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Chemical Constituents of *Kosteletzkya batensis* (Blanco) Fern.-Vill.

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Chemical investigation of the dichloromethane extracts of *Kosteletzkya batensis* (Blanco) Fern.-Vill., an endemic Philippine plant, has led to the isolation of β -sitosteryl-3 β -glucopyranoside-6'-O-fatty acid esters (**1**), phytol fatty acid esters (**2**), squalene (**3**) and chlorophyll-a (**4**) from the leaves; squalene (**3**), chlorophyll-a (**4**), a mixture of β -sitosterol (**5a**) and stigmaterol (**5b**) in a 2:1 ratio and long chain hydrocarbons (**6**) from the stems. The structures of compounds **1-6** were identified by comparison of their NMR data with literature data.

Keywords: *Kosteletzkya batensis* (Blanco) Fern.-Vill., Malvaceae, Squalene, Chlorophyll a, β -Sitosterol, Stigmaterol.

INTRODUCTION

Kosteletzkya batensis (Blanco) Fern.-Vill. (family: Malvaceae) is an endemic plant found only on the island of Luzon in the Philippines [1,2], particularly in Ilocos Norte, Ilocos Sur, Nueva Ecija, Pampanga and Rizal [2]. It grows in waste places in the lowland, under seasonal climatic conditions [2]. There are no reported studies on the chemical constituents and biological activities *K. batensis*.

This study is part of our research on the chemical constituents of plants endemic to the Philippines. We report herein the isolation of β -sitosteryl-3 β -glucopyranoside-6'-O-fatty acid esters (**1**), phytol fatty acid esters (**2**), squalene (**3**), chlorophyll-a (**4**) and saturated fatty acids from the leaves; and squalene (**3**), chlorophyll-a (**4**), a mixture of β -sitosterol (**5a**) and stigmaterol (**5b**), saturated fatty acids and long chain hydrocarbons (**6**) from the stems of *K. batensis*. To our best of knowledge, this is the first report on the isolation of these compounds from *K. batensis*.

EXPERIMENTAL

¹H (600 MHz) and ¹³C (150 MHz) NMR spectra were acquired in CDCl₃ on a 600 MHz Varian VNMR NMR spectrometer with referencing to solvent signals (δ 7.26 and 77.0 ppm). Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F₂₅₄ and the plates were visualized by spraying with vanillin/H₂SO₄ solution followed by warming.

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 CH₂Cl₂ (10 % increment) as eluents. Fifty milliliters of 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 milliliters of fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter of fractions were collected.

The leaves and twigs of *Kosteletzkya batensis* (Blanco) Fern.-Vill. were collected from San Juan, Batangas, Luzon, Philippines in February 2016. The samples were authenticated at the Botany Division, Philippine National Museum.

Isolation of chemical constituents from the leaves of *K. batensis*: The freeze-dried leaves of *K. batensis* (50.5 g) were ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (3.5 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10 % increments by volume. The 10 % acetone in CH₂Cl₂ fraction was rechromatographed using petroleum ether. The less polar fractions were combined and rechromatographed using petroleum ether to yield squalene (**3**) (2 mg). The more polar

fractions were combined and rechromatographed using 2.5 % EtOAc in petroleum ether to afford phytol fatty acid esters (**2**) (3 mg). 20 % acetone in CH₂Cl₂ fraction was rechromatographed using 15 % EtOAc in petroleum ether to afford chlorophyll-a (**4**) (7 mg) after washing with petroleum ether, followed by Et₂O. 50 % Acetone in CH₂Cl₂ fraction was rechromatographed using 20 % EtOAc in petroleum ether to afford fatty acids (7 mg). 60 % Acetone in CH₂Cl₂ fraction was rechromatographed using CH₃CN:Et₂O:CH₂Cl₂ (2:2:6, v/v) to yield β -sitosterol-3 β -glucopyranoside-6'-O-fatty acid esters (**1**) (3 mg) after trituration with petroleum ether.

