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Triterpenes and a coumarin derivative from *Kibatalia gitingensis* (Elm.) Woodson

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ABSTRACT

*Chemical investigations of the dichloromethane extracts of Kibatalia gitingensis (Elm.) Woodson afforded ursolic acid (**1**), squalene (**2**), α-amyrin acetate (**3**) and lupeol acetate (**4**) from the leaves, while the twigs yielded **1**-**4** and 6-hydroxy-7-methoxycoumarin or isoscopoletin (**5**). The structures of **1** and **5** were elucidated by extensive 1D and 2D NMR spectroscopy, while **2**-**4** were identified by comparison of their ^{13}C NMR data with those reported in the literature.*

Keywords: *Kibatalia gitingensis*, Apocynaceae, ursolic acid, squalene, α-amyrin acetate, lupeol acetate, 6-hydroxy-7- methoxycoumarin, isoscopoletin

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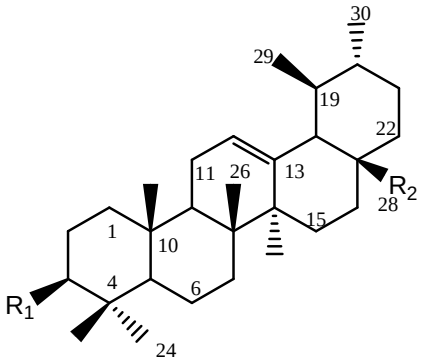
INTRODUCTION

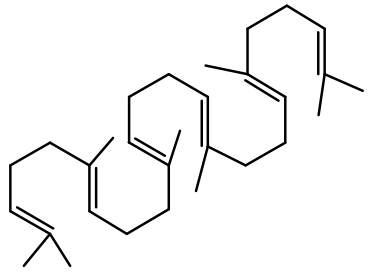
Kibatalia gitingensis (Elm.) Woodson of the family Apocynaceae is native to the Philippines, thriving well in secondary and primary forests at low and medium altitudes. Locally known as “laniti” or “laneteng-gubat”, it is listed as vulnerable in the IUCN Red List of Threatened Species [1]. It is popularly used to make building materials and decorative carvings though it has been reported that it also contains medicinally potential alkaloids [2]. Gitingensine, a new steroidal alkaloid from the leaves was found to exhibit antispasmodic activity [3, 4], ataraxic properties and a direct depressant of smooth muscles as well as a vasodilator of arteries to skeletal muscles and the splanchnic area [5]. Other studies reported that the leaves of *K. gitingensis* contain kibataline [6, 7] and 20-(epi-N-methyl)paravallarine [8]. Anazasteroidal alkaloid from the plant caused spontaneous intestinal motility and abolished serotonin contractions in mouse and dog intestines [9]. The bark of *K. gitingensis* contains a complex mixture of alkaloids, including paravallarine, N-methylparavallarine, and 20-epiparavallarine [10]. Furthermore, lanitine (2α-hydroxy-N-methylparavallarine) and its 2β-isomer were isolated from the stem bark of the plant [11].

This study is part of our research on the chemical constituents of plants endemic and native to the Philippines. We report herein the isolation and identification of ursolic acid (**1**), squalene (**2**), α-amyrin acetate (**3**) and lupeol acetate

(**4**) from the leaves; and **1**-**4** and isoscopoletin (**5**) (Fig. 1) from the twigs of *K. gitingensis*. To the best of our knowledge this is the first report on the isolation of these compounds from the tree.



1 R_1 = OH, R_2 = COOH
3 R_1 = OAc, R_2 = CH_3



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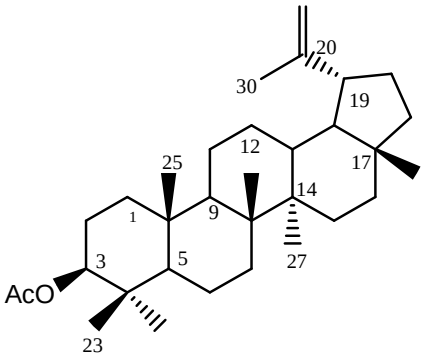

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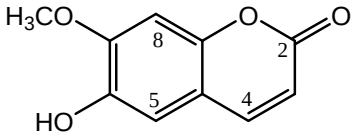

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Fig. 1. Chemical constituents from *Kibatalia gitingensis*: ursolic acid (1), squalene (2), α-amyrin acetate (3), lupeol acetate (4) and isoscopoletin (5)

MATERIALS AND METHODS

1. Isolation of the Chemical Constituents of the CH_2Cl_2 Leaf Extract

1.1 Sample Collection

Samples of leaves and twigs of *Kibatalia gitingensis* (Elm.) Woodson were collected from the De La Salle University – Science and Technology Complex (DLSU-STC) reforested area in February 2014. The samples were authenticated by one of the authors (EHM) and deposited at the De La Salle University Herbarium with voucher specimen # 908.

