



NOTE

Chemical Constituents of Leaves and Fruit of *Antidesma ghaesembilla* Gaertn.

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Chemical investigation of the dichloromethane extracts of *Antidesma ghaesembilla* Gaertn. has led to the isolation of β -friedelinol (1), lupeol (2), squalene (3), polyprenol (4), β -sitosterol (5), long-chain hydrocarbons (6) and chlorophyll-a (7) from the leaves and β -sitosterol (5) and triacylglycerols (8) from the fruit. The structure of β -friedelinol (1) was elucidated by extensive 1D and 2D NMR spectroscopy, while those of compounds 2 to 8 were identified by comparison of their NMR data with literature data.

Keywords: *Antidesma ghaesembilla*, Phyllanthaceae, β -Friedelinol, Lupeol, Squalene, Polyprenol, β -Sitosterol, Chlorophyll-a.

Antidesma ghaesembilla Gaertn. (family: Phyllanthaceae) is a plant indigenous of Philippines. Locally known as binayuyo, the young shoots are used as a vegetable and as a spice [1]. The leaves are used as a poultice to treat headaches, scurf, abdominal swellings and fevers. The stems are emmenagogue and the fruit is purgative. The fully ripe fruit can be eaten raw, cooked or made into jams and jellies [2]. A previous study reported that the crude methanol extract of *A. ghaesembilla* Gaertn. leaves exhibited moderate to strong antioxidant potential and showed significant hypoglycemic potential [3]. Recently, the methanolic extract of *A. ghaesembilla* leaves was reported to exhibit antithrombotic, cytotoxic and antibacterial activities [4]. Chemical studies on the leaves of *A. ghaesembilla* reported the isolation of a megastigmane, vomifoliol [5] and the flavone glycosides, vitexin, orientin, isovitexin and homoorientin [6].

We report herein the isolation of β -friedelinol (1), lupeol (2), squalene (3), polyprenol (4), β -sitosterol (5), long-chain hydrocarbons (6) and chlorophyll-a (7) from the leaves and β -sitosterol (5) and triacylglycerols (8) from the fruit of *A. ghaesembilla*. To the best of our knowledge this is the first report on the isolation of compounds 1-8 from *A. ghaesembilla*.

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

Sample collection: Samples of the leaves and fruits of *Antidesma ghaesembilla* Gaertn. were collected from Lupao, Nueva Ecija, Philippines in June 2016. The samples were authenticated at the Botany Division, Philippine National Museum.

Isolation procedure: A glass column 12 inches in height and 0.5 inch internal diameter was used for the chromatography. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 at 10 % increment by volume as eluents. Five milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of chemical constituents from leaves of *A. A. ghaesembilla*: The air-dried *A. ghaesembilla* leaves (323.4 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 (2.4 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10 % increment

by volume. The CH_2Cl_2 fraction was rechromatographed using petroleum ether. The less polar fractions were combined and rechromatographed using petroleum ether to afford long chain hydrocarbons (**6**) (8 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed using petroleum ether to yield squalene (**3**) (10 mg). The 10 % acetone in CH_2Cl_2 fraction from the chromatography of crude extract was rechromatographed (2 $\times$) using 1 % EtOAc in petroleum ether. Final purification was conducted by rechromatography using 5 % EtOAc in petroleum ether to afford polyprenol (**4**) (9 mg). 30 % acetone in CH_2Cl_2 fraction from the chromatography of crude extract was rechromatographed (2 $\times$) using 5 % EtOAc in petroleum ether to yield β -friedelinol (**1**) (12 mg) after washing with petroleum ether. The 40 % acetone in CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed by gradient elution using 5 % EtOAc in petroleum ether, followed by 7.5 % EtOAc in petroleum ether and finally, 10 % EtOAc in petroleum ether. The fractions eluted with 5 % EtOAc in petroleum ether were combined and rechromatographed using 5 % EtOAc in petroleum ether to provide lupeol (**2**) (7 mg) after washing with petroleum ether. The fractions eluted with 7.5 % EtOAc in petroleum ether were combined and rechromatographed using 10 % EtOAc in petroleum ether to afford β -sitosterol (**5**) (15 mg) after washing with petroleum ether. The fractions eluted with 10 % EtOAc in petroleum ether were combined and rechromatographed using 15 % EtOAc in petroleum ether to yield chlorophyll-a (**7**) (3 mg) after washing with petroleum ether, followed by Et_2O .

Isolation of the chemical constituents from fruit of *A. ghaesembilla*: The freeze-dried *A. ghaesembilla* fruit (38.2 g) was 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 (1.60 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10 % increment by volume. The CH_2Cl_2 fraction was rechromatographed (2 $\times$) using 10 % EtOAc in petroleum ether to yield triacylglycerols (**8**) (71 mg) after washing with petroleum ether. The 30 % acetone in CH_2Cl_2 fraction was rechromatographed using 10 % EtOAc in petroleum ether. Fractions from this column were combined and rechromatographed using 12.5 % EtOAc in petroleum ether to afford β -sitosterol (**5**) (39 mg) after washing with petroleum ether.

Silica gel chromatography of dichloromethane extracts of *A. ghaesembilla* afforded compounds **1-8**. The structure of β -friedelinol (**1**) was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its NMR data

with those reported in the literature for β -friedelinol [7]. The NMR spectra of lupeol (**2**) are in accordance with data reported in the literature for lupeol [8]; squalene (**3**) for squalene [9]; polyprenol (**4**) for polyprenol [10]; β -sitosterol (**5**) for β -sitosterol [11]; long-chain hydrocarbons (**6**) for long-chain hydrocarbons [12]; chlorophyll-a (**7**) for chlorophyll-a [13] and triacylglycerols (**8**) for triacylglycerols [14].

The presence of α -linolenic acid in the triacylglycerols (**8**) was deduced from the methyl triplet at δ 0.96 (t, J = 7.8 Hz), the double allylic methylenes at δ 2.78, allylic methylene protons at δ 2.00 and the olefinic protons at δ 5.34 (m) [15]. Oleic acid was also found in **8** as indicated by the resonances for the methyl triplet at δ 0.86 (t, J = 6.6 Hz), allylic methylene protons at δ 2.00 and the olefinic protons at δ 5.34 (m) [15].

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