SHORT REPORT

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Phytochemical Studies on *Polygonum barbatum* (L.) Hara var. *barbata* (Polygonaceae)

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Abstract: *Polygonum barbatum* (L.) Hara var. *barbata* (Polygonaceae), commonly known as 'bekhanjabaj', is a Bangladeshi perennial herb. A combination of the normal phase column chromatography and preparative thin layer chromatography on silica gel afforded sitosterone (**1**) from the petroleum ether fraction, and viscozulenic acid (**2**) and acetophenone (**3**) from the chloroform fraction of the methanol extract of the aerial parts of this plant. The free-radical-scavenging properties the isolated compounds **1**-**3** were evaluated by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay.

Keywords: *Polygonum barbatum* (L.) Hara var. *barbata*; Polygonaceae; acetophenone; viscozulenic acid; sitosterone; sesquiterpene; DPPH.

1. Plant Source

Polygonum barbatum (L.) Hara var. *barbata* (Polygonaceae), commonly known as 'bekhanjabaj', is a perennial herb that grows widely in marshy and aquatic places, by the sides of the rivers, seasonally flooded roadsides ditches and small ponds in Bangladesh, India and Thailand, and also in many other countries in the south-east Asia [1-3].

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The aerial parts of *Polygonum barbatum* (L.) Hara var. *barbata* were collected from Kajla, Rajshahi, Bangladesh and authenticated by Professor Naderuzzaman (Department of Botany, University of Rajshahi, Bangladesh). A voucher specimen (BKD2004-2) representing this collection has been maintained in the Herbarium of the Department of Botany, University of Dhaka, Dhaka, Bangladesh.

2. Previous Studies

The genus *Polygonum* is well known for producing pharmacologically active compounds, and also for its use in the oriental traditional medicine systems, particularly in the treatment of pain, fever and inflammatory conditions, and as a diuretic agent [4, 5].

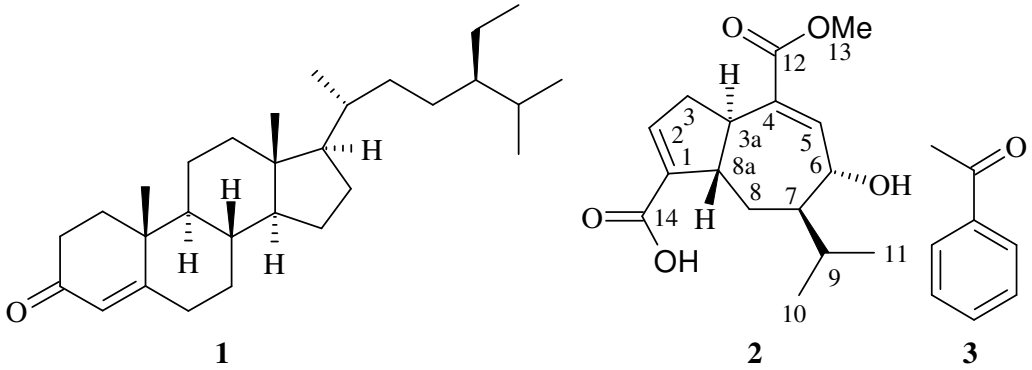
While the analgesic, anti-inflammatory and diuretic properties of the extracts of *P. barbatum* var. *barbata* in mice/rat models have been reported recently [4], to the best of our knowledge, no thorough phytochemical study has ever been carried out on this plant.

3. Present Study

The sun-dried and ground aerial parts of *P. barbatum* var. *barbata* (460 g) were extracted with methanol (MeOH, 4 L) using maceration for 5 days. The extract was concentrated by evaporation under reduced pressure at 40° C. The MeOH extract was made to 90% aq. MeOH extract and subjected to solvent partitioning with petroleum ether (PE). The resulting aqueous MeOH extract was further partitioned with chloroform ($CHCl_3$) and finally with ethyl acetate (EtOAc). All solvent extracts were concentrated by evaporation under reduced pressure at 40° C.

The above four extracts were screened by the TLC (20% EtOAc in PE). The developed plates were observed under UV lamp at 254 and 366 nm and the spots were marked. Then the plates were sprayed with 1% vanillin-sulfuric acid reagent and placed in an oven at 110 ^{o}C for 10 min. In the initial TLC analysis, the petroleum ether and chloroform extracts showed the presence of maximum number of compounds that could be isolated and identified using normal-phase chromatography. Therefore, those two extracts were analyzed further for isolation and identification of compounds.

A combination of normal column chromatography (CC on silica gel; mobile phase: PE, PE-EtOAc, EtOAc, EtOAc-MeOH, and finally 100% MeOH) and preparative TLC (on silica gel) was applied to isolate compounds sitosterone (**1**, 20.2 mg) [6, 7] from the PE extract (mobile phase, PE : EtOAc = 97: 3), and viscozulenic acid (**2**, 10.5 mg) [8] and acetophenone (**3**, 30.1 mg) [9] from the $CHCl_3$ extracts (mobile phase, PE : EtOAc = 95: 5) of *P. barbatum* var. *barbata* (Figure 1). The structures of these compounds were elucidated mainly on the basis of UV, HRMS and NMR (^{1}H and ^{13}C) spectral analyses as well as by comparison with respective published data. The free-radical-scavenging property of the isolated compounds was determined by the DPPH assay [10, 11].



