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Triterpenes and Sterol from *Artocarpus ovatus*

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ABSTRACT

Chemical investigation of the dichloromethane extract of the twigs of *Artocarpus ovatus* afforded lupeol (**1a**), α-amyrin (**1b**), β-amyrin (**1c**), lupeol fatty acid ester (**2a**), α-amyrin fatty acid ester (**2b**), β-myrin fatty acid ester (**2c**), betulin (**3a**), *3β*, 28-Dihydroxyolean-12-ene (**3b**), oleanolic acid (**4**), β-sitosterol (**5**), and chlorophyll a (**6**). The structures of **1**-**6** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature.

INTRODUCTION

Artocarpus ovatus Blanco, an endemic Philippine tree, locally known as anubing is an evergreen tree growing up to 25 meters tall. A latex obtained from the tree has potential for use as a chewing gum base (Fern, 2012). The roasted seeds are eaten, while the timber which is strong and durable is used for construction (De la Cruz, 1991).

There is no reported study on the chemical constituents of *A. ovatus*. However, congeners of the tree have been studied for their chemical constituents and biological activities. There are about 50 species of the genus *Artocarpus* (Moraceae) which are sources of edible fruit and timber and are used in folk medicines. A review of the chemical constituents, biological and pharmacological activities of *Artocarpus* has been provided (Jagtap and Bapat, 2010). *Artocarpus* species are used for treatment against inflammation, malarial fever, diarrhoea, diabetes and tapeworm infection. They are rich in phenolic compounds such as flavonoids, stilbenoids, arylbenzofurons & jacalin (Jagtap and Bapat, 2010). Compounds from *Artocarpus* species exhibit diverse biological activities including antibacterial, antitubercular, antiviral, antifungal, antiplatelet, antiarthritic, tyrosinase inhibitory and cytotoxicity (Jagtap and Bapat, 2010). This study is part of our research on the chemical constituents of the genus *Artocarpus* found in the Philippines.

We earlier reported the isolation of friedelinol, squalene, β-sitosterol, stigmasterol and phytol from the leaves of *A. camansi*, while the stems yielded polyprenol, cycloartenol and cycloartenol acetate (Tsai *et al.*, 2013). In another study, the leaves of *A. altilis* yielded β-sitosterol, triglycerides, squalene, polyprenol, lutein and fatty acids, while *A. odoratissimus* afforded β-sitosterol, triglycerides and fatty acids from the flesh of the fruit and seeds; and β-sitosterol, fatty acids and hydrocarbons from the fruit rind (Ragasa *et al.*, 2014). Furthermore, the unripe fruit of *A. heterophyllus* afforded cycloartenone, cycloartenol, and a diastereomeric mixture of 2,3-butanediols (Ragasa and Jorvina, 2004).

We report herein the fractionation by silica gel chromatography of the dichloromethane extract of the twigs of *A. ovatus*. The following compounds from the twigs were identified by NMR spectroscopy: lupeol (**1a**), α-amyrin (**1b**), β-amyrin (**1c**), lupeol fatty acid ester (**2a**), α-amyrin fatty acid ester (**2b**), β-amyrin fatty acid ester (**2b**), betulin (**3a**), *3β*, 28-dihydroxyolean-12-ene (**3b**), oleanolic acid (**4**), β-sitosterol (**5**), and chlorophyll a (**6**) (Fig. 1). To the best of our knowledge this is the first report on the identification of these compounds from *A. ovatus*.

