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A immunosuppressive triterpenoid saponin from the stems of *Epigynum griffithianum*

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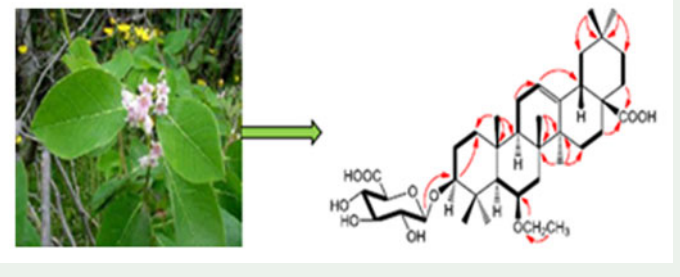
ABSTRACT

Chemical investigation of the stems of *Epigunum griffithianum* led to the isolation and identification of a new triterpenoid saponin (**1**) and two known compounds (epigynosides A (**2**) and B (**3**)). These structures were elucidated by means of spectroscopic analysis (1D and 2D NMR, MS, UV, IR) as well as comparison with the reported data. Compound 1 was evaluated in vitro for the immunosuppressive activities on proliferation of mice splenocyte and displayed significant immunosuppressive activities compared to the positive control (dexamethasone) with the concentration at 25 μM.



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Epigunum griffithianum; triterpenoid saponin; immunosuppressive activity

1. Introduction

Oleanane-type pentacyclic triterpenoids are commonly found in higher plants in the form of the free acids or more frequently as aglycones of saponins (Sparg et al. 2004). It was one of the important types of biological active compounds in natural medicinal history. In addition, there have been numerous reports of the anti-inflammatory (Ratz-Łyko et al. 2016), immunomodulatory (Singh et al. 2016; Top et al 2017), cytotoxic (Dai et al. 2017; Soberón et al. 2017), antitumor (Sun et al. 2017; Zhang et al. 2017), antimutagenic (Krizková et al. 2004), antihepatotoxic (Siddiqui et al. 2000), antidiabetic

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(Koneri et al. 1998; Singh et al. 2014; Feng et al. 2015), antibacterial (Lee et al. 2015; Gossan et al. 2017; Muhammad et al. 2016), and antiparasitic (Mongalo et al. 2015) activities of oleanolic acid and its saponins. Because of the wide spectrum of pharmaceutical activities of oleanane triterpenoids, these compounds appear to be promising drugs of natural origin. The phytochemical investigation on *Epigynum* genus plants (Apocynaceae) reported the isolation of triterpenoid saponins (Cao et al. 2003; Gao et al. 2017; Wan et al. 2017; Shao et al. 2018). In order to searching more biologically active secondary metabolites from this genus, a new triterpenoid saponin (**1**), together with two known compounds, epigynosides A (**2**) and B (**3**) (Figure 1), were isolated from this species collected from the republic of the union of myanmar. This paper describes the isolation and identification of these compounds. In addition, the immunological activities of the new compounds were evaluated against concanavalin A (Con A)-stimulated proliferation of mice splenocyte in vitro. Compound 1 exhibited

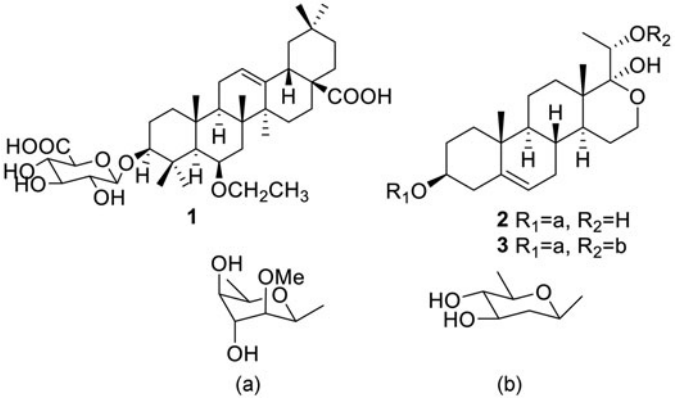

Figure 1. The chemical structure of compounds **1–3**.

