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Chemical Constituents of *Polyscias nodosa*.

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ABSTRACT

Chemical investigation of the dichloromethane extracts of *Polyscias nodosa* (Bl.) Seem. yielded squalene (**1**), phytyl fatty acid esters (**2**), lutein (**3**), and β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**4**) from the leaves; and **1**, triacylglycerols (**5**), and a mixture of stigmasterol (**6a**) and β-sitosterol (**6b**) in a 5:1 ratio from the twigs. The structures of **1**-**6b** were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Polyscias nodosa*, Araliaceae, squalene, phytyl fatty acid esters, lutein, β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate, stigmasterol, β-sitosterol

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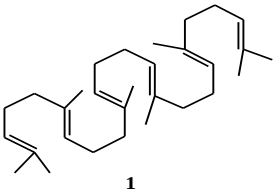
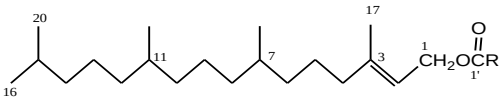
INTRODUCTION

Polyscias nodosa locally known as malapapaya is native to Tropical Asia – Indonesia, Papua New Guinea and the Philippines and the Southwestern Pacific – Solomon Islands [1]. This tree occurs throughout the Philippines where it is commercially used for making woodworks, boxes, pencil slats, chopsticks, matchsticks, ice cream spoons, plywood, native wooden shoes, lollipops, popsicle sticks, toothpicks, and similar articles [2]. Traditionally, the leaves are powdered and applied as fish poison and used medicinally against purpuric fever and as a contraceptive [2]. The only studies conducted on the chemical constituents of *P. nodosa* were the isolation of saponins from the leaves of the plant [3-5].

We report herein the isolation of squalene (**1**), phytyl fatty acid esters (**2**), lutein (**3**), and β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**4**) from the leaves; and **1**, triacylglycerols (**5**) and a mixture of stigmasterol (**6a**), β-sitosterol (**6b**) from the twigs of *P. nodosa*. To the best of our knowledge this is the first report on the isolation of these compounds from *P. nodosa.*



1



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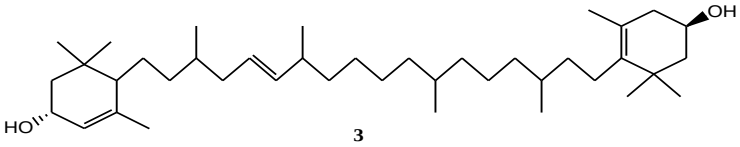

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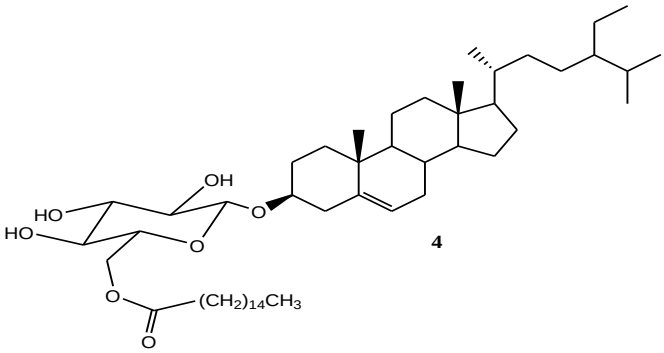

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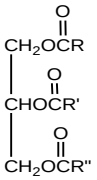
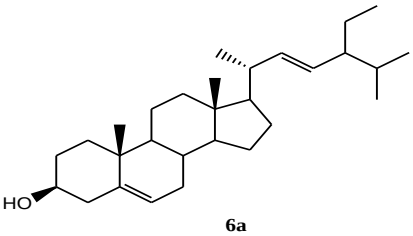
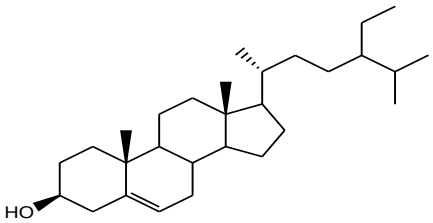


5 R, R', R'' = long chain fatty acids



6a



6b

Chemical structures of squalene (1), phytyl fatty acid esters (2), lutein (3), β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (4), triacylglycerols (5), stigmasterol (6a) and β-sitosterol (6b) from *P. nodosa.*

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_2SO_4 solution followed by warming.

