SUPPLEMENTARY MATERIAL

Antioxidant phenolic compounds from the rhizomes of *Astilbe rivularis*

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Abstract

The rhizomes of *Astilbe rivularis*, commonly known as "Thulo Okhati" are widely used in Nepal as tonic in uterine and menstrual disorders. In our preliminary study, the 70% MeOH extract of the rhizomes showed potent antioxidant activity. Hence, present study was aimed for the isolation of potent antioxidant constituents. Bergenin (**1**), 11-*O*-galloylbergenin (**2**), (+)-catechin (**3**), (-)-catechin (**4**), (-)-afzelechin (**5**), (-)-epiafzelechin (**6**) and 2-(*β*-D-glucopyranosyloxy)-4-hydroxylbenzenacetonitrile (**7**) were isolated from the rhizomes. Structures of these compounds were elucidated on the basis of spectroscopic methods. All of these isolated compounds were evaluated for their *in vitro* antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. 11-*O*-Galloylbergenin (**2**), (+)-catechin (**3**), (-)-catechin (**4**), (-)-afzelechin (**5**) and (-)-epiafzelechin (**6**) showed potent antioxidant activity.

Key words: *Astilbe rivularis*, Thulo Okhati, antioxidant activity, bergenin

Experimental

General Experimental Procedures

Optical rotations were measured with a JASCO DIP-1000KUY polarimeter. ^{1}H-, ^{13}C- and 2D-NMR spectra were measured on a JEOL α–500 (^{1}H-NMR: 500 MHz and ^{13}C-NMR: 125 MHz). Chemical shifts are given in ppm with reference to tetramethyl silane (TMS). Absorbance was recorded on Infinite 200 PRO® (Tecan Austria GmBH, Grodig, Austria). Column chromatography was carried out with MCI gel CHP20P (75 ~ 150 μm, Mitsubishi Chemical Industries Co. Ltd., Tokyo, Japan), Sephadex LH-20 (Amersham Pharmacia Biotech, Tokyo, Japan), Chromatorex ODS (30 ~ 50 μm, Fuji Silysia Chemical Co., Ltd., Aichi, Japan) and silica gel 60 (0.040-0.063 mm, Merck KGaA, Darmstadt, Germany). TLC was performed on a precoated silica gel 60 F_{254} (Aluminum sheet, Merck KGaA, Darmstadt, Germany).

Chemicals

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and Trolox were purchased from Wako Pure Chemicals, Osaka, Japan, and MES buffer was purchased from Dojindo Chemical Research, Kumamoto, Japan.

Plant material

The dried rhizomes of *A. rivularis* were purchased from crude drug market in Kathmandu, Nepal in August 2013 and identified by Prof. Takashi Watanabe, Kumamoto University. A voucher specimen (Voucher No.: KUNP20130811-05) was deposited at the Museum of Traditional Medicines, School of Pharmacy, Kumamoto University, Kumamoto, Japan.

Extraction and isolation

The dried rhizomes (235 g) were extracted three times with 70% MeOH (2 L) for 48 hours at room temperature. The combined extract was evaporated under reduced pressure to give 69.0 g extract. The extract (69.0 g) was then subjected on MCI gel CHP20P CC and eluted successively with water, 40%, 70% and 100% MeOH to give eight fractions (1~8). Fraction 2 (0.73 g, H_2O eluate) was subjected on Sephadex LH-20 CC (50% MeOH) and silica gel CC ($CHCl_3$:MeOH:H_2O = 9:1:0.1) to afford compound **7** (11.3 mg). A portion (5.0 g) of fraction 3

(22.1 g, 40% MeOH eluate) was subjected on Sephadex LH-20 CC (50% MeOH) to obtain nine fraction (3-1~3-9). Subfraction 3-8 was obtained as compound **1** (0.50 g). Subfraction 3-9 (90 mg) was subjected to ODS CC (15%, 20%, 25% MeOH) followed by Sephadex LH-20 CC (MeOH) to afford compound **3** (57.4 mg). Fraction 4 (1.66 g, 50% MeOH eluate) was subjected on Sephadex LH-20 CC (MeOH) to give seven fractions (4-1~4-7). Fraction 4-5 (0.38 g, MeOH eluate) was subjected on ODS CC (30%, 35%, 40%, 45% MeOH) followed by Sephadex LH-20 CC (MeOH) and silica gel CC ($CHCl_3$:MeOH:H_2O = 7:3:0.5) to afford compound **2** (46.8 mg)**, 4** (11.9 mg)**, 5** (6.1 mg) and **6** (46.6 mg).

