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Chemical Constituents of *Artocarpus altilis* and *Artocarpus odoratissimus.*

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ABSTRACT

Chemical investigation of the dichloromethane extract of the leaves of *Artocarpus altilis* yielded β-sitosterol (**1**), unsaturated triglycerides (**2**), squalene (**3**), polyprenol (**4**), lutein (**5**) and unsaturated fatty acids. The structures of 1-**5** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature. Chemical investigation of the dichloromethane extract of *Artocarpus odoratissimus* afforded **1**, **2**, and unsaturated fatty acids from the flesh of the fruit and seeds; and **1**, unsaturated fatty acids and hydrocarbons from the fruit rind.

Keywords: *Artocarpus altilis, Artocarpus odoratissimus,* Moraceae, β-sitosterol, squalene, polyprenol, lutein

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INTRODUCTION

Artocarpus altilis (Parkinson) Fosberg, commonly known as rimas or breadfruit is used in traditional medicine to treat various ailments. *A. altilis* leaf and bark parts are non-toxic, suggesting the safety of the extracts in therapeutic uses [1]. A recent study reported that *A. altilis* leaves are used as antihypertensive remedy. It exhibited negative chronotropic and hypotensive effects through α-adrenoceptor and Ca^{2+} channel antagonism [2]. Moreover, the wood extract of *A. altilis*, which contains artocarpin as the major constituent, reduced cell viability by inducing apoptosis and sub-G1 phase formation in human breast T47D cells in vitro [3]. Another study [4] reported that the roots of *A. altilis* showed antitubercular, cytotoxic and antimalarial activities. Bioassay guided isolation led to the identification of nine prenylated flavones. These flavones exhibited antitubercular activity against Mycobacterium tuberculosis H37Ra with a minimum inhibitory concentration (MIC) ranging from 3.12 to 100 µg/ml and showed cytotoxicity against KB, BC and Vero cell lines which were similar with the IC_{50} values of 2.9–14.7 µg/ml. Morusin, cudraflavone B, cycloartobiloxanthone, artonin E and artbiloxanthone found in the root bark exhibited moderate antiplasmodial activity with IC_{50} values of 1.9 to 4.3 µg/ml [10]. Another study reported that the major constituents of the EtOAc extract of *A. altilis* which exhibited cytoprotective effects are β-sitosterol and six flavonoids [5]. Furthermore, flavonoids isolated from *A. altilis* showed potential as antioxidants and/or skin-whitening agents [6]. A review on the traditional uses, phytochemistry and pharmacology of *Artocarpus* has been provided [7].

Artocarpus odoratissimus Blanco, locally known as marang is native to the Philippines and Borneo. In the Philippines, it has no known medicinal property. It is cultivated for its fruit which is sold commercially [8]. Studies on the nutritional composition [9], proximate analysis [10], the cytotoxicity and polyphenol diversity in selected parts of the fruits [11], and the phytochemicals and antioxidant activity of different parts of *A. odoratissimus* [12] were reported. A recent study reported the isolation of a new prenylated pyranoflavone derivative artosimmin and traxateryl acetate. Artosimmin exhibited significant cytotoxicity against cancer cell lines (HL-60 and MCF-7) and showed antioxidant properties [13].

This study was conducted as part of our research on the chemical constituents of *Artocarpus* species in the Philippines. We earlier reported the isolation of cycloartenone, cycloartenol, and a diastereomeric mixture of 2,3-butanediols from *Artocarpus heterophyllus* [14]. Recently, we reported the isolation of friedelinol, squalene, β-sitosterol, sigmasterol, phytol, polyprenol, cycloartenol and cycloartenol acetate from *Artocapus camansi* [15].

We report here the isolation of β-sitosterol (1), unsaturated triglycerides (2), squalene (3), polyprenol (4), lutein (5) (Fig. 1) and unsaturated fatty acids from *A. altilis*, while *A. odoratissimus* afforded 1, 2, and unsaturated fatty acids from the flesh of the fruit and seeds; and 1, unsaturated fatty acids and hydrocarbons from the fruit rind.