1.2 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.

1.3 General Isolation Procedure

A glass column 20 inches in height and 2.0 inches internal diameter was packed with silica gel. The crude extract from the leaves were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. One hundred 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. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

1.4 Isolation

Leaf samples of *Kibatalia gitingensis* (Elm.) Woodson were air-dried for about one week. The air-dried leaves (352 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (12.7 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The dichloromethane fraction was rechromatographed (3 ×) in petroleum ether to afford **2** (15 mg). The 20% acetone in DCM fraction was rechromatographed (4 ×) in 5% EtOAc in petroleum ether to obtain a mixture of **3** and **4** (14 mg). The 80% to 90% acetone in CH_2Cl_2 and acetone fractions were combined and rechromatographed (3 ×) in CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume ratio) to afford **1** (125 mg) after trituration with petroleum ether.

The twigs of *Kibatalia gitingensis* (Elm.) Woodson were air-dried for about one week. The air-dried twigs (329 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (3.4 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The dichloromethane fraction was rechromatographed (2 ×) in petroleum ether to afford **2** (25 mg). The 20% acetone in DCM fraction was rechromatographed (4 ×) in 5% EtOAc in petroleum ether to obtain a mixture of **3** and **4** (10 mg). The 50% acetone in CH_2Cl_2 fractions were combined and rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume ratio) to afford **1** (22 mg) after trituration with petroleum ether. The 60% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio) to afford **5** (7 mg) after trituration with petroleum ether.

Ursolic Acid (**1**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 36.98 (C-1), 28.12 (C-2), 79.04 (C-3), 38.60 (C-4), 55.22 (C-5), 18.29 (C-6), 32.96 (C-7), 39.47 (C-8), 47.53 (C-9), 38.75 (C-10), 23.29 (C-11), 125.86 (C-12), 137.93 (C-13), 42.01 (C-14), 27.22 (C-15), 24.18 (C-16), 47.89 (C-17), 52.71 (C-18), 39.05 (C-19), 38.82 (C-20), 30.60 (C-21), 36.68 (C-22), 28.00 (C-23), 15.47 (C-24), 15.59 (C-25), 17.07 (C-26), 23.55 (C-27), 180.79 (C-28), 17.00 (C-29), 21.16 (C-30).

Squalene (**2**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.68 (C-1), 131.24 (C-2), 124.30 (C-3), 26.68 (C-4), 39.72 (C-5), 134.89 (C-6), 124.40 (C-7), 26.77 (C-8), 39.75 (C-9), 135.01 (C-10), 124.30 (C-11), 28.27 (C-12), 17.67 (C-13), 16.03 (C-14), 16.00 (C-15).

α-Amyrin acetate (**3**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 38.46 (C-1), 23.60 (C-2), 80.97 (C-3), 37.70 (C-4), 55.25 (C-5), 18.23 (C-6), 32.86 (C-7), 40.02 (C-8), 47.64 (C-9), 36.78(C-10), 23.36 (C-11), 124.31 (C-12), 139.62 (C-13), 42.07 (C-14), 26.59 (C-15), 28.08 (C-16), 33.74 (C-17), 59.05 (C-18), 39.64 (C-19), 39.60 (C-20), 31.24 (C-21), 41.52 (C-22), 28.74 (C-23), 16.86 (C-24), 15.73 (C-25), 16.73 (C-26), 23.22 (C-27), 28.74 (C-28), 17.50 (C-29), 21.39 (C-30), 171.00, 21.31 (OAc).

Lupeol acetate (**4**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 38.4 (C-1), 27.4 (C-2), 80.95 (C-3), 38.5 (C-4), 55.3 (C-5), 18.0 (C-6), 34.2 (C-7), 55.3 (C-8), 50.3 (C-9), 37.1 (C-10), 20.9 (C-11), 25.1 (C-12), 38.0 (C-13), 42.1 (C-14), 27.4 (C-15), 35.6 (C-16), 47.8 (C-17), 48.28 (C-18), 48.0 (C-19), 151.0 (C-20), 29.69 (C-21), 40.0 (C-22), 28.1 (C-23), 15.7 (C-24), 16.2 (C-25), 16.17 (C-26), 14.5 (C-27), 18.2 (C-28), 109.3 (C-29), 18.2 (C-30), 171.0, 21.4 (OAc).