Isolated compounds

bergenin (**1**): $[\alpha]_D^{25}$ −44.9° (*c* 0.34, MeOH),

11-*O*-galloylbergenin (**2**): $[\alpha]_D^{25}$ +15.0° (*c* 0.31, MeOH),

(+)-catechin (**3**): $[\alpha]_D^{25}$ +21.4° (*c* 0.29, MeOH),

(−)-catechin (**4**): $[\alpha]_D^{25}$ −10.9° (*c* 0.31, MeOH),

(−)-afzelechin (**5**): $[\alpha]_D^{25}$ −8.8° (*c* 0.31, MeOH),

(−)-epiafzelechin (**6**): $[\alpha]_D^{25}$ −48.3° (*c* 0.28, MeOH) and

2-(*β*-D-glucopyranosyloxy)-4-hydroxyl-benzenacetonitrile (**7**): $[\alpha]_D^{25}$ −5.8° (c 0.49, MeOH)

Free Radical Scavenging Activity

The DPPH radical-scavenging activity of extract and isolated compounds was examined using the method reported previously (Joshi et al. 2014) with slight modifications. Briefly, 50 μL of 200 mM MES [2-(*N*-morpholino) ethanesulphonic acid] buffer (pH 6.0), 100 μL of samples with different concentrations (in DMSO:Ethanol = 1:1) and 50 μL of 800 μM DPPH in ethanol solution were mixed in a 96-well plate and kept in dark at room temperature for 20 minutes. 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×(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. From these data, curve was plotted and effective concentration (EC_{50}) value was calculated which is defined

as the concentration (µg/mL or μM) of the extract and compounds required for 50% reduction of the DPPH radical absorbance.

Table S1. ^{1}H- and ^{13}C-NMR Data of Compounds **1** and **2** in CD_3OD

Position	**1**		**2**	
	δ_C	δ_H, mult.(*J* in Hz)	δ_C	δ_H, mult.(*J* in Hz)
2	82.9	3.87, m	81.1	3.86, m
3	71.8	3.47, t (9.0)	71.7	3.56, m
4	75.5	4,03, m	75.3	3.95, m
4a	81.3	4.07, m	80.6	4.10, m
6	165.7	-	165.6	-
6a	119.3	-	119.3	-
7	110.0	7.08, s	111.2	7.08, s
8	152.2	-	152.2	-
9	142.2	-	142.2	-
10	149.3	-	149.1	-
10a	117.2	-	116.9	-
10b	74.2	4.95, d (10.7)	74.3	5.01, d (10.6)
11	62.6	3.72, m	64.6	4.39-4.84, m
12 -OCH_3	60.8	3.88, s	61.0	3.89, s
14	-	-	168.1	-
1'	-	-	121.0	-
2', 6'	-	-	110.3	7.11, s
3', 5'	-	-	146.4	-
4'	-	-	139.9	-

Table S2. ^{13}C- NMR Data of Compounds **3**, **4**, **5**, **6** and **7**

Position	**3** (in CD_3OD)	**4** (in CD_3OD)	**5** (in CD_3OD)	**6** (in CD_3OD)	**7** (in D_2O)
1	-	-	-	-	112.1
2	82.8	82.0	82.9	80.0	130.9
3	68.8	67.8	68.9	67.5	110.4
4	28.4	27.6	28.9	29.4	157.1
5	157.6	156.5	157.6	157.7	103.4
6	96.4	96.7	96.4	96.5	155.2
7	157.7	156.1	157.9	157.5	17.6
8	95.6	95.8	95.6	96.0	120.4
9	156.8	156.1	157.0	157.5	-
10	100.9	101.3	101.0	100.1	-
1'	132.2	131,6	131.6	131.7	100.6
2'	115.3	115.5	129.6	129.2	73.0
3'	146.2	145.3	116.1	115.8	75.9
4'	146.2	145.4	158.4	158.1	69.6
5'	116.2	116.8	116.1	115.8	76.4
6'	120.1	120.5	129.6	129.2	60.8

Table S3. ^{1}H- NMR Data of Compounds **3**, **4**, **5**, **6** and **7**

Position	**3** (in CD_3OD)	**4** (in CD_3OD)	**5** (in CD_3OD)	**6** (in CD_3OD)	**7** (in D_2O)
2	4.58, d (7.5)	4.58, d (7.6)	4.59, d	4.58, brs	7.11, d (8.7)
3	3.97, m	3.98, m	3.98, m	4.18, m	6.49, d (2.3)
4	2.50, m	2.52, m	2.50, m	2.74, m	-
	2.84, m	2.86, m	2.87, m	2.88, m	-
5	-	-	-	-	6.60, dd (2.3,
6	5.94, d (2.3)	5.94, brs	5.84, d	5.94, d	-
7	-	-			3.67, s
8	5.87, d (2.3)	5.87, d (1.5)	5.93, d	5.91, d	-
1'	--	-	-	-	4.97, (7.6)
2'	6.83, d (1.9)	6.84, brs	7.22, d	7.31, d	3.20－3.81
3'	-	-	6.78, d	6.77, d	3.20－3.81
4'	-	-	-	-	3.20－3.81
5'	6.77, d (8.3)	6.77, d (7.9)	6.78, d	6.77, d	3.20－3.81
6'	6.71, dd (1.9,	6.73, dd (1.5,	7.22, d	7.31, d	3.20－3.81

References

Joshi KR, Devkota HP, Watanabe T, Yahara S. 2014. Thotneosides A, B and C: potent antioxidants from Nepalese crude drug, leaves of *Aconogonon molle*. Chem. Pharm. Bull. 62: 191–95.