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Research Article

# Triterpene and Sterols from *Premna nauseosa* Blanco

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**ABSTRACT**
Chemical investigation of the dichloromethane extract of *Premna nauseosa* Blanco afforded squalene (**1**) and a mixture of β-sitosterol (**2**) and stigmasterol (**3**) in about 6:1 ratio. The structures of **1**-**3** were identified by comparison of their NMR data with literature data.

**Keywords**: *Premna nauseosa* Blanco, Verbenaceae, squalene, β-sitosterol, stigmasterol.

## INTRODUCTION

*Premna nauseosa* of the family Verbenaceae, commonly known as alagau-gubat is an endemic Philippine plant found in low altitude places in Luzon, Philippines. The leaves of *P. nauseosa* are said to be a cure for stomach problems[1]. There are no reported studies on the chemical constituents of *Premna nauseosa*. In an earlier study, the leaf crude ethanolic extract of *P. nauseosa* exhibited an $IC_{50}$ value of 12.06 µg/mL when tested *in vitro* on colorectal carcinoma (HCT-116) using the MTT assay. Furthermore, the ethanolic extract possessed 78% free radical scavenging activity based on DPPH assay[2].

In this study, the dichloromethane extract of *P. nauseosa* leaves yielded squalene (**1**) and a mixture of β-sitosterol (**2**) and stigmasterol (**3**). The structures of **1**-**3** are presented in Fig. 1. This is the first report on the isolation of these compounds from *P. nauseosa.*

## 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 *Premna nauseosa* Blanco were collected from Brgy. Tulay, Odiongan, Romblon, Philippines in January 2016. The samples were authenticated at the Botany Division, Philippine National Museum.

*General Isolation Procedure*

A glass column 12 inches in height and 0.5 inch internal diameter was used for the chromatography. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in $CH_2Cl_2$ at 10% increment by volume as eluents. Five 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. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

*Isolation of the Chemical Constituents from the Leaves of Premna nauseosa*.

The air-dried *Premna nauseosa* leaves (298.6 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 (3.8 g) which was chromatographed using increasing proportions of acetone in $CH_2Cl_2$ at 10% increment by volume. The $CH_2Cl_2$ fraction was rechromatographed using petroleum ether to afford **1** (4 mg). The 30% acetone in $CH_2Cl_2$ fraction was rechromatographed using 10% EtOAc in petroleum ether. Fractions collected from this column were combined and rechromatographed using 15% EtOAc in petroleum ether

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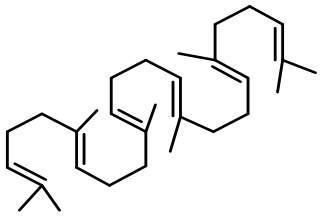
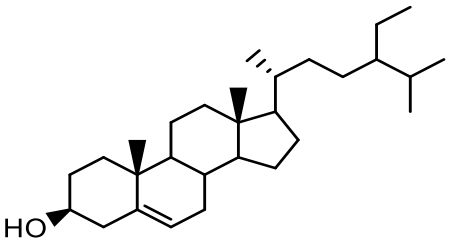


1

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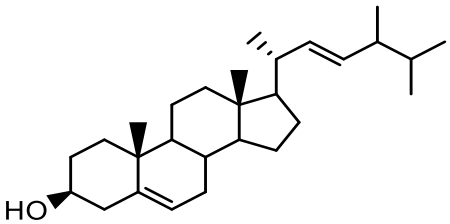


3

Figure 1: Chemical structures of squalene (**1**), β-sitosterol (**2**) and stigmasterol (**3**) from *Premna nauseosa*.

to yield a mixture of **2** and **3** (5 mg) after washing with petroleum ether.

## RESULTS AND DISCUSSION

Silica gel chromatography of the dichlorometnahe extract of the leaves of *P. nauseosa* afforded squalene (**1**), β-sitosterol (**2**) and stigmasterol (**3**). The NMR spectra of **1** are in accordance with data reported in the literature for squalene[3]; **2** for β-sitosterol[4]; and **3** for stigmasterol[4]. The 6:1 ratio of the mixture of **2** and **3** was deduced from the integrations of the $^1$H NMR resonances for the olefinic protons of **2** at δ 5.33 (dd, *J* = 1.8, 4.8 Hz, H-6)[4] and **3** at δ 5.33 (dd, *J* = 1.8, 4.8 Hz, H-6), 5.13 (dd, *J* = 9.0, 15.0 Hz, H-22) and 5.00 (dd, *J* = 9.0, 15.0 Hz, H-23)[4].

Although no biological tests were conducted on the isolated compounds, a literature search of **1**-**3** 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[5]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties[6]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells[7]. The preventive and therapeutic potential of squalene containing compounds on tumor promotion and regression have been reported[8]. A recent review on the bioactivities of squalene has been provided[9].

β-Sitosterol (**2**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells[10]. It was shown to be effective for the treatment of benign prostatic hyperplasia[11]. It was also reported to attenuate β-catenin and PCNA expression, as well as quench radical *in vitro*, making it a potential anticancer drug for colon carcinogenesis[12]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake[13]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells[14].

Stigmasterol (**3**) shows therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions[15]. It lowers plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Winstar as well as WKY rats[16]. Other studies reported that stigmasterol showed cytostatic activity against Hep-2 and McCoy cells[17], markedly inhibited tumour promotion in two stage carcinogenesis experiments[18], exhibited antimutagenic[19], topical anti-inflammatory[20], antiosteoarthritic[21] and antioxidant[22] activities.

## CONCLUSION

In an earlier study, the ethanolic extract of *P. nauseosa* exhibited high cytotoxic activity against colorectal carcinoma (HCT-116) and free radical scavenging activity based on DPPH assay[2]. The dichloromethane extract of *P. nauseosa* yielded squalene (**1**), β-sitosterol (**2**) and stigmasterol (**3**). Compounds **1**[5] and **2**[12] were reported to possess chemopreventive activity against colon carcinogenesis, while **2** was also shown to quench radical *in vitro*[12]. Thus, **1** and **2** could contribute to the cytotoxic activity of *P. nauseosa* against HCT-116. Furthermore, **2** could be partly responsible for the free radical scavenging activity of *P. nauseosa.*

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