ABSTRACT

Chemical investigation of the dichloromethane extract of the leaves of Hoya pubicalyx Merr. yielded taraxerol (1), β-sitosterol (2) and stigmasterol (3). The structures of 1–3 were identified by comparison of their NMR data with those reported in the literature.

Keywords: Apocynaceae, Hoya pubicalyx, β-sitosterol, stigmasterol, taraxerol

INTRODUCTION

Hoya is the largest genus in the family Apocynaceae which is also considered as one of the largest families of flowering plants [1]. Hoya pubicalyx is one of at least 109 species of Hoya found in the Philippines. The original plant described was collected from Mauban, Quezon (formerly Tayabas) province, Luzon island in the Philippines and is believed to be endemic [2]. As a scandent vine with fleshy, oblong to oblong-ovate leaves, it produces globose umbels of flowers ranging in color from pink to purplish to dark red to reddish maroon that last up to 8 days. It has been commercialized as an ornamental plant world-wide due to its floriferousness and cold-hardiness in temperate countries. However, no report on its chemistry or ethnobotany has been found.

This study is part of our research on the chemical constituents of Philippine native hoyas. We earlier reported the isolation of lupenone and lupeol from the roots; lupeol, squalene and β-sitosterol from the leaves; and betulin from the stems of H. mindorensis Schlechter [3]. In another study, we reported the isolation of α-amyrin, β-amyrin, lupeol acetate, α-amyrin acetate, and β-amyrin acetate from the stems; and α-amyrin, bauceronol, squalene, lutein, β-sitosterol, and stigmasterol from the leaves of H. multiflora Blume [4]. Moreover, the isolation of β-amyrin cinnamate and taraxerol from the stems; and taraxerol, triglycerides, chlorophyll a, and a mixture of β-sitosterol and stigmasterol from the leaves of H. wayetii Kloppenb. has been reported [5]. Furthermore, the isolation of taraxerol, taraxerone, β-sitosterol, stigmasterol, α-amyrin cinnamate and β-amyrin cinnamate from the stems; taraxerol,
taraxerone, and β-sitosterol from the roots; α-amyrin cinnamate and β-amyrin cinnamate from the flowers; and squalene, β-sitosterol, and saturated hydrocarbons from the leaves of *H. buotii* has been reported [6]. We also reported the isolation of β-amyrin cinnamate, squalene, β-sitosterol, β-amyrin, α-amyrin, lupeol and saturated hydrocarbons from the leaves; and squalene, taraxerol, lupeol cinnamate, β-sitosterol and stigmasterol from the stems of *H. diversifolia* [7]. Recently, the isolation of taraxerol, taraxeryl acetate, α-amyrin acetate, and β-amyrin acetate was reported from the stems of *H. paziae* Kloppenb. [8].

In this study, the dichloromethane extract of *H. pubicalyx* yielded taraxerol (1), β-sitosterol (2) and stigmasterol (3). The chemical structures of 1–3 are presented in Fig. 1. To the best of our knowledge this is the first report on the isolation of 1–3 from *C. pubicalyx*.

**Fig. 1.** Chemical structures of taraxerol (1), β-sitosterol (2) and stigmasterol (3) from Hoya pubicalyx.

**MATERIALS AND METHODS**

**General Experimental Procedure**

NMR spectra were recorded on a Varian VNMRS spectrometer in CDCl$_3$ at 600 MHz for $^1$H NMR and 150 MHz for $^{13}$C NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography, was performed with plastic backed plates coated with silica gel F$_{254}$ and the plates were visualized by spraying with vanillin/H$_2$SO$_4$ solution followed by warming.

**Sample Collection**

Vines of *H. pubicalyx* were harvested from a cultivated plant designated as H.PFR. It is authenticated by one of us (FBA) under Material Transfer Agreement No. 2015-10.

**General Isolation Procedure**

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. Fifty milliliter fractions were collected. Fractions with components possessing the same R$_f$ values by TLC were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. All fractions were monitored by thin layer chromatography. A glass column 12 inches in height and 0.5 inch internal diameter was used for further purification. Five milliliter fractions were collected. Rechromatography and final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

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Isolation
The air-dried stems (53.1 g) of *H. pubicalyx* were ground in an Osterizer blender, soaked in CH$_2$Cl$_2$ for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (1.3 g) which was chromatographed by gradient elution with CH$_2$Cl$_2$ and increasing amounts of acetone by 10% increments by volume. The 10% acetone in CH$_2$Cl$_2$ fraction was rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to yield 1 (2 mg) after washing with petroleum ether. The 30% acetone in CH$_2$Cl$_2$ fraction was rechromatographed (3 ×) using CH$_2$Cl$_2$ to afford a mixture of 2 and 3 (5 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION
Silica gel chromatography of the dichloromethane extracts of *H. pubicalyx* yielded compounds 1–3. The structures of 1–3 were identified by comparison of their NMR data with literature data. The NMR spectra are in accordance with data reported in the literature for taraxerol (1) [9]; β-sitosterol (2) [10], and stigmasterol (3) [10].

These results indicate that *H. pubicalyx* shares similar chemical characteristics with other members of the genus *Hoya*: *H. wayetii* [5], *H. buotii* [6], *H. diversifolia* [7] and *H. paziae* [8] which yielded taraxerol (1); and *H. mindorensis* [3], *H. multiflora* [4], *H. wayetii* [5], *H. diversifolia* [7] and *H. buotii* [6] which contained β-sitosterol (2) and stigmasterol (3).

Although no biological activity tests were conducted on the isolated compounds, a literature search of 1–3 revealed that these have diverse bioactivities.

Taraxerol (1) was reported to exhibit anti-inflammatory activity by selective COX-1 inhibition [11]. Another study reported that 1 downregulates the expression of proinflammatory mediators in macrophages by preventing NF-κB activation [12]. Furthermore, 1 was shown as a glucose transport inhibitor and stimulator of glycogen synthesis [13]. Moreover, 1 inhibited the growth of Hela and BGC-823 with IC$_{50}$ of 73.4 µmol/L$^{-1}$ and 73.3 µmol/L$^{-1}$, respectively [14].

β-Sitosterol (2) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [15]. It was shown to be effective for the treatment of benign prostatic hyperplasia [16]. It was also reported to attenuate β-catenin and PCNA expression, as well as quench free radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis [17]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [18]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [19].

Stigmasterol (3) shows therapeutic efficacy against Ehrlich ascites carcinoma in mice while conferring protection against cancer induced altered physiological conditions [20]. It lowers plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Winstar and WKY rats [21]. Other studies reported that stigmasterol showed cytostatic activity against Hep-2 and McCoy cells [22], markedly inhibiting tumour promotion in two stage carcinogenesis experiments [23], exhibited antimutagenic [24], topical anti-inflammatory [25], antiosteoarthritic [26] and antioxidant [27] activities.

CONCLUSION
The dichloromethane extracts of *H. pubicalyx*, a plant endemic to the Philippines, afforded taraxerol (1), β-sitosterol (2), and stigmasterol (3) which have been reported to exhibit diverse biological activities.

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REFERENCES