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# Prenylated flavonol glycosides from *Epimedium grandiflorum*: Cytotoxicity and evaluation against inflammation and metabolic disorder

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ABSTRACT

Two new prenylated flavonol glycosides, epimedigrandiosides A and B (**1** and **2**), and 28 previously known compounds including prenylated flavonol derivatives, flavonol glycoside, megastigmanes, phenyl alkanoids, sesquiterpenoid glycoside, lignan, and hexene glucoside were isolated from the methanol extract of *Epimedium grandiflorum*. Structure elucidation was achieved by means of spectroscopic and spectrometric techniques including 1D and 2D NMR and HRESIMS. The absolute configuration of sugars was determined by chemical methods Structure elucidation of 3‴-carbonyl-2″-β-L-quinovosyl icariin (**19**) was not previously described, so its $^{1}H$ and $^{13}C$ NMR data were reported for the first time. The methanol extract and the isolated compounds were evaluated for their activity towards several targets related to inflammation and metabolic disorder including NF-κB, iNOS, PPARα and PPARγ. Moreover, their cytotoxic activity against four cancer cell lines (SK-MEL, KB, BT-549, SK-OV-3) and two noncancerous kidney cell lines (LLC-PK1 and Vero) were also evaluated.

## 1. Introduction

The genus *Epimedium* (Berberidaceae) has various names such as horny goat weed, rowdy lamb herb, bishop's hat, fairy wings, barrenwort, randy beef grass, and yin yang huo and various of its species have been used traditionally as a tonic, aphrodisiac, and antirheumatic for centuries in China, Japan and Korea (Ye and Chen, 2001). A number of biological activities have been associated with this genus, such as anti-osteoporosis, antitumor, antidepressant, antimicrobial, anti-oxidant, anti-inflammatory, antihepatotoxic, antiradiation, anti-aging, immunomodulatory, cardiovascular and hormone regulatory, as well as neuroprotective (Ma et al., 2011; Sze et al., 2010). *Epimedium* plants are distributed in China, North Africa, North Italy, Korea, Japan, and some countries in South East Asia. There are 55 species in the genus *Epimedium,* and 47 species were found in China (Mabberley, 2008). More than 15 species in this genus have been marketed as crude drugs (Guo and Xiao, 2003) but only five, *E. brevicornum* Maxim., *E. sagittatum* Maxim., *E. koreanum* Nakai, *E. pubescens* Maxim., and *E. wushanense* T.S. Ying are officially recorded in the Chinese Pharmacopoeia (Commmission, 2010). Over 260 compounds, mainly the flavonoids, have been reported from different species of *Epimedium*. Other reported compounds from *Epimedium* are polysaccharides, lignans, sesquiterpenoids, alkaloids, and phenylalkanoids (Ma et al., 2011). Among the isolated compounds, prenylflavonoid glycosides are recognized as the major bioactive constituents of the *Epimedium* (Ma et al., 2011; Sze et al., 2010). Over fifty secondary metabolites have been reported from *Epimedium grandiflorum* (Ma et al., 2011). The Chinese crude medicine, containing *E. grandiflorum* as a part of the composition, has been reported to possess anti-inflammatory and cardiovascular effects (Li, 2016; Yu, 2006). In an effort to find potential bioactive candidates, thirty secondary metabolites of *E. grandiflorum* including two new prenylated flavonoid glycosides (**1** and **2**) were isolated and characterized. This paper describes a phytochemical investigation of *E. grandiflorum* and activity evaluation of the crude extract and the isolated compounds against inflammation and metabolic disorder, as well as their cytotoxicity.

## 2. Results and discussion

Isolation and characterization of two new flavonoids, icaritin 3-*O*-β-D-(4‴, 6‴-di-*O*-acetyl) glucopyranosyl-(1 → 3)-α-L(4″-*O*-acetyl) rhamnopyranoside (epimedigrandioside A, **1**) and desmethylicaritin 3-*O*-β-D-(3‴, 6‴-di-*O*-acetyl) glucopyranosyl-(1 → 3)-α-L(4″-*O*-acetyl)rhamnopyranosyl-7-*O*-β-D-glucopyranoside (epimedigrandioside B, **2**) and 28 known compounds (Fig. 1) were carried out for a MeOH extract of *E. grandiflorum*. Various chromatographic procedures using Sephadex LH-20, silica gel, RP-18 silica, and preparative TLC were applied to purify the compounds. Their structures were determined on the basis of

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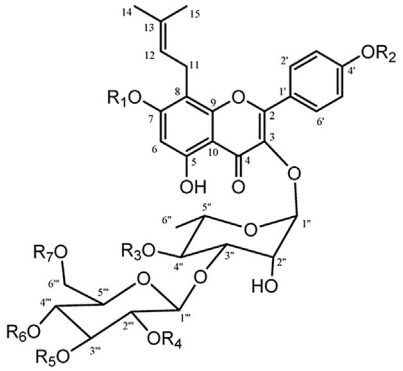
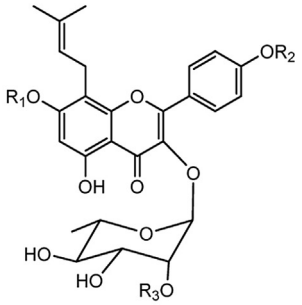
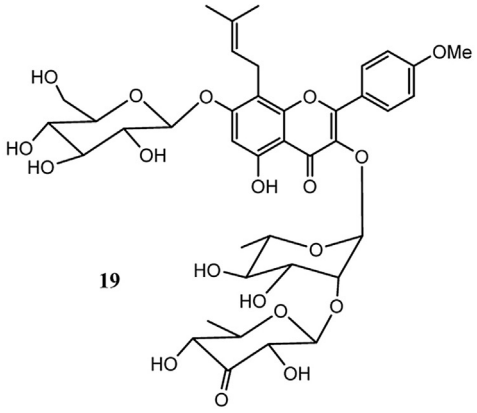


