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Pentacyclic Triterpenoids and Steroids from *Voacanga megacarpa.*

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ABSTRACT

Chromatographic purification of the crude DCM-methanolic extract of the Philippine endemic medicinal plant, *Voacanga megacarpa*, afforded a 1:1 mixture of lupeol acetate (**1**) and β-amyrin acetate (**1**), and a 1:1 mixture of stigmasterol (**3**) and β-sitosterol (**4**). The compounds were identified through analysis of their NMR spectroscopic data and by comparison with reported literature data. This is the first report on the isolation and identification of these compounds from *V. megacarpa*. The crude DCM-methanolic and alkaloid extracts, and fractions (1 and 2) showed moderate inhibitory activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MIC_{50} = 64 μg/mL).

Keywords: *Voacanga megacarpa,* triterpenoids, steroids, lupeol acetate, β-amyrin acetate, stigmasterol, β-sitosterol, antitubercular.

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INTRODUCTION

The *Voacanga* Thouars, (family Apocynaceae) is a small taxon comprised of twelve species. The genus is distributed in tropical Africa followed by Malesia. The Philippine indigenous *Voacanga* species (*V. megacarpa* and *V. globosa*) are trees, up to 15-25 m high, in secondary forest or scrub. Both plant species are characterized with white corolla and the stamens are inserted [1]. *Voacanga megacarpa* Merr. is found in the lowland forests of Camarines Sur province. The calyces of *V. megacarpa* are almost free and the lobes are much longer than the tube. The corolla lobes are elliptic, rounded to emarginated at apex and the tubes 22-40 mm long. The leaves and the decoction of the bark are traditionally used as analgesic and local anaesthetics [1-2]. Preliminary studies reported the identification of two monoterpenoid indole alkaloids namely, vobtusine and voacamine [1]. So far, no studies describing the biological activities of *V. megacarpa* have been reported. The alkaloids of a related endemic Philippine species, *V. globosa*, have shown antituberculosis and anti-cholinesterase activity [3].

As part of our continued interest in exploring the antitubercular activity of Philippine medicinal plants [4-13], we herein disclose the first isolation and identification of a mixture of the triterpenoids lupeol acetate (**1**) and β-amyrin acetate (**2**), and mixture of the sterols, stigmasterol (**3**) and β-sitosterol (**4**) from the leaves of *V. megacarpa* (Figure 1).

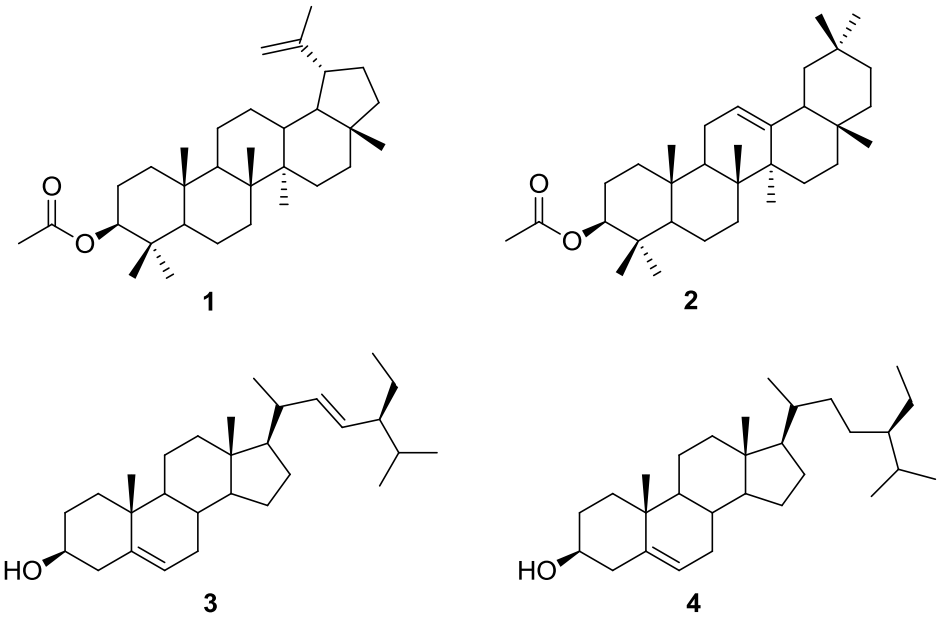

Figure 1: Triterpenoids and steroids from *V. megacarpa*.

