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Chemical Constituents of *Ficus minahassae* (Teijsm. & de Vriese) Miq.

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ABSTRACT

*Chemical investigation of the dichloromethane extract of the leaves of Ficus minahassae (Teijsm. & de Vriese) Miq. has led to the isolation of 2-hydroxyethyl benzoate (**1**), phytyl fatty acid ester (**2**), squalene (**3**), and β-sitosterol (**4**). The structures of **1**-**4** were identified by comparison of their NMR data with literature data.*

Keywords: *Ficus minahassae*, Moraceae, 2-hydroxyethyl benzoate, phytyl fatty acid ester, squalene, β-sitosterol

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INTRODUCTION

Ficus minahassae (Teijsm. & de Vriese) Miq., locally known as hagimit, is found in primary forsts throughout the Philippines [1]. The leaves are used topically as antirheumatic. A decoction of the roots enhances milk production in lactating mothers and is used to relieve muscle pains. Roasted leaves, pounded and mixed with oil, are applied directly to the skin to heal boils and bruises [2]. The sap is used as a beverage [1], while the bark is employed as material for clothing and rope [3]. A study reported that the percentage of rubber in the bark of the stems of *F. minahassae* was 0.52% [4]. Phytochemical screening of *F. minahassae* leaves indicated the presence of tannins, steroids, terpenoids, cardiac glycosides and flavonoids [5]. There are no reported studies on the isolation and identification of chemical constituents of *F. minahassae*.

This study was conducted as part of our research on the chemical constituents of *Ficus* species found in the Philippines. Ten *Ficus* species, six of which are endemic to the Philippines have been studied [6-14]. We earlier reported the isolation of a new neohopane triterpene [6], and furanocoumarin derivatives, bergapten and oxypeucedanin hydrate [7] which exhibited antimicrobial properties from *F. pumila*. In another study, we reported the isolation of squalene, polyprenol, β-amyrin fatty acid ester, α-amyrin acetate, β-amyrin acetate, lupeol fatty acid

ester, lupenone, oleanone, and ursenone from the leaves of *F. pseudopalma* and lutein, lupeol acetate, β-carotene, phytol, α-amyrin fatty acid ester, squalene, polyprenol, β-amyrin fatty acid ester, α-amyrin acetate, β-amyrin acetate, β-sitosterol and stigmasterol from the leaves of *F. ulmifolia* [8]. Chemical investigation of the dichloromethane extracts of the leaves of two *Ficus species* led to the isolation of 11α,12α-epoxyurs-14-en-3β-yl acetate, β-amyrin, α-amyrin, squalene, β-sitosterol, stigmasterol, polyprenol, linoleic acid and lutein from *F. linearifolia*; and ergosta-6,22-dien-3,5,8-triol, ergosterol, taraxerol, hop-22(29)-ene, squalene, β-sitosterol, stigmasterol, polyprenol, linoleic acid and lutein from the leaves of *F. triangularis* [9]; and 3,5,4'-trihydroxy-6",6"-dimethylpyrano[2",3":7,6]flavanone, α-amyrin fatty acid ester, β-amyrin fatty acid ester, lupeol fatty acid ester, stigmast-4-en-3-one, β-sitosterol and stigmasterol from the stems of *F. triangularis* [10]. *F. odorata* afforded β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate, squalene, lutein, α-amyrin acetate, lupeol acetate, and β-carotene. β-Sitosteryl-3β-glucopyranoside-6'-*O*-palmitate exhibited cytotoxicity against AGS cell line with 60.28% growth inhibition [11]. Recently, the isolation of lupenone, β-friedelinol, squalene, β-sitosterol, cycloeucalenol, lupeol, α-amyrin, and β-amyrin from *F. nervosa* [12]; ursolic acid, oleanolic acid, butyrospermol cinnamate and lutein from *F. ampelas* [13]; 4-(2-hydroxyethyl)-2-methoxyphenol, β-sitosterol, *meso*-2,3-butanediol, (2*R*,3*R*)-2,3-butanediol and (2*S*,3*S*)-2,3-butanediol from *F. nota* [14]; and β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters, α-amyrin fatty acid esters, β-sitosterol, stigmasterol, β-amyrin, and long-chain saturated fatty alcohols from *F. septica* [15] have been reported.