1 **2** **3**

Figure 1. Compounds (**1**-**3**) from *P. barbatum*

Sitosterone (**1**): white amorphous solid (20.2 mg). *HRFABMS: m/z* 435.3603 (calculated for $C_{29}H_{48}ONa$ 435.3602); *^{1}H NMR (400 MHz, $CDCl_3$)* and *^{13}C NMR (100 MHz, $CDCl_3$):* as published data [6, 7]

Viscozulenic acid (**2**): brown amorphous solid (10.5 mg). *Optical rotation:* $[\alpha]^{25}_D$ – 185.1° (*c* 0.35, $CHCl_3$); *UV λ_{max} (MeOH):* 217, 257 nm; *HRFABMS: m/z* 317.1364 (calculated for $C_{16}H_{22}O_5Na$ 317.1365); *^{1}H NMR (400 MHz, CD_3OD):* δ 7.14 (1H, bd, *J* = 5.4 Hz, H-2), 6.80 (1H, d, *J* = 3.1 Hz, H-5), 4.16 (1H, dd, *J* = 8.2, 3.1 Hz, H-6), 3.74 (3H, s, OMe-13), 2.45 (1H, bd, *J* = 12.0 Hz, H-3a), 2.36 (1H, m, H_2-3), 2.22 (1H, m, H-8a), 2.22 (1H, m, H-8a), 2.18 (1H, m, H_2-3), 2.16 (1H, m, H-9), 1.94 (1H, m, H_2-8), 1.46 (1H, m, H-7), 1.40 (1H, m, H_2-8), 1.11 (3H, d, *J* = 7.0 Hz, H_3-10), 1.05 (3H, d, *J* = 7.0 Hz, H_3-11); *^{13}C NMR (100 MHz, CD_3OD)*: δ 170.0 (C-14), 168.8 (C-12), 143.9 (C-5), 143.4 (C-2), 134.8 (C-4), 132.1 (C-1), 69.4 (C-6), 52.3 (C-13, OMe), 49.6 (C-7), 37.4 (C-8a), 35.3 (C-3a), 28.4 (C-9), 26.7 (C-8), 26.0 (C-3), 20.3 (C-11), 20.0 (C-10) [8].

Acetophenone (**3**): colorless liquid (30.1 mg). *HREIMS: m/z* 120.0577 (calculated for C_8H_8O 120.0575); *^{1}H NMR (400 MHz, $CDCl_3$)* and *^{13}C NMR (100 MHz, $CDCl_3$):* as published data [9].

To the best of our knowledge this is the first report on the isolation of compounds **1**-**3** from the aerial parts of *P. barbatum*. Compounds **1** and **3** are rather wide-spread in the plant kingdom. However, within the genus *Polygonum*, **2** was previously reported only from *P. bistorta* [12]. The sesquiterpene **3** was previously isolated only from another *Polygonum* species, *P. viscosum* [8], which is also endemic to Bangladesh. The co-occurrence of compound **2** and **3** in the species of *Polygonum* might have some chemotaxonomic implications.

This is the first report on the evaluation of the free-radical-scavenging property of compounds **1**-**3** using the DPPH assay. None of the compounds (**1**-**3**) showed any significant free-radical-scavenging activity in the DPPH assay. The level of activity showed by these compounds was rather low, and the RC_{50} values [concentration at which a test sample reduces 50% of the DPPH (80 μg/mL) absorbance at 517 nm], were within the range of 1.8 x 10^{-1} and 1.2 x 10^{-1} mg/mL, and that of the positive control Trolox® was 2.6 x 10^{-3} mg/mL (Table 1). Although the free-radical-scavenging activity of acetophenone (**3**) itself was not that great, several naturally occurring hydroxylated acetophenones are known to have prominent free-radical-scavenging activity because of the presence of phenolics hydroxyl functionalities [13, 14]. The free radical scavenging property of phenolic natural products is mainly owing to their ability to act as reducing agents, hydrogen donors and singlet oxygen quenchers, and to some extent, could also be due to their metal chelation potential [10, 11]. Acetophenone (**3**) is used extensively as a food additive to create various fruit fragrances, e.g. almond, cherry, honeysuckle, jasmine, and strawberry. This compound appears to posses hypnotic and anticonvulsant property. Viscozulenic acid (**2**), isolated from *P. viscosum* [8], was previously assessed for its analgesic, anticholinergic, anti-inflammatory, CNS depressant, cytotoxic and anti-HIV-1 activities [15, 16].

Table 1. Free-radical-scavenging properties of compounds **1**-**3** in the DPPH assay

Compounds	RC_{50} value (mg/mL)
Sitosterone (**1**)	2.1.1 x 10^{-1}
Viscozulenic acid (**2**)	1.2 x 10^{-1}
Acetophenone (**3**)	1.8 x 10^{-1}
Trolox® (positive control)	2.6 x 10^{-3}

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