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Secondary Metabolites from *Hoya pubicalyx* Merr.

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ABSTRACT

Chemical investigation of the dichloromethane extract of the leaves of *Hoya pubicalyx* afforded 2-hydroxyethyl benzoate (**1**), a mixture of fatty acid methyl esters (**2**) and lupenone (**3**) in a 3:1 ratio, and a mixture of β-sitosterol (**4**) and stigmasterol (**5**) in a 3:1 ratio. The structures of **1**-**5** were identified by comparison of their NMR data with those reported in the literature.

Keywords: Apocynaceae, fatty acid methyl esters, *Hoya pubicalyx*, 2-hydroxyethyl benzoate, lupenone, β-sitosterol, stigmasterol

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INTRODUCTION

Apocynaceae is one of the largest families of flowering plants and *Hoya* is considered the largest genus in this family [1]. Among the endemic species of the Philippines, *Hoya pubicalyx* is one of at least 109 species of *Hoya* native to the country. It was described from a plant originally collected in Mauban, Quezon (formerly Tayabas) province, Luzon island [2]. This species is a scandent vine with fleshy, oblong to oblong-ovate leaves. The plant produces globose umbels of flowers which range in color from pink to purplish to dark red to reddish maroon and last up to 8 days. Its ability to produce many flowers and withstand cool temperatures in temperate countries are the characteristics that help its successful commercialization as an ornamental plant world-wide. In an earlier study, we reported the isolation of taraxerol, β-sitosterol and stigmasterol from the stems of *H. pubicalyx* [3].

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 [4]. 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, bauerenol, squalene, lutein, β-sitosterol, and stigmasterol from the leaves of *H. multiflora* Blume [5]. 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 [6]. 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 [7]. 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* [8]. Recently, the isolation of taraxerol, taraxeryl acetate, α-amyrin acetate, and β-amyrin acetate was reported from the stems of *H. paziae* Kloppenb. [9].

In this study, we obtained 2-hydroxyethyl benzoate (**1**), a mixture of fatty acid methyl esters (**2**) and lupenone (**3**), and another mixture of β-sitosterol (**4**) and stigmasterol (**5**) from the leaves of *H. pubicalyx.* The chemical structures of **1**-**5** are presented in Fig. 1. To the best of our knowledge, this is the first report on the isolation of **1**-**3** from *H. pubicalyx.*

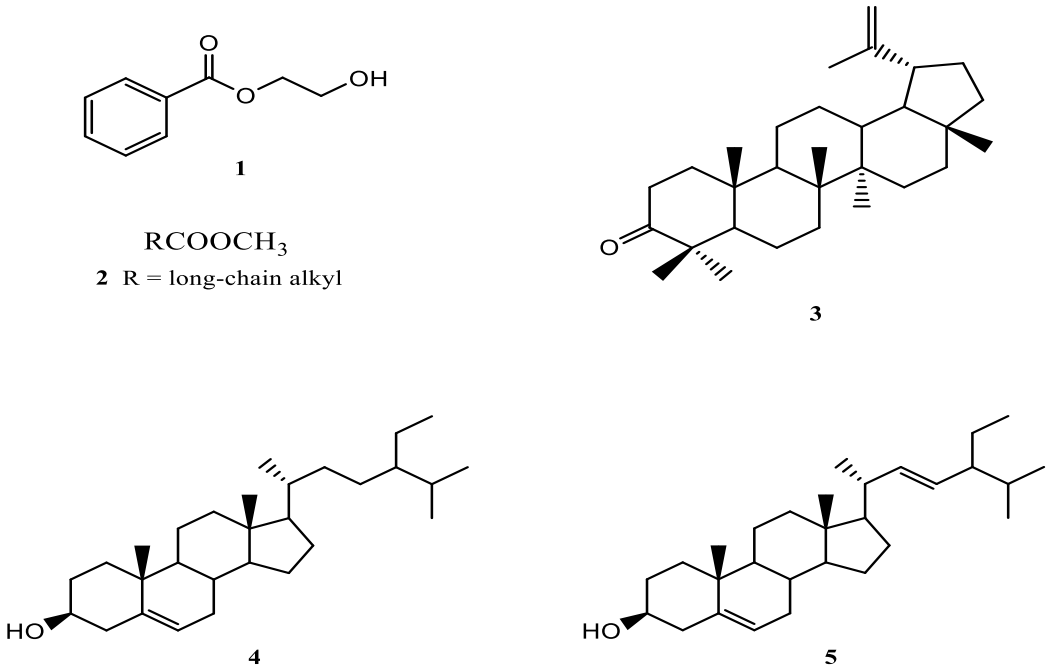

Fig. 1. Chemical structures of 2-hydroxyethyl benzoate (1), fatty acid methyl esters (2), lupenone, β-sitosterol (4), and stigmasterol (5) from the leaves of *H. pubicalyx.*

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MATERIALS AND METHODS

General Experimental Procedure

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Ascend 400 in $CDCl_3$ at 400 MHz. Column chromatography was performed with silica gel 60 (70-230 mesh, Merck). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} (Merck) and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming. All solvents used are analytical grade.

Sample Collection

Vines of *H*. *pubicalyx* were harvested from a plant being cultivated at the Philippine Nuclear Research Institute *Hoya* Germplasm Collection under Material Transfer Agreement No. 2015-10. It is designated as H.PFR which has been propagated by stem cutting from a plant that traced its origin from Quezon province, Luzon island, Philippines. It is authenticated by one of us (FBA).

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 CH_2Cl_2 (10%increment) as eluents. Fifty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the chemical constituents of the leaves of H. pubicalyx

The air-dried leaves of *H. pubicalyx* (80.9 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (2.4 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 in 10% increments. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 2.5% EtOAc in petroleum ether to afford a mixture of **2** and **3** (5 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 10% EtOAc in petroleum ether to yield a mixture of **4** and **5** (6 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to yield **1** (2 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Chemical investigation of the dichloromethane extract of the leaves of *H. pubicalyx* yielded **1**-**5**. The NMR spectra of **1** are in accordance with data reported in the literature for 2-hydroxyethyl benzoate [10]; **2** for fatty acid methyl esters [11]; **3** for lupenone [12]; **4** for β-sitosterol [13]; and **5** for stigmasterol [13].

H. pubicalyx shares similar chemical characteristics with other members of the Philippine native *hoyas*: *H. mindorensis* [4] which contained lupenone (**3**); *H. mindorensis* [4], *H. multifora* [5], *H. wayetii* [6], *H. buotii* [7], and *H. diversifolia* [8] which afforded β-sitosterol (**4**); and *H. multiflora* [5], *H. wayetii* [6], *H. buotii* [7], and *H. diversifolia* [8], which contained stigmasterol (**5**)~~;~~. The leaves of *H. pubicalyx* contained **4** and **5,** just like its stems [3].

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