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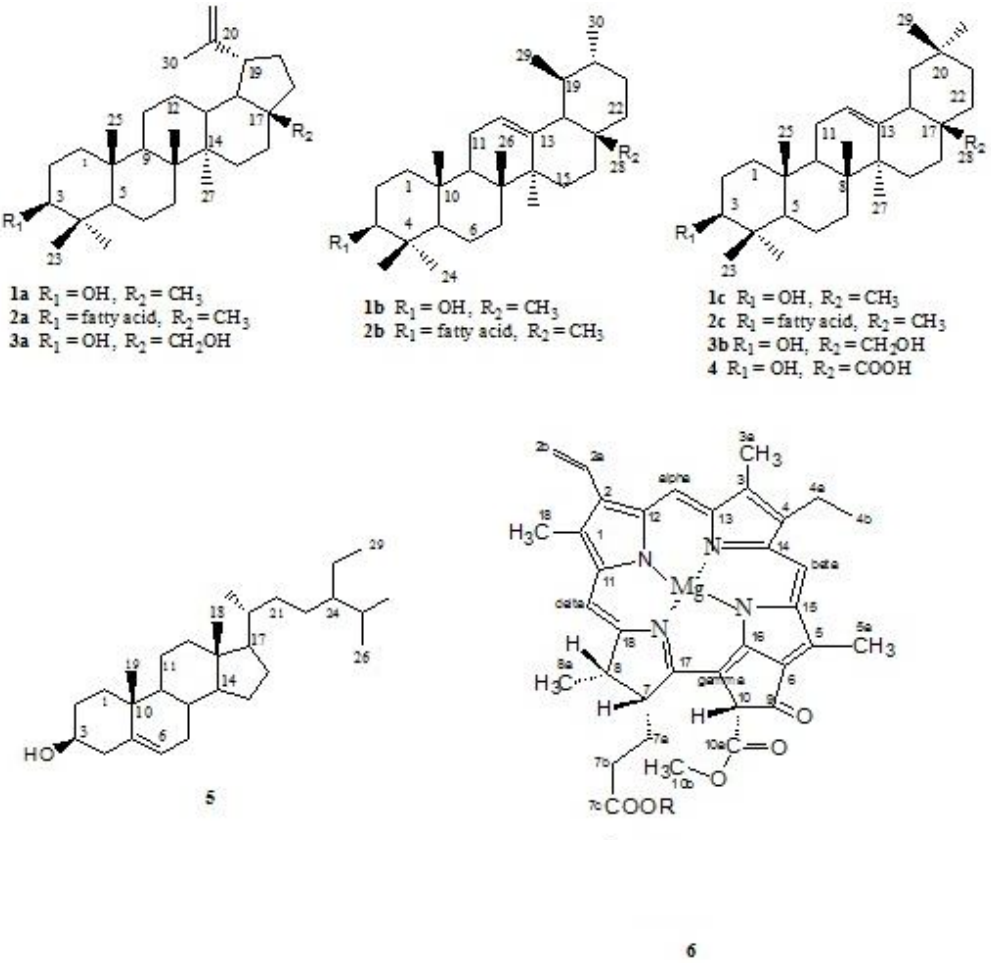

Fig. 1: Chemical constituents of the twigs of *Artocarpus ovatus*: lupeol (**1a**), α-amyrin (**1b**), β-amyrin (**1c**), lupeol fatty acid ester (**2a**), α-amyrin fatty acid ester

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

General Isolation Procedure

A glass column 18 inches in height and 1.0 inche 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 dichloromethane (10% increment) as eluents. Fifty milliliter fractions were collected.

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. 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

The air-dried twigs of *A. ovatus* (278.7 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 (8.1 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (5 ×) in 5% EtOAc in petroleum ether to afford a mixture of **2a-2c** (5 mg) after washing with petroleum ether. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) in 15% EtOAc in petroleum ether to afford a mixture of **1a-1c** (1 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed

(3 ×) in 20% EtOAc in petroleum ether to afford **5** (6 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) in 20% EtOAc in petroleum ether to afford a mixture of **3a** and **3b** (2 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) in CH_3CN:Et_2O:CH_2Cl_2 (1:1:8) by volume ratio to afford **4** (4 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2 fraction was rechromatographed (4×) in CH_3CN:$Et_2O$$CH_2Cl_2$ (1:1:8) by volume ratio to afford **6** (5 mg) after washing with petroleum ether, followed by Et_2O.

Lupeol (**1a**)

colorless solid. ^{1}H NMR (600 MHz, $CDCl_3$): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.68 (H-29b), 4.55 (H-29a), 3.18 (H-3), 1.68 (s, H_3-30), 0.96 (s, H_3-23), 0.78 (s, H_3-24), 0.83 (s, H_3-25), 0.94 (s, H_3-26), 1.06 (s, H_3-27), 0.91 (s, H_3-28), 1.68 (s, H_3-30).