We report herein the isolation of 2-hydroxyethyl benzoate (**1**), phytyl fatty acid ester (**2**), squalene (**3**), and β-sitosterol (**4**) from the leaves of *F. minahassae.* The chemical structures of **1**-**4** are presented in Fig. 1. To the best of our knowledge this is the first report on the isolation of **1**-**4** from *F. minahassae.*

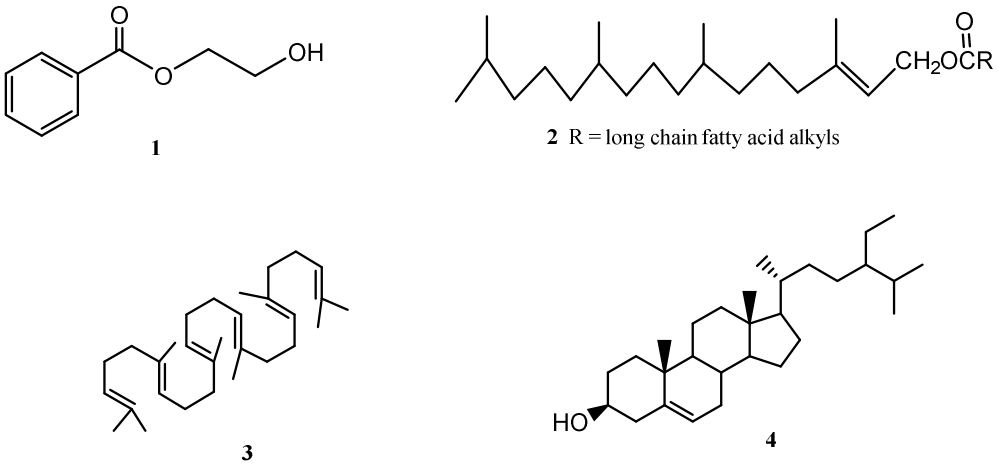

Fig. 1. Chemical structures of 2-hydroxyethyl benzoate (1), phytyl fatty acid ester (2), squalene (3), and β-sitosterol (4) from *F. minahassae*

MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H 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_2SO_4 solution followed by warming.

Sample Collection

The leaves of *F. minahassae* were collected from the riparian forest along Carmona River in the vicinity of the De La Salle University – Science and Technology Complex (DLSU-STC), Binan, Laguna in May 2015. The samples (collection #950) were authenticated by one of the authors (EHM).

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 spots of the same R_f values 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. Two milliliter fractions were collected.

Isolation of the Chemical Constituents of the Leaves of *F. minahassae*

The air-dried leaves (68 g) of *F. minahassae* were ground in an Osterizer blender, soaked in CH_2Cl_2 for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.8 g) which was chromatographed by gradient elution with CH_2Cl_2, followed by increasing amounts of acetone by 10% increments by volume. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford **3** (4 mg). The 20% acetone in CH_2Cl_2 fractions was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to afford **2** (3 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 10% EtOAc in petroleum ether to afford **4** (3 mg) after washing with petroleum ether. The 80% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 20% EtOAc in petroleum ether to afford **1** (2 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *F. minahassae* yielded **1**-**4**. The NMR spectra of **1** are in accordance with data reported in the literature for 2-hydroxyethyl benzoate [16]; **2** for phytyl fatty acid ester [17]; **3** for squalene [18]; and **4** for β-sitosterol [18].

Although no biological tests were conducted on the isolated compounds, a literature search of **3** and **4** revealed that these have diverse bioactivities.

Squalene (**1**) was reported to significantly suppress colonic ACF formation and crypt multiplicity which strengthened the hypothesis that it possesses chemopreventive activity against colon carcinogenesis [19]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [20]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells [21]. The preventive and therapeutic potential of **1** on tumor promotion and regression have been reported [22]. A recent review on the bioactivities of **1** has been provided [23].

β-Sitosterol (**2**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [24]. It was shown to be effective for the treatment of benign prostatic hyperplasia [25]. It was also reported to attenuate β-catenin and PCNA expression, as well as quench the radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis [26]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [27]. It has also been reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [28].

CONCLUSION

F. minahassae shares similar chemical characteristics with other members of the genus *Ficus* found in the Philippines: *F. pseudopalma, F. linearifolia, F. triangularis, F. odorata,* and *F. nervosa* which contained squalene (**3**); and *F. ulmifolia, F. linearifolia, F. triangularis, F. nervosa, F. nota* and *F. septica* which afforded β-sitosterol (**4**). This is the first report on the isolation of 2-hydroxyethylbenzoate (**1**) and phytyl fatty acid ester (**2**) from Philippine *Ficus* species,

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