MATERIALS AND METHODS

General

NMR spectra were recorded on a Bruker Avance 300 (300.13 MHz) spectrometer using the solvent peak as internal reference ($CDCl_3$: δ H 7.26; δ C: 77.0). Normal-phase column chromatography was performed with silica gel 60 (Merck Art. 1.07734.1000 and 1.09385.1000). Thin-layer chromatography was performed on aluminum plates coated by silica gel 60 HF_{254} (Merck Art. 1.07739.1000). The plates were visualized by fluorescence quenching under UV (254 nm and 356 nm) and by spraying with vanillin-sulfuric acid, followed by warming.

Plant Material

The leaves of *Voacanga megacarpa* were collected from Mt. Buhi, Camarines Sur, Philippines on April, 2013. The plant sample (PNH #37975) was authenticated by Mr. Noe Gapas of the Botanical Division, Philippine National Museum in Intramuros, Manila where a voucher specimen was also deposited.

Extraction and Isolation

The ground, air-dried leaves *V. megacarpa* (4.9 kg) were extracted with DCM-MeOH (1:1, 47.5 L, 3x overnight) and the percolates were concentrated using a rotary evaporator at 40 ^{o}C. The crude extract (208.3 g) obtained after concentration was subjected to acid-base extraction to yield the alkaloid extract (129.3 g). The crude alkaloid extract was subjected to vacuum liquid chromatography using gradient elution (10% increment) of EtOAc-MeOH afforded six fractions. Chromatographic purification (3x) of the fraction eluted with 10% MeOH in EtOAc (fraction 1) using 1% MeOH in hexane yielded the compound mixtures **2** & **3** (1:1, 67.6 mg) and **4** & **5** (1:1, 61.1 mg).

Lupeol acetate (**1**) [14]: ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 4.68 (H-29β), 4.56 (H-29α), 4.48 (H-3), 2.37(H-19), 2.03($COCH_3$), 1.69(H-30), 1.03(H-26), 0.94(H-27), 0.85(H-25), 0.84(H-24), 0.83(H-23), 0.78 (H-28). **β-amyrin acetate** (**2**) [15]: 5.13 (H-12), 4.54 (H-3α), 2.04 ($COCH_3$), 1.27 (H-27), 1.03 (H-23), 0.99 (H-24), 0.89 (H-25), 0.86 (H-28). 0.82 (H-29), 0.80 (H-30), 0.78 (H-26). White powder (67.6 mg).

Stigmasterol (**3**) and β-**Sitosterol** (**4**) [16]: ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.20 (H-3), 5.26 (H-6), 5.19(H-23), 4.68 (H-22), 3.63 (H-3), 2.38 (H-20), 1.8-2.0, 1.35-1.62, 0.91-1.05, 0.69-0.89. White amorphous powder (61.1 mg).

Antituberculosis Activity

Microplate Alamar Blue Assay (MABA). The method given in reference [17] was used for testing *Mycobacterium tuberculosis* H_{37}Rv susceptibility. The standard TB drug rifampin (RMP) was used as positive drug control. MIC_{50} is defined as the minimum concentration of test sample that exhibited greater or equal to 50% inhibition against the test organism.

Table 1: % Inhibition and minimum inhibitory concentration of the extracts, fractions and compounds from *V. megacarpa*.

Test Sample	% inhibition at 64 μg/mL	Minimum Inhibitory Concentration (MIC_{50})
Crude Extract	55	64
Alkaloid Extract	51	64
Organic Extract	7	> 64
Fraction 1	53	64
Fraction 2	65	64
Fraction 3	49	> 64
Fraction 4	17	> 64
Fraction 5	0	> 64
Fraction 6	15	> 64
Rifampin	99%	0.098

RESULTS AND DISCUSSION

The crude DCM-methanolic and alkaloid extracts of *V. megacarpa* exhibited moderate inhibitory activity against *M. tuberculosis* H_{37}Rv. Vacuum liquid chromatography yielded two fractions (1 and 2) with MIC_{50} of 64 μg/mL. Chromatographic purification of fraction one afforded a mixture of the triterpenoids, lupeol acetate (**1**) and β-amyrin acetate (**2**), and a mixture of the sterols, stigmasterol (**3**) and β-sitosterol (**4**). The structure of these compounds were deduced based on their ^{1}H NMR spectral data and confirmed by comparison of their NMR spectroscopic data with reported literature data [14-16].

This is the first report on the identification of these compounds from *V. megacarpa*. Literature survey of other alkaloid-containing Apocynaceae genera viz. *Alstonia* and *Tabernaemontana* [18-19] indicates the characteristic presence of lupane and oleanene triterpenoids such as **1** and **2**, respectively.

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CONCLUSION

This study underscored the isolation and identification of metabolites **1-4** from *V. megacarpa*. Antitubercular screening of extracts and fractions indicated moderate inhibitory activity.

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