Isoscopoletin (**5**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.25 (d, *J* = 8.0 Hz, H-3), 7.57 (d, *J* = 8.0 Hz, H-4), 6.83 (1H, s, H-5), 6.90 (1H, s, H-8), 3.94 (OCH_3, s); ^{13}C NMR (150 MHz, $CDCl_3$): δ 161.43 (C-2), 113.43 (C-3), 143.27 (C-4), 111.49 (C-4a), 107.6 (C-5), 149.66 (C-6), 143.97 (C-7), 103.19 (C-8), 150.25 (C-8a), 56.40 (OCH_3).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *Kibatalia gitingensis* afforded ursolic acid (**1**), squalene (**2**) [12], and a mixture of α-amyrin acetate (**3**) [13] and lupeol acetate (**4**) [14] in a 4:1 ratio from the leaves; and **1**, **2**, a mixture of α-amyrin acetate (**3**) and lupeol acetate (**4**) in a 3:1 ratio and isoscopoletin (**5**) [15] from the twigs. The structures of **1** and **5** were elucidated by extensive 1D and 2D NMR spectroscopy. The structures of **2**-**4** were identified by comparison of their ^{13}C NMR data with those reported in the literature [12-14], while **5** was confirmed by comparison of its ^{1}H NMR resonances with literature data [15]. The ratios of **3** and **4** were deduced from the integration of the ^{1}H NMR resonances for the olefinic protons of **3** at δ 5.10 (t, *J* =3.6 Hz) and **4** at δ 4.55 (d, *J* =2.4 Hz) and 4.66 (d, *J* =2.4 Hz).

Although no biological activity tests were conducted on the isolated compounds (**1**-**5**), literature search revealed that these have diverse bioactivities as follows.

Ursolic acid (**1**) was found to induce apoptosis in tumor cells by activation of caspases and modulation of other pathways involved in cell proliferation and migration. It decreases proliferation of cells and induces apoptosis, thereby inhibiting growth of tumor cells both *in vitro* and *in vivo* [16]. An earlier study reported that **1** exhibited anti-tumor activity against human colon carcinoma cell line HCT15 [17]. Moreover, **1** inhibits the growth of colon cancer-initiating cells by targeting STAT3 [18]. Furthermore, **1** has potential therapeutic use in prostate cancer through its antiproliferative and apoptotic effects [19]. A recent study reported that **1** inhibited cell growth and proliferation of Jurkat leukemic T-cells, as well as suppressed PMA/PHA induced IL-2 and TNF-α production in a concentration and time dependent manner [20]. Another study reported that ursolic acid-activated autophagy induced cytotoxicity and reduced tumor growth of cervical cancer cells TC-1 in a concentration-dependent manner [21].

Squalene (**2**) was reported to significantly suppress colonic ACF formation and crypt multiplicity which strengthened the hypothesis that it possesses chemopreventive activity against colon carcinogenesis [22]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [23]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells [24]. The preventive and therapeutic potential of squalene containing compounds on tumor promotion and regression have been reported [25]. A recent review on the bioactivities of squalene has been provided [26].

α-Amyrin acetate (**3**) at 100 mg/kg showed significant ($p < 0.05$) inhibition of egg albumen-induced paw edema with 40 % inhibition at the 5th hour. β-Amyrin acetate and **3** isolated from the *Alstonia boonei* stem bark exhibited profound anti-inflammatory activity [27]. β-Amyrin acetate and **3** were also reported to exhbit sedative, anxiolytic and anticonvulsant properties [28]. The anti-inflammatory effect of lupeol acetate (**4**) involves the opioid system, as indicated by the complete blockade of the opioid antagonist naloxone [29]. Isoscopoletin (**5**) showed substantial inhibition in a cell proliferation assay using human CCRF-CEM leukaemia cells with IC_{50} value of 4.0 μM [30]. It also exhibited antimicrobial properties against *Bacillus cerius* and *Staphylococcus aureus* [31].

CONCLUSION

Previous studies on *K. gitingensis* reported the isolation of a number of alkaloids from the tree. In this study, the dichloromethane extracts of the leaves and twigs of *K. gitingensis* yielded ursolic acid (**1**), squalene (**2**) and isoscopoletin (**5**) which were reported to exhibit anticancer properties, while α-amyrin acetate (**3**) and lupeol acetate (**4**) were reported to possess anti-inflammatory activities.

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