

ORIGINAL RESEARCH ARTICLE

Amentoflavone and Kaempferol Glycosides from the Aerial Parts of *Cissampelos pareira*

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Abstract

Aerial parts of *Cissampelos pareira* L. (Family: Menispermaceae), commonly known as “Batulpate” in Nepal, are used for the treatment of fever, indigestion and to stop bleeding after child birth. In this study, a biflavonoid, amentoflavone; and two kaempferol glycosides, kaempferol 3-O- β -D-glucopyranoside and kaempferol 3-O- β -D-glucuronopyranoside were isolated from 70% MeOH extract of the aerial parts. Structures of these compounds were elucidated on the basis of ^1H - and ^{13}C -NMR spectral data. To the best of our knowledge, it is the first report on the isolation of these compounds from a plant belonging to family Menispermaceae.

Keywords: *Cissampelos pareira*; Menispermaceae; Batulpate; Flavonoids; Amentoflavone

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Introduction

Cissampelos pareira L. (Family: Menispermaceae), commonly known as “Batulpate” in Nepal, is a climbing herb with perennial root-stock. It is distributed throughout Nepal to about 3000 m altitude. The aerial parts and roots are widely used as traditional medicines in various ailments. The juice of the plant is administered orally to stop bleeding after child birth and to counteract the loss of blood. It is also used as tonic, diuretic and for the treatment of fever and indigestion. The juice is also applied locally to treat skin diseases and the decoction is used to relieve the pain of dislocated bones. The roots are used for the treatment of indigestion, constipation, cough and cold and applied locally in snake bite [1].

Previous studies have reported antiviral [2], antiprotozoal [3] and antifertility [4] activities of the extracts of aerial parts of *C. pareira*. Regarding chemical constituents, alkaloids are reported as major constituents in leaves [5] and roots [6-10]. Ramirez et al. [3] reported a chalcone-flavone dimer, cissampeloflavone as an antiprotozoal agent from the aerial parts and Ganguly et al. [4] also reported the presence of flavonoids in the leaves of *C. pareira*. However, there are no other reports about individual flavonoids present in *C. pareira* aerial parts. Thus, in present study, we focused on the phenolic constituents in the aerial parts and isolated one biflavonoid [1] and two flavonoid glycosides [2, 3], which are reported in this paper.

Materials and Methods

General Experimental Procedures

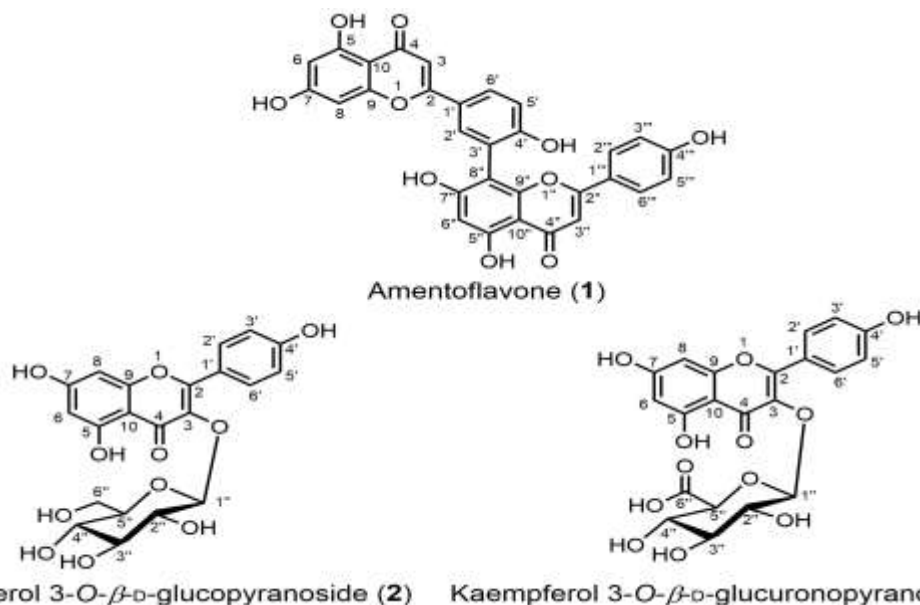
Optical rotations were measured with a JASCO DIP-1000KUY polarimeter. ^1H -, ^{13}C -Nuclear magnetic resonance (NMR) spectra were measured on a JEOL α -500 spectrometer. Chemical shifts are given in ppm with reference to tetramethyl silane (TMS). Column chromatography was carried out with MCI gel CHP20P (75 ~ 150 μm , Mitsubishi Chemical Industries Co. Ltd., Tokyo, Japan), Sephadex LH-20 (Amersham Pharmacia Biotech, Tokyo, Japan) and silica gel 60 (0.040-0.063 mm, Merck KGaA, Darmstadt, Germany). Thin layer chromatography (TLC) was performed on a precoated silica gel 60 F254 (Aluminum sheet, Merck KGaA, Darmstadt, Germany) and 10% sulfuric acid spray followed by heating was used for derivatization.