α-Amyrin (**1b**)

Colorless solid, ^{1}H NMR (600 MHz, $CDCl_3$): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.15 (H-3), 0.67 (H-5), 5.15 (H-12), 0.94 (s, H_3-23), 0.76 (s, H_3-24), 0.75 (s, H_3-25), 0.89 (s, H_3-26), 1.02 (s, H_3-27), 0.94 (s, H_3-28), 0.85 (d, *J* = 6.0 Hz, H_3-29), 0.74 (d, *J* = 7.0 Hz, H_3-30).

β-*Amyrin* (**1c**)

Colorless solid. ^{1}H NMR (600 MHz, $CDCl_3$): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.15 (H-3), 0.67 (H-5), 5.22 (H-12), 0.77 (s, H_3-23), 0.90 (s, H_3-24), 0.74 (s, H_3-25), 0.94 (s, H_3-26), 1.16 (s, H_3-27), 1.06 (s, H_3-28), 0.86 (s, H_3-29), 0.79 (s, H_3-30).

Lupeol fatty acid ester (**2a**)

Colorless solid. ^{13}C NMR (150 MHz, $CDCl_3$): 38.62 (C-1), 23.42 (C-2), 80.49 (C-3), 38.61 (C-4), 55.48 (C-5), 18.23 (C-6), 34.71 (C-7), 40.97 (C-8), 50.99 (C-9), 38.62 (C-10), 21.06 (C-11), 25.17 (C-12), 38.62 (C-13), 42.64 (C-14), 27.49 (C-15), 35.49 (C-16), 43.95 (C-17), 48.36 (C-18), 47.90 (C-19), 150.89 (C-20), 29.36 (C-21), 39.94 (C-22), 27.49 (C-23), 16.93 (C-24), 14.82 (C-27), 18.23 (C-28), 109.39 (C-29), 19.35 (C-30), 173.69 (C-1'), 34.87 (C-2'), 31.92 (C-3'), 22.69, 25.17, 29.18-29.70 $(CH_2)_n$, 14.12 (CH_3).

α-Amyrin fatty acid ester (**2b**)

Colorless solid. ^{13}C NMR (150 MHz, $CDCl_3$): 38.68 (C-1), 22.69 (C-2), 80.49 (C-3), 37.14 (C-4), 55.48 (C-5), 18.37 (C-6), 32.48 (C-7), 39.68 (C-8), 47.89 (C-9), 37.11 (C-10), 23.31 (C-11), 124.66 (C-12), 138.73 (C-13), 42.17 (C-14), 28.70 (C-15), 26.56 (C-16), 33.75 (C-17), 59.04 (C-18), 39.59 (C-19), 39.68 (C-20), 31.25 (C-21), 41.51 (C-22), 28.06 (C-23), 16.92 (C-26), 23.42 (C-27), 28.06 (C-28), 17.46 (C-29), 21.38 (C-30), 173.69 (C-1'), 34.87 (C-2'), 31.92 (C-3'), 22.69, 25.17, 29.18-29.70 $(CH_2)_n$, 14.12 (CH_3).

β-*Amyrin fatty acid ester* (**2c**)

Colorless solid. ^{13}C NMR ($CDCl_3$): 38.62 (C-1), 22.69 (C-2), 80.49 (C-3), 37.14 (C-4), 55.48 (C-5), 18.37 (C-6), 32.48 (C-7), 39.68 (C-8), 47.22 (C-9), 37.14 (C-10), 23.66 (C-11), 121.95 (C-12), 144.30 (C-13), 41.51 (C-14), 26.08 (C-15), 26.91 (C-16), 32.48 (C-17), 47.83 (C-18), 46.76 (C-19), 31.25 (C-20), 34.87 (C-21), 37.14 (C-22), 28.06 (C-23), 16.92 (C-24), 16.92 (C-26), 26.08 (C-27), 28.06 (C-28), 33.31 (C-29), 23.66 (C-30), 173.69 (C-1'), 34.87 (C-2'), 31.92 (C-3'), 22.69, 25.17, 29.18-29.70 $(CH_2)_n$, 14.12 (CH_3).

