).**[10]

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