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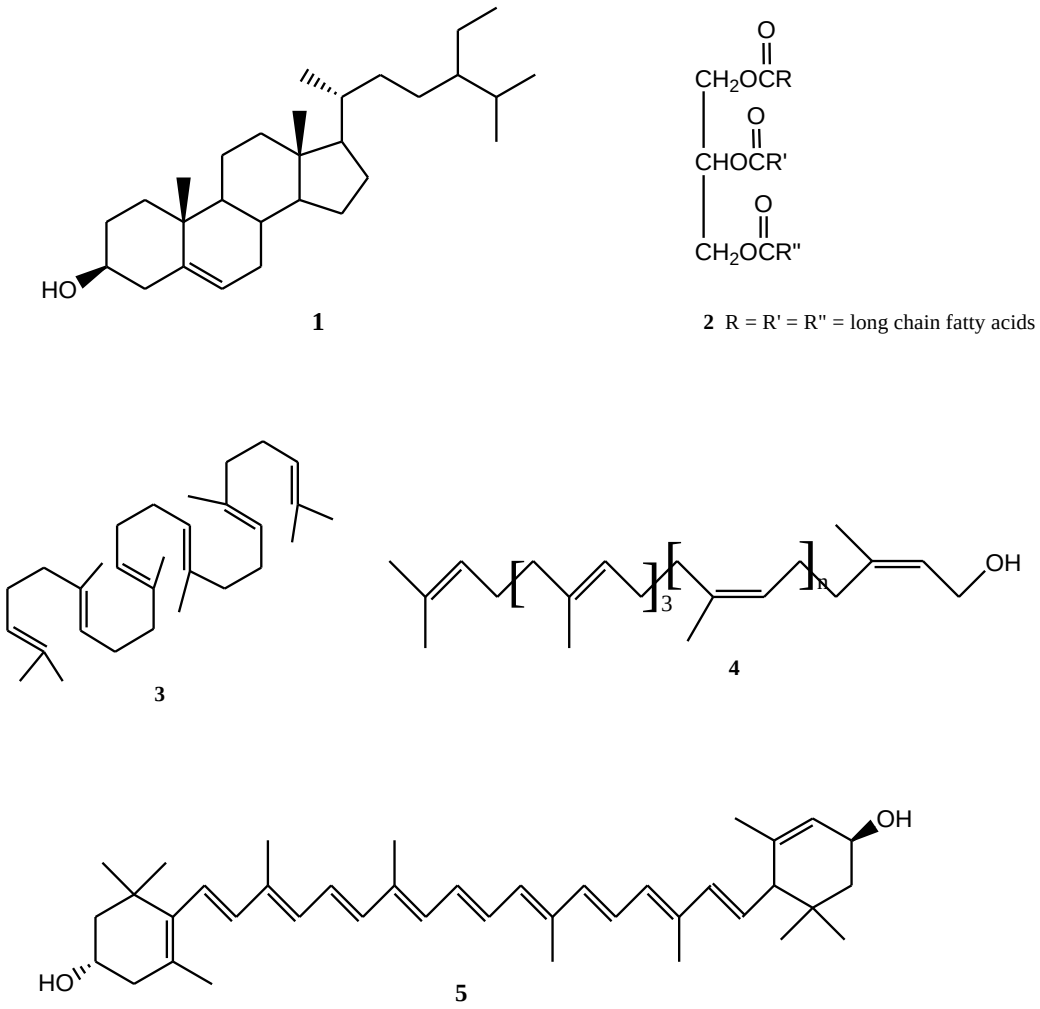

Figure 1: Chemical constituents of *A. altilis*: β-sitosterol (1), unsaturated triglycerides (2), squalene (3), polyprenol (4), lutein (5) and *A. odoratissimus* (1-2).

MATERIALS AND METHODS

General Experimental Procedures

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 followed by warming.

A glass column (18 inches in height and 1.0 inch internal diameter) was packed with silica gel. The crude extract was fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10 % increments) as eluents. 100 mL fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *Rf* values were combined and rechromatographed. A glass column (12 inches in height and 0.5 inch internal diameter) was used for the rechromatography. 5mL fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. 1 mL fractions were collected.

Sample Collection

The leaves of *A. altilis* was collected from the De La Salle-Manila Campus in January 2013. It was identified as *Artocarpus altilis* (Parkinson) Fosberg at the Bureau of Plant Industry, Manila, Philippines.

The fruit of *A. odoratissimus* were collected from Antipolo City, Philippines in August 2013. It was identified as *Artocarpus odoratissimus* Blanco at the Bureau of Plant Industry, Manila, Philippines.

Isolation of Chemical Constituents of Artocarpus altilis

The air-dried leaves (348.4 g) of *A. altilis* were soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (12.5 g) which was chromatographed in increasing proportions of acetone in CH_2Cl_2 at 10% increments by volume. The CH_2Cl_2 fraction was rechromatographed (3 ×) in petroleum ether to afford 3 (4 mg). The 20% to 30% acetone in CH_2Cl_2 fractions were combined and rechromatographed by gradient elution in 5% EtOAc in petroleum ether, followed by 7.5% EtOAc in petroleum ether, then finally 10% EtOAc in petroleum ether. The fractions eluted with 5% EtOAc in petroleum ether were combined and rechromatographed (3 ×) in 7.5% EtOAc in petroleum ether to afford 4 (3 mg). The fractions eluted with 7.5% EtOAc in petroleum ether were combined and rechromatographed (2 ×) in the same solvent to afford 2 (5 mg) after washing with petroleum ether. The fractions eluted with 10% EtOAc in petroleum ether were combined and rechromatographed (4 ×) in the same solvent to afford unsaturated fatty acids (7 mg). The 40% acetone in CH_2Cl_2 fractions were combined and rechromatographed (4 ×) in 15% EtOAc in petroleum ether to afford 1 (2 mg). The 50% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) in CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume ratio) afford 5 (6 mg) after washing with petroleum ether, followed by Et_2O.