Betulin (**3a**)

Colorless solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.18 (H-3), 0.96 (s, H_3-23), 0.74 (s, H_3-24), 0.81 (s, H_3-25), 0.98 (s, H_3-26), 1.00 (s, H_3-27), 3.33 (d, H-28a), 3.79 (d, H-28b), 4.55 (d, H-29a), (d, 4.68 H-29b), 1.66 (s, H_3-30).

***3β*, 28-Dihydroxyolean-12-ene** (**3b**)

Colorless solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.18 (H-3), 5.18 (d, H-12), 0.96 (s, H_3-23), 0.74 (s, H_3-24), 0.87 (s, H_3-25), 0.98 (s, H_3-26), 1.15 (s, H_3-27), 0.95 (H_3-29), 0.88 (H_3-29), 3.20 (d, H-28a), 3.52 (d, H-28b).

Oleanolic acid (**4**)

Colorless solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.20 (dd, *J* = 4.2, 11.4 Hz, H-3α), 5.26 (t, *J* = 3.6 Hz, H-12), 2.81 (dd, *J* = 4.2, 13.8 Hz, H-18), 0.96 (s, H_3-23), 0.73 (s, H_3-24), 0.89 (s, H_3-25), 0.75 (s, H_3-26), 1.11 (s, H_3-27), 0.91 (s, H_3-29), 0.88 (s, H_3-30); ^{13}C NMR (150 MHz, $CDCl_3$): δ 38.38 (C-1), 27.17 (C-2), 77.02 (C-3), 38.74 (C-4), 55.20 (C-5), 18.28 (C-6), 32.59 (C-7), 39.25 (C-8), 47.62 (C-9), 37.07 (C-10), 23.38 (C-11), 122.63 (C-12), 143.58 (C-13), 41.57 (C-14), 27.67 (C-15), 22.90 (C-16), 46.51 (C-17), 40.96 (C-18), 45.86 (C-19), 30.67 (C-20), 33.78 (C-21), 32.42 (C-22), 28.09 (C-23), 15.53 (C-24), 15.31 (C-25), 17.12 (C-26), 25.93 (C-27), 183.29 (C-28), 33.06 (C-29), 23.56 (C-30).

β-Sitosterol (**5**)

^{1}H NMR (600 MHz, $CDCl_3$): δ 3.51 (m, H-3), 2.26, 2.21 (H_2-4), 5.33 dd (*J* = 3.0, 2.4 Hz, H-6), 0.66 (s, H_3-18), 0.99 (s, H_3-19), 0.90 (d, *J* = 6.6 Hz, H_3-21), 0.79 (d, *J* = 7.2 Hz, H_3-26), 0.82 (d, *J* = 6.6 Hz, H_3-27), 0.85 (t, *J* = 7.8 Hz, H_3-29).

Chlorophyll a (**6**)

^{1}H NMR (600 MHz, $CDCl_3$): δ 3.39 (s, H-1), 7.98 (dd, *J* = 18, 12 Hz, H-2a), 6.26 (dd, *J* = 18, 1.2 Hz, H-2b), 6.26 (dd, *J* = 12, 1.2 Hz, H-2b), 3.22 (s, H-3a), 3.67 (s, H-4a), 1.69 (t, *J* = 7.2, H-4b), 3.76 (s, H-1α or H-5α), 4.17 (m, H-7), 2.15, 2.46 (m, H_2-7a), 2.32, 2.60 (m, H_2-7b), 4.42 (m, H-8), 1.78 (d, *J* = 7.2 Hz, H_3-8a), 6.24 (s, H-10), 3.86 (s, H_3-10b), 9.38 (s, H-α or H-β), 9.51 (s, H-α or H-β), 8.54 (s, H-δ), 4.46 (m, P1), 5.11 (t, *J* = 1.2 Hz, P2), 1.56 (br s, P17), 0.82 (d, *J* = 6.6 Hz, P18 and P19), 0.76 (d, *J* = 6.6 Hz, P16), 0.74 (d, *J* = 6.6 Hz, P20).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the twigs of *A. ovatus* afforded lupeol (**1a**) (Prakash and Prakash), α-amyrin (**1b**) (Raga *et al.*, 2011a), β-amyrin (**1c**) (Raga *et al.*, 2011a), lupeol fatty acid ester (**2a**) (Raga *et al.*, 2011b), α-amyrin fatty acid ester (**2b**) (Miranda *et al.*, 2006), β-amyrin fatty acid ester (**2c**) (Barreiros *et al.*, 2012), betulin (**3a**) (Tijjani *et al.*, 2012), *3β*, 28-Dihydroxyolean-12-ene (**3b**) (Rahman *et al.*, 2007), oleanolic acid (**4**) (Ragasa and Lim, 2005), β-sitosterol (**5**) (Cayme and Ragasa, 2004), and chlorophyll a (**6**) (Ragasa and de Jesus, 2014). The structures of **1**-**6** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature.