Isolation of the chemical constituents from the stems of *K. batensis*: The freeze-dried stems of *K. batensis* (51.3 g) were ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (3.2 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10 % increments by volume. 10 % Acetone in CH₂Cl₂ fraction was rechromatographed using petroleum ether to yield long-chain hydrocarbons (**6**) (2 mg) after washing with petroleum ether. The 20 % acetone in CH₂Cl₂ fraction was rechromatographed using petroleum ether to afford **3** (2 mg). The 30 % acetone in CH₂Cl₂ fraction was rechromatographed using 15 % EtOAc in petroleum ether, followed by 20 % EtOAc in petroleum ether. The fractions eluted with 15 % EtOAc in petroleum ether were combined and rechromatographed using 15 % EtOAc in petroleum ether to yield a mixture of β -sitosterol (**5a**) and stigmasterol (**5b**) (4 mg) after washing with petroleum ether. The fractions eluted with 20 % EtOAc in petroleum ether were combined and rechromatographed using 20 % EtOAc in petroleum ether to afford chlorophyll-a (**4**) (5 mg) after washing with petroleum ether, followed by Et₂O. The 60 % acetone in CH₂Cl₂ fraction was rechromatographed using 20 % EtOAc in petroleum ether to afford saturated fatty acids (4 mg).

RESULTS AND DISCUSSION

The NMR spectra of β -sitosterol-3 β -glucopyranoside-6'-O-fatty acid esters (**1**) are in accordance with data reported in the literature for β -sitosterol-3 β -glucopyranoside-6'-O-fatty acid esters [3]; **2** for phytol fatty acid esters [4]; **3** for squalene [5]; **4** for chlorophyll-a [6]; **5a** for β -sitosterol [7]; **5b** for stigmasterol [7] and **6** for long-chain hydrocarbons [8].

Although no biological activity tests were conducted on the isolated compounds, a literature search of **1** and **3-5b** revealed that these have diverse bioactivities. β -Sitosterol-3 β -glucopyranoside-6'-O-palmitate (**1**) was exhibited cytotoxicity against Bowes (melanoma), MCF7 (breast) cancer cell lines [9] and human stomach adenocarcinoma (AGS) [3] and showed potent anti-complement activity [10]. Squalene (**3**) possessed chemopreventive activity against colon carcinogenesis [11], showed cardioprotective [12] and anti-proliferative effects on breast cancer cells [13] and exhibited preventive and therapeutic potential on tumor promotion and regression [14]. A recent review on the bioactivities of squalene has been provided [15]. Chlorophyll-a (**4**) and its various derivatives are used in traditional medicine and for therapeutic purposes [16]. They have been studied for wound healing [17], anti-inflammatory properties [18], control of calcium oxalate crystals [19], utili-

zation as effective agents in photodynamic cancer therapy [20-22] and chemopreventive effects in humans [23,24]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been reported by Ferruzzi and Blakeslee [25]. β -Sitosterol (**5a**) inhibited human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [26], used for the treatment of benign prostatic hyperplasia [27], chemopreventive potential against colon cancer [28], attenuated β -catenin and PCNA expression, inhibited the expression of NPC1L1 [29], induced apoptosis mediated by the activation of ERK and down regulated Akt in MCA-102 murine fibrosarcoma cells [30]. Stigmasterol (**5b**) showed therapeutic efficacy against Ehrlich ascites carcinoma in mice [31], lowered plasma cholesterol levels, inhibited intestinal cholesterol and plant sterol absorption, suppressed hepatic cholesterol and classic bile acid synthesis in Wistar and WKY rats [32], exhibited cytostatic activity against Hep-2 and McCoy cells [33], markedly inhibited tumour promotion in two stage carcinogenesis experiments [34] and exhibited antimutagenic [35], topical antiinflammatory [36], antiosteoarthritic [37] and antioxidant [38] activities.

Conclusion

Chemical investigations of leaves and stems of *Kosteletzkya batensis* (Blanco) Fern.-Vill. afforded β -sitosterol-3 β -glucopyranoside-6'-O-fatty acid esters (**1**), phytol fatty acid esters (**2**), squalene (**3**), chlorophyll a (**4**), β -sitosterol (**5a**), stigmasterol (**5b**), saturated fatty acids and long chain hydrocarbons. Compounds **1** and **3-5b** were reported to exhibit diverse biological activities. This is the first report on the chemical constituents of *K. batensis* and the potential biological activities of the plant based on the isolated compounds.

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