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Chemical Constituents of *Petersianthus quadrialatus* (Merr.).

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ABSTRACT

The dichloromethane extract of the twigs of *Petersianthus quadrialatus* (Merr.) afforded stigmasterol (**1**) and taraxerol (**2**), while the leaves yielded **1**, unsaturated triglycerides (**3**) and a mixture of β-amyrin fatty acid ester (**4a**) and α-amyrin fatty acid ester (**4b**) in a 2:1 ratio. The structures of **1**-**4b** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature.

Keywords: *Petersianthus quadrialatus,* taraxerol, β-amyrin fatty acid ester, α-amyrin fatty acid ester, stigmasterol

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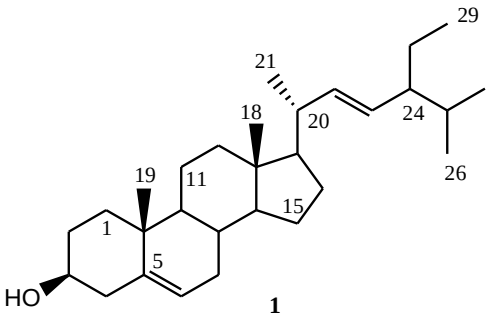
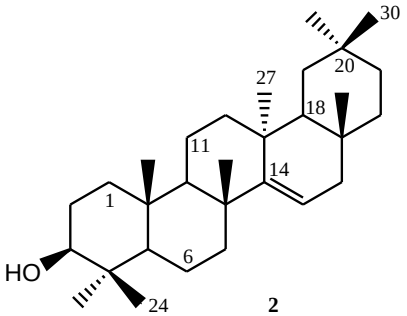
INTRODUCTION

Petersianthus quadrialatus Merr. of the family Lecythidaceae, locally known as toog is an endemic Philippine tree. The wood is commercially marketed as Philippine rosewood. There are only two known species of the genus *Petersianthus.* The other species is *Petersianthus macrocarpus* which is found in tropical West Africa [1]. Earlier studies reported the isolation of triterpenoid saponins from the bark of *P. macrocarpus* [2, 3]. The ethanolic extracts of *P. macrocarpus* strongly inhibited the growth of the human colonic cancer cell line CaCo-2 *in vitro* [4] and exhibited potent antimalarial effects [5]. In the Philippines, the wood of *P. quadrialatus* is mainly used for the production of face veneer, fancy plywood, pulp and paper [1]. The gluability of rotary-cut veneers from the tree was reported [6]. An earlier study reported that *P. quadrialatus* contained 55.4% halocellulose [7]. There are no other reported studies on the chemical constituents and biological activities of *P. quadrialatus.* This study was conducted as part of our research on the chemical constituents of Philippine endemic trees.

We report herein the isolation of stigmasterol (**1**) and taraxerol (**2**) from the twigs; and **1**, unsaturated triglycerides (**3**), β-amyrin fatty acid ester (**4a**) and α-amyrin fatty acid ester (**4b**) from the leaves of *P. quadrialatus*. To the best of our knowledge this is the first report on the isolation of these compounds from *P. quadrialatus*.



1



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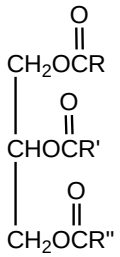
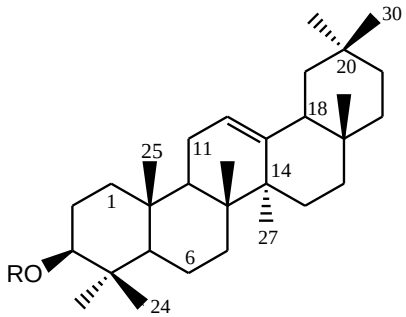
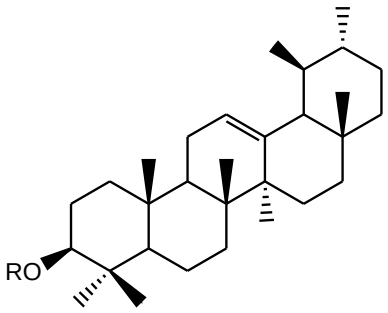


3 R = R' = R" = long chain fatty acids



4a R = long chain fatty acids



4b R = long chain fatty acids

Figure 1: Chemical constituents of *P. quadrialatus*: stigmasterol (1), taraxerol (2), unsaturated triglycerides (3), β-amyrin fatty acid ester (4a) and α-amyrin fatty acid ester (4b).