Although bioassays were not conducted on the isolated compounds, there were previous studies that reported on their biological activities. Lupeol (**1a**) exhibited antiurolithiatic and diuretic activity (Vidya *et al.*, 2002). It prevented the formation of vesical calculi and reduced the size of the preformed stones in rats (Anand et al. 1994). α-Amyrin (**1b**) and β-amyrin (**1c**) were reported to possess anti-inflammatory activity (Recio et al. 1995; Madeiros et al. 2007), while α-amyrin, β-amyrin, and the 3-O-acyl derivatives of α-amyrin (**2b**) and β–amyrin (**2c**) exhibited analgesic property (Otuki et al. 2005; Soldi et al. 2008).

Betulin (**3a**) inhibited cell growth and induction of apoptosis in human melanoma cell line (Orchel *et al.*, 2014). It also induces apaptosis of *Leishmania donovani*. Treatment of promastigotes with betulin led to mitochondrial membrane damage, activation of caspase-like proteases, and DNA fragmentation in *L. donovani* promastigotes (Saudagar and Dubey, 2014).

An earlier study reported that ursolic acid and oleanolic acid (**4**) exhibited anti-tumor activity against human colon carcinoma cell line HCT15 with ursolic acid showing stronger activity than oleanolic acid (Li *et al.*, 2002). It exhibited anti-inflammatory effects by inhibiting hyperpermeability, the expression of CAMs, and the adhesion and migration of leukocytes (Lee *et al.*, 2013). It showed anti-inflammatory activities through the inhibition of the HMGB1 signaling pathway (Yang *et al.*, 2012). It has anti-inflammatory, hepatoprotective, gastroprotective, anti-ulcer, and immunoregulatory effect (Vachalkova *et al.*, 2004), gastroprotective effect on experimentally induced gastric lesions in rats and mice (Astudillo *et al.*, 2002), inhibits mouse skin tumor (Oguro ,1998), protects against hepatotoxicants and is used in China to treat hepatitis (Lui *et al.*, 1993), and significant antitumor activity on human colon carcinoma cell line HCT 15 (Li *et al.*, 2002).

β-Sitosterol (**5**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells (Awad *et al.*, 2007). It was shown to be effective for the treatment of benign prostatic hyperplasia (Jayaprakasha *et al.*, 2007). 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 (Baskar *et al.*, 2010). It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake (Jesch et al, 2009). It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells (Moon *et al.*, 2007).

Chlorophyll (**6**) and its various derivatives are used in traditional medicine and for therapeutic purposes (Edwards, 1954). Natural chlorophyll and its derivatives have been studied for wound healing (Kephart, 1955), anti-inflammatory properties (Larato and Pfao, 1970), control of calcium oxalate crystals (Tawashi *et al.*, 1980), utilization as effective agents in photodynamic cancer therapy (Sternberg *et al.*, 1998; Nourse *et al.*, 1988; Henderson *et al.*, 1997), and chemopreventive effects in humans (Egner *et al.*, 2001; Egner *et al.*, 2003). A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided (Hardwick *et al.*, 1997).

Thus, the dichloromethane extract of the twigs of *Artocarpus ovatus* afforded compounds (**1**-**6**) with diverse biological activities.

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