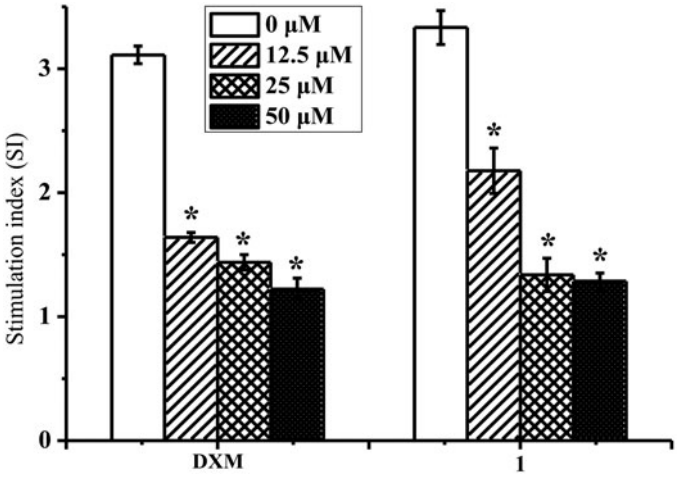

Figure 2. Effect of compound **1** on Con A-stimulated splenocyte proliferation in vitro. DXM was used as the positive control. The values were presented as mean ± SD of triplicate replicates. Statically significant difference was determined by ANOVA and Tukey test ($^{*}p < 0.05$, compared with the control group).

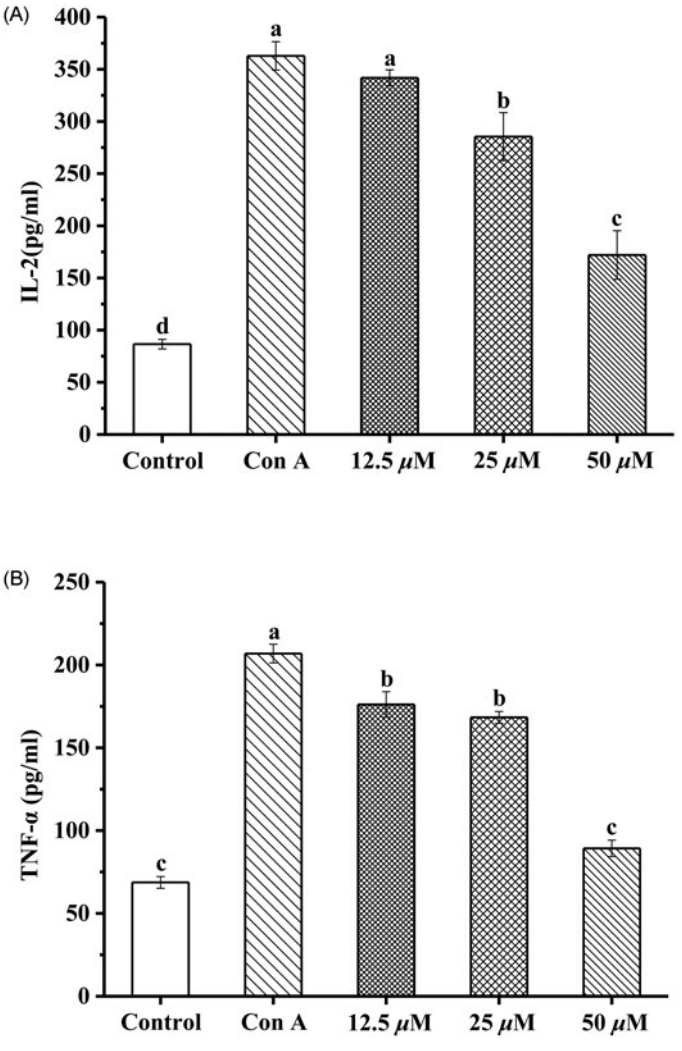

Figure 3. Investigation of the immunosuppressive effect of **1** on splenocytes of male *Balb/c* mice in Con A-activated T cells. The concentritions of IL-2 and TNF-α in the supernatant of treatment with different concentrations of detected compound for 48 h. In figure, compound **1** at concentritions of 12.5 μM, 25 μM and 50μM. The values were presented as mean ± SD of triplicate replicates. Different capital letters indicate significant differences ($p < 0.05$).