Plant Material

Fresh aerial parts of *C. pareira* were collected on June 2010, from Lumle, Kaski District, Nepal and shade dried for two weeks. A voucher specimen has been deposited at Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

Extraction and Isolation

The shade dried aerial parts (58.0 g) were extracted with 70% methanol (MeOH) (1.5 L x 2 times) at room temperature and the combined extracts were evaporated under reduced pressure to obtain dried

**Figure 1:** Structures of the isolated compounds**Table 1:** ¹H- and ¹³C-NMR data of compound **1** in DMSO-*d*₆

C No	δ _c	δ _H , mult. (J in Hz)	C No.	δ _c	δ _H , mult. (J in Hz)
2	163.9*		2''	163.7	
3	102.5	6.82, s	3''	102.4	6.77, s
4	181.6		4''	182.0	
5	161.3		5''	160.4	
6	98.8	6.18, d (2.1)	6''	98.7	6.36, s
7	163.5*		7''	161.4	
8	93.8	6.44, d (2.1)	8''	103.9	
9	157.2		9''	154.6	
10	103.9		10''	103.9	
1'	121.2		1'''	121.2	
2'	127.6	8.02, d (2.4)	2'''	128.0	7.58, d (8.8)
3'	121.2		3'''	115.7	6.70, d (8.8)
4'	159.5		4'''	160.4	
5'	116.1	7.12, d (8.5)	5'''	115.7	6.70, d (8.8)
6'	131.2	7.99, dd (2.4, 8.5)	6'''	128.0	7.58, d (8.8)

extract (10.0 g). The extract was then suspended in water and separated into water soluble and

insoluble fractions. The water soluble fraction (7.9 g) was subjected to MCI gel CHP20P column chromatography (CC) and eluted successively with water, 40%, 70% and 100% MeOH to obtain total 8 fractions (Fr. 1~8). A flavonoid rich fraction, Fr. 6 (200 mg, 70% MeOH eluate) was subjected to Sephadex LH-20 CC (MeOH) to obtain 5 subfractions (Subfr. 6-1~6-5). Subfractions 6-3 and 6-4 were combined (30 mg) and subjected to silica gel CC (CHCl₃:MeOH:H₂O = 8:2:0.1) to obtain kaempferol 3-O-β-D-glucopyranoside; compound **2** (5.0 mg) and kaempferol 3-O-β-D-glucuronopyranoside; compound **3** (2.0 mg). The water insoluble fraction (2.1 g) was subjected to Sephadex LH-20 CC (MeOH) to obtain 8 fractions (Fr. 11~18). Among them, Fr. 17 (74 mg) was subjected to silica gel CC (CHCl₃:MeOH = 20:1) to obtain amentoflavone; compound **1** (2.4 mg).

Amentoflavone (1)

A pale yellow amorphous powder; ¹H- and ¹³C-NMR (in DMSO-*d*₆), **Table 1**.

Kaempferol 3-O-β-D-glucopyranoside (2)

A pale yellow amorphous powder; [α]_D²⁰ -35.8° (c 0.75, pyridine); ¹H- and ¹³C-NMR (in CD₃OD+D₂O) **Table 2**.

Kaempferol 3-O-β-D- glucuronopyranoside (3)

A pale yellow amorphous powder; [α]_D²⁰ -51.2° (c

0.52, pyridine:water = 1:1); ^1H - and ^{13}C -NMR (in DMSO- d_6), **Table 2**.

Table 2: ^1H - and ^{13}C -NMR data of compounds **2** and **3**

C No.	2 (in CD ₃ OD+D ₂ O)		3 (in DMSO- d_6)	
	δ_c	$\delta_{\text{H, mult.}}$ (J in Hz)	δ_c	$\delta_{\text{H, mult.}}$ (J in Hz)
1				
2	157.0		156.2	
3	135.1		133.0	
4	179.0		177.3	
5	161.6		161.0	
6	100.0	6.26, d (1.8)	98.6	6.02, d (1.8)
7	164.6		164.7	
8	95.3	6.40, d (1.8)	93.8	6.22, d (1.8)
9	159.2		156.3	
10	105.6		103.5	
1'	122.6		121.0	
2', 6'	132.0	7.97, d (8.5)	130.9	8.00, d (8.5)
3', 5'	116.2	6.96, d (8.5)	115.0	6.83, d (8.5)
4'	160.2		160.0	
1''	103.7	5.02, d (6.7)	100.8	5.53, d (7.6)
2''	74.9	3.17–3.90	74.1	3.30–3.80
3''	77.4	3.17–3.90	75.4	3.30–3.80
4''	70.5	3.17–3.90	72.1	3.30–3.80
5''	77.0	3.17–3.90	76.4	3.30–3.80
6''	61.8	3.17–3.90	172.2	

Results and Discussion

In this study, the aerial parts of *C. pareira*, traditionally used for the treatment of various disorders in folk medicine were investigated for their chemical constituents. Three compounds (1-3) were isolated from the 70% MeOH extract by repeated column chromatography on MCI gel CHP20P, Sephadex LH20 and silica gel. The structures of these compounds were identified as amentoflavone [11], kaempferol 3-*O*- β -D-glucopyranoside [12] and kaempferol 3-*O*- β -D-glucuronopyranoside [13] (**Figure 1**) on the basis of NMR spectral data and comparison with reported values in literatures.

To the best of our knowledge, it is the first report on the isolation of these compounds from a plant belonging to family Menispermaceae. Flavonoids and biflavonoid are one of the most widely studied chemical classes of natural products for their distribution in plant families, chemical diversity and various health beneficial effects. Amentoflavone has been isolated and identified from more than 120 plant species and reported to have several pharmacological effects including potent antioxidant and anti-inflammatory activities [14]. Similarly, kaempferol and/or its glycosides were also reported from more than 400 plant species and showed potent antioxidant, anti-inflammatory, antitumor and antimicrobial activities [15].

In conclusion, three compounds, amentoflavone [1], kaempferol 3-*O*- β -D-glucopyranoside [2] and kaempferol 3-*O*- β -D-glucuronopyranoside [3] were isolated from the aerial parts of *C. pareira*. These compounds can be utilized as chemotaxonomic markers for the identification of crude drugs based on the aerial parts of *C. pareira*. Further studies are needed to find the possible relationship between these constituents and traditional uses and reported bioactivities of the extracts.

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