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Chemical Constituents of *Arenga tremula.*

Consolacion Y Ragasa[1,2*], Dalton R Fortin[2] and Chien Chang Shen[3].

[1]Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines,
[2]Chemistry Department, De La Salle University 2401 Taft Avenue, Manila 1004, Philippines
[3]National Research Institute of Chinese Medicine, 155-1, Li-Nong St., Sec. 2, Taipei 112, Taiwan

ABSTRACT

The dichloromethane extract of the twigs of *Arenga tremula* subsp. *tremula* afforded squalene (**1**), chlorophyll a (**2**), monoglycerides (**3**) and triglycerides (**4**), while the leaves yielded **2**, **4**, lutein (**5**), and a mixture of β-sitosterol (**6**) and stigmasterol (**7**). The structures of **1**-**7** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature.

Keywords: *Arenga tremula,* Arecaceae, sugar palm, squalene, β-sitosterol, stigmasterol, lutein, chlorophyll a

Corresponding author

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INTRODUCTION

Arenga tremula subsp. *tremula* is an ornamental Philippine endemic dwarf sugar palm, locally known as dumayaka [1-3]. The genus *Arenga* comprises 22 species which are good sources of sugar and starch, used for thatch and basket production and have high potential as ornamental plants [2]. There are two subspecies of *Arenga tremula*, namely, *tremula* which is endemic to the Philippines and *longistamina* Mogea which is found in Hainan, Taiwan and Ryukyu islands [2]. In the Philippines, the petioles and midribs are used to make baskets, while the leaves are used for thatching and wickerwork. In Hainan, it is a source of starch. The young tops are edible although consumption of large quantities may produce toxic effects [2]. The fruit is toxic and the active principle is raphides (calcium oxalate crystals) which is found in the pericarp, while the fruit juice causes skin irritation and blisters [3]. There is no known medicinal use and no reported chemical constituents of *A. tremula.*

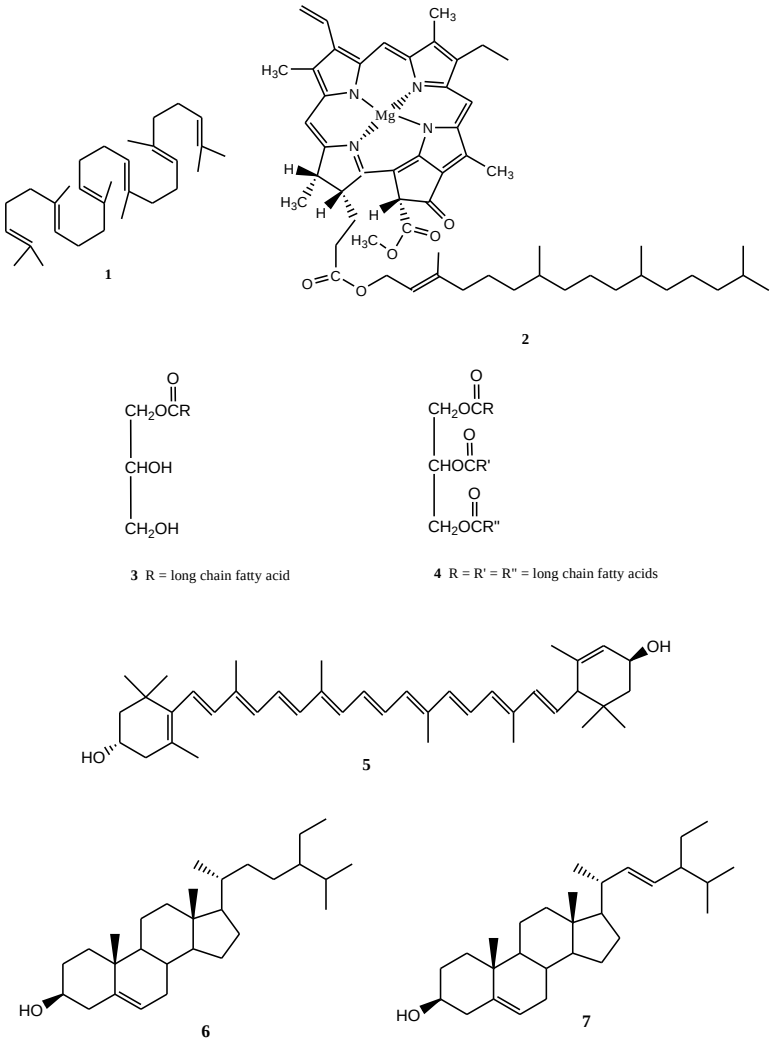

Figure 1: Chemical constituents of *A. tremula*: squalene (1), chlorophyll a (2), monoglycerides (3) and triglycerides (4), lutein (5), β-sitosterol (6), and stigmasterol (7).