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EXPERIMENTAL

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 F254 and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

Sample Collection

The leaves of *Petersianthus quadrialatus* Merr. used in this study were collected from Mt. Makiling Forest Reserve, Los Baños, Laguna, Philippines in May 2013. The sample was authenticated at the University of the Philippines, Los Baños, College, Laguna.

Isolation

The air-dried leaves (292 g) of *P. quadrialatus* was ground in a blender, soaked in CH_2Cl_2 for three days and then filtered. The twigs were chopped into small pieces, air-dried, then ground using mortar and pestle. The twigs (35 g) were soaked in CH_2Cl_2 for three days and then filtered. The filtrates were concentrated under vacuum to afford the crude extracts: leaves (10 g) and twigs (0.5 g) which were chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume as eluents. A glass column 18 inches in height and 1.0 inch internal diameter was used for the fractionation of the crude extracts. Five milliliter fractions were collected. Fractions with spots of the same *Rf* 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.

The 20% to 40% acetone in CH_2Cl_2 fractions from the chromatography of the crude leaf extract were combined and rechromatographed in 10% EtOAc in petroleum ether, followed by 12.5% EtOAc in petroleum ether, and finally 15% EtOAc in petroleum ether. The fractions eluted with 12.5% EtOAc in petroleum ether were rechromatographed (3 ×) in 15% EtOAc in petroleum ether to afford **2** (7 mg) after washing with petroleum ether. The fractions eluted with 15% EtOAc in petroleum ether were rechromatographed in CH_2Cl_2 to afford **1** (9 mg) after washing with petroleum ether.

The CH_2Cl_2 and 10% acetone in CH_2Cl_2 fractions from the chromatography of the crude twig extract were combined and rechromatographed in 2.5% EtOAc in petroleum ether, followed by 5% EtOAc in petroleum ether. The fractions eluted with 2.5% EtOAc in petroleum ether were combined and rechromatographed (5 ×) in 5% EtOAc in petroleum ether to afford a mixture of **4a** and **4b** (3 mg) after washing with petroleum ether. The fractions eluted with 5%

EtOAc in petroleum ether were combined and rechromatographed (4 ×) in 5% EtOAc in petroleum ether to afford **3** (2 mg).

Stigmasterol (**1**): colorless solid. ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.3 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.7 (C-12), 42.2 (C-13), 56.9 (C-14), 24.4 (C-15), 28.9 (C-16), 56.0 (C-17), 12.0 (C-18), 19.4 (C-19), 40.5 (C-20), 21.1 (C-21), 138.3 (C-22), 129.3 (C-23), 51.2 (C-24), 31.9 (C-25), 21.2 (C-26), 19.0 (C-27), 25.4 (C-28), 12.2 (C-29).

Taraxerol (**2**): colorless solid. ^{13}C NMR (150 MHz, $CDCl_3$): δ 38.0 (C-1), 27.1 (C-2), 79.1 (C-3), 39.0 (C-4), 55.5 (C-5), 18.8 (C-6), 35.1 (C-7), 38.7 (C-8), 48.7 (C-9), 37.5 (C-10), 17.5 (C-11), 35.8 (C-12), 37.7 (C-13), 158.1 (C-14), 116.9 (C-15), 36.6 (C-16), 37.7 (C-17), 49.3 (C-18), 41.3 (C-19), 28.8 (C-20), 33.7 (C-21), 33.1 (C-22), 28.0 (C-23), 15.4 (C-24), 15.4 (C-25), 29.8 (C-26), 25.9 (C-27), 29.9 (C-28), 33.3 (C-29), 21.3 (C-30).

Triglycerides (**3**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.12 (H-1/H-3, 2H, dd, *J* = 7.2, 14.4 Hz); 4.27 (H-1/H-3, 2H, dd, *J* = 4.8, 14.4 Hz); 5.25 (H-2, m); 2.29 (H_2-2'); 1.60 (H_2-3'); 1.23-1.28 $(CH_2)_n$, 5.34 (CH=CH); 2.78 (=CCH_2C=) 0.85 (CH_3, t, *J* = 6 Hz); 0.96 (CH_3, t, *J* = 6 Hz).

