SHORT REPORT

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Chemical Constituents and their DPPH Radical Scavenging Activity of Nepalese Crude Drug *Begonia picta*

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Abstract: Vitexin (**1**), isovitexin (**2**), orientin (**3**), isoorientin (**4**) and 1,3–dihydroxy–6, 7–dimethoxyxanthone (**5**) were isolated from the whole plant of *Begonia picta*, a Nepalese crude drug commonly known as "Magarkaanche". Structures were elucidated on the basis of chemical and spectroscopic methods. All of these compounds were isolated for the first time from *B. picta* and their *in vitro* antioxidant activity was evaluated by diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Compounds **3** and **4** showed significant free radical scavenging activity.

Keywords: *Begoina picta;* vitexin; orientin; Flavonoid; Xanthone.

1. Plant Source

Begonia picta Sm. (Family: Begoniaceae) is succulent herb with tuberous rootstock up to 20 cm tall. It is locally called as "Magarkaanche" in Nepal and distributed throughout Nepal, Pakistan, India and Bhutan between 600-2800 m. Traditionally in Nepal, roots are taken to cure stomach trouble and leaves are used as a remedy for colic and dysentery. Decoction of plant is given in colic and dyspepsia [1]. The plant is used for headache, conjunctivitis and peptic ulcer. The crushed leaves are used as a poultice for sore nipples [2].

The fresh plant was collected from Bhalu Pahad, Syangja, Nepal in August 2013 and identified by Prof. Takashi Watanabe, Kochi University of Technology, Japan. The voucher specimen (Voucher

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2. Previous Studies

As far our knowledge, no previous chemical isolation has been performed on *B. picta* till date. However, different classes of anthocyanins were isolated from the flowers of different horticultural *Begonia* species [3]. Different steroids and flavonoids were also reported from *Begonia evansiana* [4] and *Begonia malabarica* [5]. Thus, the propose of this study was the isolation and identification of chemical constituents and evaluation of their DPPH free radical scavenging activity.such as kaurenoic acid, 3-acetyloxykaurenoic acid and cermamide have been isolated from *H. cameroonenses* [2].

3. Present Study

The shade dried whole plant of *B. picta* (120 g) was extracted successively with 70% MeOH (4.5 L) at 55°C (5 hours) and room temperature (about 20°C, for 6 days), and MeOH (4.5 L) at 55°C (5 hours) and room temperature (about 20°C, for 22 hours). The combined extract was evaporated under reduced pressure to give 12.3 g of extract. The extract was suspended in water (400 mL) to give water soluble (11.0 g) and water insoluble fraction (1.3 g). The water soluble fraction (11.0 g) was subjected on MCI gel CHP20P CC and eluted successively with water, 40%, 60%, 80% and 100% MeOH to give ten fractions (1~10). Fraction 4 (154 mg, 40% MeOH eluate) was subjected to Sephadex LH-20 CC (50% MeOH) to afford five subfractions (4-1~4-5). Fraction 4-4 (87 mg) was subjected on ODS CC (20% MeOH) to afford compound **4** (29 mg) and **3** (13 mg). Fraction 6 (120 mg, 40% MeOH eluate) was subjected to Sephadex LH-20 CC (50% MeOH) to afford compound **2** (8 mg) and **3** (16 mg). Fraction 8 (552 mg, 70% MeOH eluate) was subjected to Sephadex LH-20 CC (MeOH) to afford four subfractions (8-1~8-4). Fraction 8-2 (229 mg) was subjected on ODS CC (50% MeOH) to afford compound **1** (30 mg). Fraction 10 (709 mg, MeOH eluate) was subjected to Sephadex LH-20 CC (MeOH) and ODS CC (60% MeOH) to afford compound **5** (6 mg).

The structures of these compounds were elucidated as: vitexin (**1**) [6], isovitexin (**2**) [7], orientin (**3**) [8], isoorientin (**4**) [7,8], and 1,3–dihydroxy–6, 7–dimethoxyxanthone (**5**) [7] (Fig. 1) on the basis of spectral analysis (Table 1 and 2) and comparison with literature values. All of the compounds were isolated for the first time from this plant.

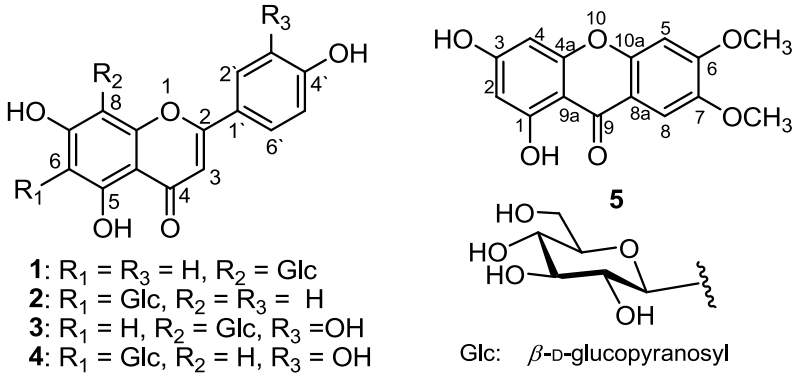

Figure 1. Structures of compouns **1-5**

Table 1. ^{1}H-NMR data of compounds **1**-**5**.