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This study was conducted as part of our research on the chemical constituents of plants endemic to the Philippines [4-24]. We earlier reported the chemical constituents of *Tectona philippinensis* [4, 5], *Diospyros blancoi* [6], *Dillenia philippinensis* [7], *Pycnarrhena manillensis* [8], *Broussonetia luzonicus* [9], *Atalantia retusa* [10], and *Myristica philippensis* [11], The following endemic plants were also investigated: *A. pyramidalis* Cav. Pers. [12], *A. cf. elliptica* [13], and *A. squamulosa* [14] from the genus *Ardisia*; *C. cebuense* [15, 16], *C. griffithii* [17], *C. rupestre, C. nanophyllum* [18], *C. utile* [19], *C. iners* [20], and *C. trichophyllum* [21] from the genus *Cinnamomum*; and *F. pseudopalma, F. ulmifolia* [22], *F. odorata* [23], *F. linearifolia,* and *F. triangularis* [24] from the genus *Ficus.*

We report herein the isolation of squalene (**1**), chlorophyll a (**2**), monoglycerides (**3**) and triglycerides (**4**) from the twigs; and **2**, **4**, lutein (**5**), and a mixture of β-sitosterol (**6**) and stigmasterol (**7**) from the leaves of *A. tremula.* To the best of our knowledge this is the first report on the isolation of these compounds from *A, tremula.*

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

The sample was collected from Bataan, Philippines in October 2013. It was identified as *Arenga tremula* subsp. *tremula* at the Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman, Quezon City.

Isolation of the Chemical Constituents of A. tremula

The air-dried twigs (101 g) of A. tremula were cut into small pieces, ground using mortar and pestle, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (2.8 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *Rf* values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. The CH_2Cl_2 fraction was rechromatographed in petroleum ether, followed by 1% EtOAc in petroleum ether. The fractions eluted with 1% EtOAc in petroleum ether were combined and rechromatographed (3×) in the same solvent to afford **1** (2 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed in petroleum ether, followed by 1% EtOAc in petroleum ether.

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The fractions eluted with petroleum ether were combined and rechromatographed (2×) using 1% EtOAc in petroleum ether as eluent to afford triglycerides (5 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (4×) using 10% EtOAc in petroleum ether as eluent to afford **2** (3 mg) after washing with petroleum ether, followed by Et_2O. The 70% acetone in CH_2Cl_2 fraction was rechromatographed (3×) using Et_2O:CH_3CN:CH_2Cl_2 (0.5:0.5:9 by volume) as eluent to afford **3** (4 mg).

The air-dried leaves (402 g) of *A. tremula* were ground in a blender, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (16 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 30% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether, followed by 12.5% EtOAc in petroleum ether, and finally 15% EtOAc in petroleum ether as eluents. The fractions eluted with 10% EtOAc in petroleum ether were combined and rechromatographed (2×) in the same solvent to afford **4** (6 mg). The fractions eluted with 12.5% EtOAc in petroleum ether were combined and rechromatographed (3×) in the same solvent to afford a mixture **6** and **7** (7 mg) after washing with petroleum ether. The fractions eluted with 15% EtOAc in petroleum ether were combined and rechromatographed (2×) in the same solvent to afford **5** (4 mg) after washing with petroleum ether, followed by Et_2O. The 80% acetone in dichloromethane fraction was rechromatographed (3×) using Et_2O:CH_3CN:CH_2Cl_2 (0.5:0.5:9 by volume) as eluent to afford **2** (3 mg) after washing with petroleum ether, followed by Et_2O.

RESULTS AND DISCUSSION

The dichloromethane extract of the twigs of *Arenga tremula* subsp. *tremula* afforded squalene (**1**) [25], chlorophyll a (**2**) [26], monoglycerides (**3**) [27] and triglycerides (**4**) [28], while the leaves yielded **2**, **4**, lutein (**5**) [29], β-sitosterol (**6**) [30], and stigmasterol (**7**) [9]. The structures of **1**-**7** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature [25-30].

Although there are no reported bioactivities for *A. tremula,* the compounds isolated from the plant have shown diverse bioactivities [31- 53].

Squalene (**1**) significantly suppresses colonic ACF formation and crypt multiplicity. This strengthens the hypothesis that squalene possesses chemopreventive activity against colon carcinogenesis [31]. Squalene has cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [32].

Chlorophyll (**2**) and its various derivatives are used in traditional medicine and for therapeutic purposes [33]. Natural chlorophyll and its derivatives have been studied for wound healing [34], anti-inflammatory properties [35], control of calcium oxalate crystals [36], utilization as effective agents in photodynamic cancer therapy [37-39], and chemopreventive effects in humans [40-41]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided [42].

Antimicrobial tests on the monoglyceride (**3**) and triglyceride (**4**) indicated that they exhibited antimicrobial activity against *S. aureus, P. aeruginosa, B. subtilis, C. albicans* and *T. mentagrophytes* [27]. Another study reported that triglycerides showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation [43].

Dietary lutein (**5**), especially at 0.002%, inhibited tumor growth by selectively modulating apoptosis, and by inhibiting angiogenesis [44]. Another study reported that the chemopreventive properties of all-*trans* retinoic acid (ATRA) and lutein may be attributed to their differential effects on apoptosis pathways in normal *versus* transformed mammary cells [45]. A previous study reported that very low amounts of dietary lutein (0.002%) can efficiently decrease mammary tumor development and growth in mice [46].

β-Sitosterol (**6**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [47]. It was shown to be effective for the treatment of benign prostatic hyperplasia [48]. 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 [49]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [59]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [51].

Stigmasterol (**7**) shows therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions [52]. 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 [53].

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