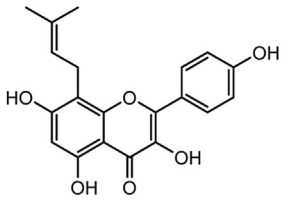
| | R1 | R2 | R3 | R4 | R5 | R6 | R7 |
|---|---|---|---|---|---|---|---|
| **1** | H | Me | Ac | H | H | Ac | Ac |
| **2** | Glc | H | Ac | H | Ac | H | Ac |
| **3** | H | Me | Ac | H | Ac | H | Ac |
| **4** | H | Me | Ac | Ac | H | H | Ac |
| **5** | H | Me | Ac | H | H | H | H |
| **6** | H | Me | Ac | H | H | H | Ac |
| **7** | Glc | Me | Ac | H | Ac | H | Ac |
| **8** | Glc | Me | Ac | H | H | H | H |
| **9** | Glc | Me | Ac | Ac | H | H | Ac |



| | R1 | R2 | R3 |
|---|---|---|---|
| **10** | H | Me | H |
| **11** | H | Me | Xyl |
| **12** | H | Me | Rha |
| **13** | H | Me | Glc |
| **14** | Glc | Me | Rha |
| **15** | Glc | Me | Xyl |
| **16** | Glc | Me | Glc |
| **17** | Glc | H | H |
| **18** | Glc | Me | Rha |



**19**



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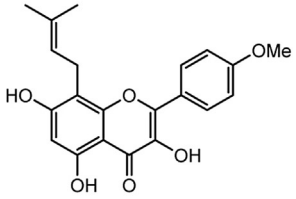

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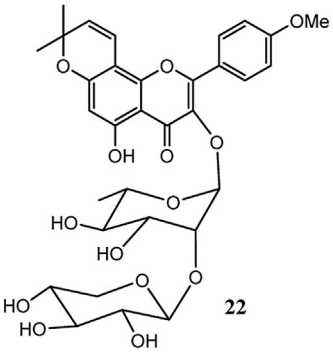

**22**

**Fig. 1.** Structures of isolated prenylated flavonol derivatives.

spectroscopic, spectrometric, and/or chemical methods.

Compound **1** was obtained as a yellow powder and its molecular formula $C_{39}H_{46}O_{18}$ was determined by HRESIMS from a protonated ion $[M+H]^+$ at *m/z* 803.2789 (calcd. for $C_{39}H_{47}O_{18}$, 803.2762) and $^{13}C$ NMR data. The UV spectrum of **1** showed absorptions at 270, 313, and 355 nm. The IR spectrum showed bands for hydroxyl, ester carbonyl, and chelated carbonyl groups at 3366, 1728, and 1650 $cm^{-1}$, respectively. The $^{13}C$ NMR spectrum of **1** showed 39 carbon resonances, of which 15 were assignable to a flavonoid skeleton, one to a methoxy group, five to a prenyl moiety, six to three acetyl groups, and twelve to two sugar units. A DEPT NMR experiment was used to differentiate the $^{13}C$ NMR resonances as seven methyl, two methylene, sixteen methine, and fourteen non-protonated carbons. The $^1H$ and $^{13}C$ NMR spectra exhibited characteristic resonances for a methoxy group [$\delta_H/\delta_C$ 3.85(s)/56.0 (4′-OMe)], an aromatic methine [$\delta_H/\delta_C$ 6.30 (s)/98.9 (CH-6)], a set of $A_2B_2$ *ortho*-coupled protons of 1,4-disubtituted aromatic ring [$\delta_H/\delta_C$ 7.83 (d, $J$ = 8.9 Hz)/130.8 (CH-2′ and 6′), and 7.14 (d, $J$ = 8.9 Hz)/114.6 (CH-3′ and 5′)], and a prenyl group [$\delta_H/\delta_C$ 3.35 (overlap)/21.6 ($CH_2$-11), 5.14 (t, $J$ = 6.6 Hz)/122.7 (CH-12), 1.59 (s)/25.9 ($CH_3$-14), 1.64 (s)/18.2 ($CH_3$-15) and $\delta_C$ 131.4 (C-13)] (Tables 1 and 2). Furthermore, the $^{13}C$ NMR spectrum showed characteristic resonances attributed to a flavonol skeleton for a conjugated oxo group [$\delta_C$ 178.2 (C-4)], two aromatic non-protonated carbons [$\delta_C$ 104.5 (C-10) and 122.6 (C-1′)], and six aromatic oxygenated carbons [$\delta_C$ 157.2 (C-2), 134.2 (C-3), 159.3 (C-5), 162.5 (C-7), 154.3 (C-9), and 162.0 (C-4′)] and $^1H$ NMR spectrum exhibited a characteristic singlet of 5-OH at $\delta_H$ 12.43 (s). The assignment of $^1H$ and $^{13}C$ NMR spectroscopic data for **1** (Tables 1 and 2) was based on $^1H$–$^1H$ COSY couplings and HSQC and HMBC correlations (Fig. 2). The HMBC correlations of H-2′/H-6′, H-3′/H-5′, and 4′-O*CH*$_3$ with C-4′ suggested that C-4′ was substituted by a