β-*Amyrin fatty acid ester* (**4a**): colorless solid. ^{13}C NMR ($CDCl_3$) δ 38.2 (C-1), 22.7 (C-2), 80.6 (C-3), 37.7 (C-4), 55.3 (C-5), 18.3 (C-6), 32.6 (C-7), 39.8 (C-8), 47.2 (C-9), 37.1 (C-10), 23.7 (C-11), 121.6 (C-12), 145.2 (C-13), 41.5 (C-14), 26.1 (C-15), 27.0 (C-16), 32.5 (C-17), 47.6 (C-18), 46.5 (C-19), 31.2 (C-20), 34.9 (C-21), 37.1 (C-22), 28.1 (C-23), 16.5 (C-24), 15.5 (C-25), 16.8 (C-26), 26.0 (C-27), 28.0 (C-28), 33.3 (C-29), 23.7 (C-30), 173.7 (C-1'), 34 9 (C-2'), 31.9 (C-3'), 22.7, 25.2, 29.2-29.7 $(CH2)_n$, 129.8-130.2 (CH=CH), 14.1 (CH_3).

α-Amyrin fatty acid ester (**4b**)**:** colorless solid. ^{13}C NMR (150 MHz, $CDCl_3$): δ 38.4 (C-1), 22.7 (C-2), 80.6 (C-3), 37.7 (C-4), 55.3 (C-5), 18.3 (C-6), 32.9 (C-7), 39.8 (C-8), 47.5 (C-9), 37.0 (C-10), 23.2 (C-11), 124.3 (C-12), 139.6 (C-13), 42.1 (C-14), 28.7 (C-15), 26.6 (C-16), 33.7 (C-17), 59.1 (C-18), 39.6 (C-19), 39.6 (C-20), 31.2 (C-21), 41.5 (C-22), 28.1 (C-23), 15.7 (C-24), 15.7 (C-25), 16.8 (C-26), 23.4 (C-27), 28.1 (C-28), 17.5 (C-29), 21.4 (C-30), 173.7 (C-1'), 34 9 (C-2'), 31.9 (C-3'), 22.7, 25.2, 29.2-29.7 $(CH2)_n$, 129.7-130.2 (CH=CH), 14.1 (CH_3).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the twigs of *Petersianthus quadrialatus* (Merr.) afforded stigmasterol (**1**) [8] and taraxerol (**2**) [9], while the leaves yielded **1**, unsaturated triglycerides (**3**) [10] and a mixture of β-amyrin fatty acid ester (**4a**) [11] and α-amyrin fatty acid ester (**4b**) [11] in a 2:1 ratio. The structures of **1**, **2**, **4a** and **4b** were identified by comparison of their ^{13}C NMR data with those reported in the literature [8, 9, 11], while the structure of **3** was compared with the ^{1}H NMR of unsaturated triglycerides [10].

Although bioassays were not conducted on the isolated compounds, there were previous studies that reported on their biological activities. Stigmasterol (**1**) shows therapeutic

efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions [12]. 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 [13]. Taraxerol (**2a**) was reported to exhibit antiparasitic activities against *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense* [14]. It has inhibitory effects on AGS cell growth through inducing G(2)/M arrest and promotion of cell apoptosis [15] and displayed potent NO-reducing activity in microglial cells [16]. 3β-Taraxerol restored the DEX induced inhibition of glucose uptake and the expression of insulin signaling markers GLUT4 and PI3K, thus reversing insulin resistance [17]. It is a potent anti-inflammatory, anticancer, antimicrobial and cardioprotective agent [18]. A triglyceride (**3**), trilinolein exhibited protective effects against cardiovascular disorders [19]. It also inhibits schemia induced ventricular arrhythmias and it exhibits anti-oxidant effect [20]. It was also reported to inhibit the growth of human non-small cell lung carcinoma A549 and induce apoptosis in a dose- and time- dependent manner [21]. Another study reported that triglycerides (**3**) showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation. Trilinolenin (18:3; μ-3) was toxic only after prolonged incubation [22]. α-Amyrin, β-amyrin, and the 3-O-acyl derivatives of β-amyrin (**4a**) and α-amyrin (**4b**) exhibited analgesic property [23, 24].

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