significantly inhibitory effect on the Con A-stimulated mice splenocyte proliferation in vitro (Figures 2 and 3).

2. Result and discussion

Compound **1** was obtained as a white amorphous powder; [α]25D-31.0 (c 0.09, MeOH). Its molecular formula was assigned as $C_{38}H_{60}O_{10}$ based on the sodium adduct high resolution electrospray ionization mass spectroscopy (HRESIMS) ion at m/z 699.4079 $[M + Na]^+$, calcd 699.4084 and NMR data. The IR spectrum revealed the presence of hydroxy, carbonyl and olefinic functional groups from absorption maxima at

3448, 1701, 1639 cm^{-1}. Acid hydrolysis of **1** afforded a D-glucuronic acid, based on gas chromatography analysis following treatment with L-cysteine methyl ester hydrochloride and trimethylsilylimidazole (TMS) derivatization, and coupling pattern of the anomeric proton (d, J = 8.0 Hz) indicated a β configuration for the glucuronopyranosyl unite. Besides, the ^{1}H and ^{13}C NMR spectroscopic data (Table S1) revealed one oxymethine signal (δ_H 3.38, 1H, dd, J = 11.6, 4.2 Hz; δC 89.4), a trisubstituted olefin system (δ_H 5.55, 1H, t, J = 3.4 Hz; δC 144.2, 122.9), as well as seven singlet methyl proton signals at δ_H (1.49, 1.66, 1.51, 1.55, 1.34, 1.00, and 0.95 The above observations displayed signals characteristic of an oleanane triterpene glycoside. The β-D-glucuronopyranosyl moiety connected at C-3 was supported by heteronuclear multiple bond correlation (HMBC) correlations from H-3 (δ_H 3.38, 1H, dd, J = 11.6, 4.2 Hz) to C-1′ (δ_C 107.4) and H-1′ (δ_H 5.03, 1H, d, J = 8.0 Hz) to C-3 (δ_C 89.4) (Figure S1). The coupling pattern of H-3 (dd, J = 11.6, 4.2 Hz) suggested the α-orientation of this proton, which was proven by the ROESY correlation from H-3 (δ_H 3.38) to H-5 (δ_H 0.89). There was one carbonyl carbon linked at C-17, as supported by the correlations from H-16 (δ_H 2.12), H-18 (δ_H 3. 33), H2-22 (δ_H 2.04, 1.81) to C-17 (δ_C 180.1) in the HMBC spectrum. The HMBC correlations of H-31(δ_H 4.26) with C-6 (δ_C 67.3) and H-32 (δ_H 1.17) with C-31 (δ_C 61.2) suggested that there was a ethyoxyl linked at C-6, and the correlation of H-6 (δH 4.76) with H-5 (δ_H 0.89) indicated a β configuration for the ethyoxylunit. Finally, compounds 1was determined as 3β-hydroxyoleane-6β-oxethyl-12-en-28-oic acid 3-O-β-D-glucuronic acid. The known compounds were identified as epigynosides A and B (**2** and **3**) (Wan et al. 2017) and by comparison with the reported data

3. Conclusions

Compounds **1** was determined as 3β-hydroxyoleane-6β-oxethyl-12-en-28-oic acid 3-O-β-D-glucuronic acid which displayed significant immunosuppressive activities compared to the positive control (dexamethasone) with the concentration at 25 μM.

Acknowledgement

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Disclosure statement

We declare that there is no conflict of interest existed in this paper.

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