Sample Collection

Polyscias nodosa (Bl.) Seem. was collected from the De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna, Philippines in April 2014. The sample was authenticated at the Botany Division of the Philippine National Museum with control no. 830.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extract from the twigs 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

The air-dried leaves of *P. nodosa* (149.3 g) was 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 (3.5 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 (3 ×) in petroleum ether to afford **1** (5 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed using 5% EtOAc in petroleum ether (2 ×) to afford **2** (2 mg). The 50% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:1 by volume ratio) to afford **3** (3 mg) after washing with petroleum ether, followed by Et_2O. The 60% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6 by volume ratio) to afford **4** (2 mg) after trituration with petroleum ether.

Isolation of the Chemical Constituents of the Twigs

The air-dried twigs of *P. nodosa* (123 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 (1.5 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 (3 ×) in petroleum ether to afford **1** (3 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 5% EtOAc in petroleum ether (2 ×) to afford **5** (4 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed using 20% EtOAc in petroleum ether (3 ×) to afford a mixture of **6a** and **6b** (5 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *P. nodosa* afforded squalene (**1**) [6], phytyl fatty acid esters (**2**) [7], lutein (**3**) [8], and β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**4**) [9] from the leaves; and **1**, triacylglycerols (**5**) [6], and a mixture of stigmasterol (**6a**) [10] and β-sitosterol (**6b**) [10] in a 5:1 ratio from the twigs. The structures of **1**-**6b** were identified by comparison of their ^{1}H NMR data with those reported in the literature [6-10].

Although no biological activity tests were conducted on the isolated compounds, a literature search of **1**, **3**, **4**, **6a** and **6b** revealed that these have a range 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 [11]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [12]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells [13]. The preventive and

therapeutic potential of squalene containing compounds on tumour promotion and regression have been reported [14]. A recent review on the bioactivities of squalene has been provided [15].

Dietary lutein (**3**), especially at 0.002%, inhibited tumor growth by selectively modulating apoptosis, and by inhibiting angiogenesis [16]. Another study reported that the chemopreventive properties of all-*trans* retinoic acid and lutein may be attributed to their differential effects on apoptosis pathways in normal *versus* transformed mammary cells [17]. Moreover, very low amounts of dietary lutein (0.002%) can efficiently decrease mammary tumor development and growth in mice [18]. Another study reported that lutein and zeaxanthine reduces the risk of age related macular degeneration [19].

β-Sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**4**) was reported to exhibit cytotoxicity against Bowes (melanoma) and MCF7 (breast) cancer cell lines with IC_{50} values of 152 mM and 113 mM, respectively [9]. Furthermore, **4** exhibited cytotoxicity against human stomach adenocarcinoma (AGS) cell line with 60.28% growth inhibition [20]. In search of substances that inhibit the hemolytic activity of human serum against erythrocytes, **4** was evaluated on its anti-complement activity. Compound **4** was found to exhibit potent anti-complement activity (IC_{50} = 1.0 ± 0.1 μM) on the classical pathway of the complement, as compared to the positive control, tiliroside (IC_{50} = 76.5 ± 1.1 μM) [21].

Stigmasterol (**6a**) shows therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions [22]. 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 [23]. Other studies reported that **6a** showed cytostatic activity against Hep-2 and McCoy cells [24], markedly inhibited tumour promotion in two stage carcinogenesis experiments [25], exhibited antimutagenic [26], topical anti-inflammatory [27], anti-osteoarthritic [28] and antioxidant [29] activities.

β-Sitosterol (**6b**) was reported to exhibit growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [30]. It was shown to be effective for the treatment of benign prostatic hyperplasia [31]. It attenuated β-catenin and PCNA expression, as well as quenched radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis [32]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [33]. It can inhibit the expression of NPC1L1 in the
enterocytes to reduce intestinal cholesterol uptake [34].

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