Position	**1** [a]	**2** [a]	**3** [b]	**4** [b]	**5** [a]
	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)
2					6.48, s
3	6.75, s	6.77, s	6.48, s	6.50, s	
4					6.91, s
5					6.13, s
6	6.26, s		6.24, s		
8		6.52, s		6.45, s	7.30, s
6,7 OCH_3					3.88, s
1`					
2`	8.01, d (8.2)	7.92, d (8.8)	7.45, brs	7.33, brs	
3`	6.89, d (8.2)	6.93, d (8.8)			
5`	6.89, d (8.2)	6.93, d (8.8)	6.89, d (8.2)	6.87, d (8.5)	
6`	8.01, d (8.2)	7.92, d (8.8)	7.37, brd (8.2)	7.35, brd (8.5)	
Glc1	4.68, d (10.0)	4.61, d (9.7)	4.98, d (9.7)	4.90, d (9.5)	
Glc2	3.17-3.88	4.05, dd (9.7, 8.8)	4.12, dd (9.5, 9.2)	4.16, dd (9.5, 9.2)	
Glc3	3.17-3.88	3.16-3.24	3.31-3.98	3.31-3.46	
Glc4	3.17-3.88	3.16-3.24	3.31-3.98	3.31-3.46	
Glc5	3.17-3.88	3.16-3.24	3.31-3.98	3.48, dd (5.2, 3.4)	
Glc6	3.17-3.88	3.68-3.71 3.42-3.45	3.31-3.98	3.88, dd (12.2, 2.4) 3.74, dd (12.2, 5.2)	

[a]DMSO-d_6, [b]CD_3OD

DPPH radical scavenging activity: The DPPH radical scavenging activity of **1**-**5** was measured by the method as previously described by Joshi *et al*., 2014 [9]. Briefly, 80 µL of each compound at various concentrations (in DMSO:EtOH = 1:1) was mixed with 40 µL of MES buffer (200 mM, pH 6.0) and 40 µL of DPPH solution (800 µM in EtOH) in a 96-well plate. The reaction mixture was shaken vigorously and left for 30 min. at room temperature in the dark. The anti-oxidative activity corresponding to the scavenging of DPPH radicals was measured at 510 nm with UV spectrophotometer using following formula: Radical scavenging activity (%) = 100 x (A-B)/A. Where, A is the control absorbance of DPPH radicals without samples and B is the absorbance after reacting with samples. Trolox was used as the positive control. The result is expressed as mean of four experiments. From these data, curve was plotted and concentration (μM) of the sample required for 50% reduction of the DPPH radical absorbance (IC_{50}) was calculated. All these isolated compounds were evaluated for their DPPH free radical scavenging activity. Orientin (**3**) (IC_{50} 54.0 μM) and isoorientin (**4**) (IC_{50} 53.4 μM) showed potent DPPH free radical scavenging activity as compared to positive control Trolox (IC_{50} 96.1 μM).

Table 2. ^{13}C-NMR data of compounds **1-5**.

Position	**1** [a]	**2** [a]	**3** [b]	**4** [b]	**5** [a]
	δ_C	δ_C	δ_C	δ_C	δ_C
1					163.7
2	164.0	163.6	166.0	166.2	98.8
3	102.4	102.9	103.7	103.9	164.2
4	182.1	181.9	183.9	184.0	94.2
4a					157.2
5	160.4	160.7	161.5	162.0	103.6
6	98.1	109.0	98.5	109.1	161.4
7	163.0	163.9	165.0	164.8	139.9
8	104.6[c]	93.9	108.8	95.2	104.4
8a					120.4
9	156.0	156.0	158.5	158.7	182.8
9a					103.8
10	104.0[c]	103.3	104.8	105.2	
10a					148.7
6,7 OCH_3					56.4
1`	121.6	121.2	123.4	123.5	
2`	129.0	128.6	114.2	114.2	
3`	116.0	116.1	146.4	147.0	
4`	161.1	161.6	150.6	151.0	
5`	116.0	116.1	117.0	116.8	
6`	129.0	128.6	120.6	120.3	
Glc1	73.4	73.0	74.8	75.3	
Glc2	70.8[c]	70.6[c]	72.0	72.6[c]	
Glc3	78.7	79.0	79.7	80.1	
Glc4	70.5[c]	70.3[c]	71.3	72.6[c]	
Glc5	82.0	81.6	82.1	82.6	
Glc6	61.3	61.6	61.3	62.9	

[a]DMSO-d_6, [b]CD_3OD
[c] assignment in the same column may be reversed

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