Isolation of Chemical Constituents of Artocarpus odoratissimus

The fruit was separated into flesh of the fruit, seeds and rind and processed separately. The flesh of the fruit, seeds and rind were cut into small pieces and then freeze-dried. The flesh of the fruit (759 g), seeds (304 g) and rind (116 g) were separately ground in a blender, soaked in CH_2Cl_2 for three days and then filtered. The filtrates were concentrated under vacuum to afford the crude extracts: fruit (67.5 g), seeds (46.3 g) and rind (1.5 g).

The crude extract from the flesh of the fruit was chromatographed in increasing proportion of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (4 ×) in 10% EtOAc in petroleum ether to afford unsaturated fatty acids (9 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed in 10% EtOAc in petroleum ether, followed by 15% EtOAc in petroleum ether. The fractions eluted with 10% EtOAc in petroleum ether were rechromatographed (3 ×) in 7.5% % EtOAc in petroleum ether to afford 2 (7 mg). The fractions eluted with 15% EtOAc in petroleum ether were rechromatographed (3 ×) in the same solvent to afford 1 (6 mg) after washing with petroleum ether.

The crude extract from the seeds was chromatographed in increasing proportion of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (3 ×) in 10% EtOAc in petroleum ether to afford unsaturated fatty acids (15 mg). The 10% and 20% acetone in CH_2Cl_2 fractions were combined and rechromatographed in 10% EtOAc in petroleum ether, followed by 15% EtOAc in petroleum ether. The fractions eluted with 10% EtOAc in petroleum ether were rechromatographed (3 ×) in 7.5% % EtOAc in petroleum ether to afford 2 (12 mg). The fractions eluted with 15% EtOAc in petroleum ether were rechromatographed (2 ×) in the same solvent to afford 1 (4 mg) after washing with petroleum ether.

The crude extract from the rind of the fruit was chromatographed in increasing proportion of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed in petroleum ether, followed by 5% EtOAc in petroleum ether, and finally 10% EtOAc in petroleum ether. The petroleum ether fraction was rechromatographed (2 ×) in petroleum ether to afford hydrocarbons (3 mg). The 10% EtOAc in petroleum ether fractions were combined and rechromatographed in 10% EtOAc in petroleum ether to afford unsaturated fatty acids (5 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed in 10% EtOAc in petroleum ether, followed by 15% EtOAc in petroleum ether. The fractions eluted with 15% EtOAc in petroleum ether were rechromatographed (2 ×) in the same solvent to afford **1** (2 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the leaves of *Artocarpus altilis* yielded β-sitosterol (1) [16], unsaturated triglycerides (2) [17], squalene (3) [18], polyprenol (4) [19], lutein (5) [20] and unsaturated fatty acids. The structures of 3-5 were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature [16-20].

Silica gel chromatography of the dichloromethane extract of the fruit and seed of *A. odorarissimus* afforded 1 [16], 2 [17], and unsaturated fatty acids [21] from the flesh of the fruit and seeds; and 1, unsaturated fatty acids and hydrocarbons [22] from the fruit rind. The structures of 1, 2, unsaturated fatty acids and hydrocarbons were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature [16-17, 21-22]. Although no biological activity tests were conducted on the isolated compounds, literature search revealed that these have known bioactivities.

β-Sitosterol (1) was reported to exhibit growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [23]. It was shown to be effective for the treatment of benign prostatic hyperplasia [24]. It attenuated β-catenin and PCNA expression, as well as quenched radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis [25]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [26]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [27].

Triglyceride (2) exhibited antimicrobial activity against *S. aureus, P. aeruginosa, B. subtilis, C. albicans* and *T. mentagrophytes* [17]. Another study reported that triglycerides showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation [28].

Squalene (3) significantly suppresses colonic ACF formation and crypt multiplicity which strengthens the hypothesis that it possesses chemopreventive activity against colon carcinogenesis [29]. Squalene has cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [30].

Polyprenols (4) act as co-enzymes of membrane active transport systems for polysaccharides, peptidoglycans and carbohydrate containing biopolymers [31]. Polyprenol from *Ginkgo biloba* L exhibited hepatoprotective effects against CCl_4-induced hepatotoxicity in rats [32]. Polyprenols from *Ginkgo biloba* L. exhibited antitumor activity [33]. The antidyslipidemic activity of polyprenol from *Coccinia grandis* in high-fat diet-fed hamster model was also reported [34].

Dietary lutein (5), especially at 0.002%, inhibited tumor growth by selectively modulating apoptosis and by inhibiting angiogenesis [35]. 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 [36]. Very low amounts of dietary lutein (0.002%) can efficiently decrease mammary tumor development and growth in mice [37].

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