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Research Article

Chemical Constituents of *Melanolepis multiglandulosa* (Reinw. Ex Blume)

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ABSTRACT

Chemical investigation of the dichloromethane extracts of *Melanolepis multiglandulosa* (Reinw. ex Blume) yielded taraxeryl fatty acid esters (**1**), squalene (**2**), (*E*)-3-alkenoic acids (**3**), β-carotene (**4**), a mixture of β-sitosterol (**5a**) and stigmasterol (**5b**), long-chain fatty alcohols (**6**), and long-chain hydrocarbons (**7**) from the leaves; and **7**, triacylglycerols (**8**), and long-chain saturated fatty acid esters (**9**) from the twigs. The structures of **1**-**9** were identified by comparison of their NMR data with literature data.

Keywords: *Melanolepis multiglandulosa* (Reinw. ex Blume), Euphorbiaceae, taraxeryl fatty acid esters, squalene, (*E*)-3-alkenoic acids, β-carotene, β-sitosterol, stigmasterol, long-chain fatty alcohols, long-chain hydrocarbons, triacylglycerols, long-chain saturated fatty acid esters

INTRODUCTION

Melanolepis multiglandulosa (Reinw. ex Blume) of the family Euphorbiaceae and locally known in the Philippines as alim, is found in East Asia – southern Japan, Taiwan, Thailand, Malaysia, Indonesia, Philippines to Papua New Guinea, Mariana Islands, and Solomon Islands[1]. The leaves of *M. multiglandulosa* are used in different ways: as a poultice against different kinds of scurf, as an anthelmintic against parasitic worms, as an anti-cough decoction, as a drink to treat constipation and tuberculosis, and, mixed with some bark and flowers, as temporary relief for chest pain and fever[2]. An earlier study reported that the methanolic extracts of the roots of *M. multiglandulosa* contained friedelin, oleanolic acid, olean-12-en-3β,28-diol, β-amyrin acetate, 6β- hydroxystigmast-4-en-3-one, stigmast-4-en-3-one, stigmast-4,22-dien-3-one, 5α-stigmast-3,6-dione, phytosterols [campesterol (11.44%); stigmasterol (52.00); β-sitosterol (36.56%)], phytosterol glycosides [campesterol-3-*O*-β-D-glucoside (14.91%); stigmasterol-3-*O*-β-D-glucoside (47.54%); β-sitosterol-3-*O*-β-D-glucoside (37.55%), and D-sucrose][3].

We report herein the isolation of taraxeryl fatty acid ester (**1**), squalene (**2**), (*E*)-3-alkenoic acid (**3**), β-carotene (**4**), a mixture of β-sitosterol (**5a**) and stigmasterol (**5b**), long-chain fatty alcohols (**6**), and long-chain hydrocarbons (**7**) from the leaves; and **7**, triacylglycerols (**8**), and long-chain saturated fatty acid esters (**9**) from the twigs of *M. multiglandulosa*. The structures of **1**–**5b** and **8** are presented in Fig. 1. To the best of our knowledge, this is the first report on the isolation of **1**–**4** and **6**–**9** from *M. multiglandulosa* and the first reported study on the chemical constituents of the leaves and twigs of *M. multiglandulosa*.

MATERIALS AND METHODS

General Experimental Procedure

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Ascend 400 in $CDCl_3$ at 400 MHz. Column chromatography was performed with silica gel 60 (70-230 mesh, Merck). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} (Merck) and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming. All solvents used are analytical grade.

Sample Collection

Samples of the leaves and twigs of *Melanolepis multiglandulosa* (Reinw. ex Blume) were collected from the reforested areas of the De La Salle University – Science and Technology Complex (DLSU-STC), Leandro V. Locsin Campus, Biñan City, Laguna, Philippines in

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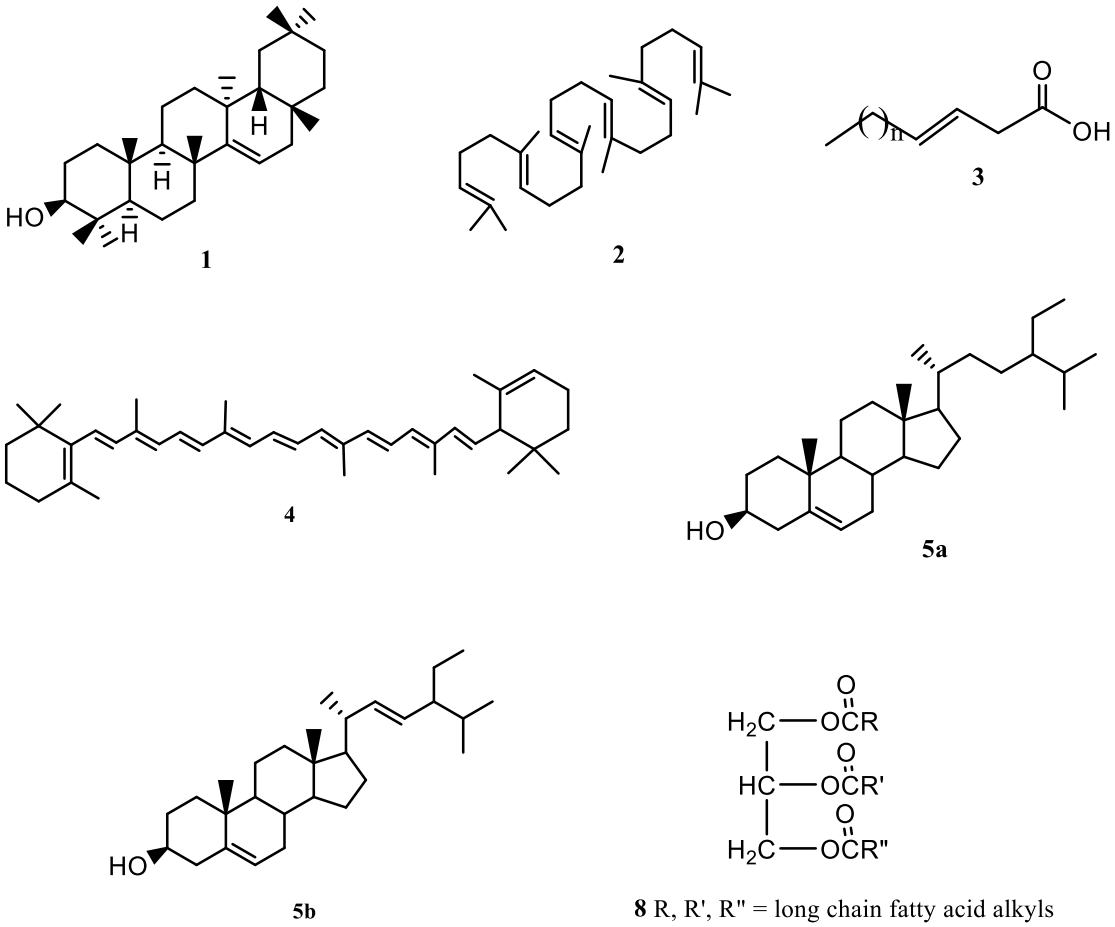

Figure 1: Chemical structures of taraxeryl fatty acid ester (**1**), squalene (**2**), (*E*)-3-alkenoic acid (**3**), β-carotene (**4**), β-sitosterol (**5a**), stigmasterol (**5b**), long-chain fatty alcohols (**6**), long-chain hydrocarbons (**7**), triacylglycerols (**8**), and long-chain saturated fatty acid esters (**9**) from *M. multiglandulosa*.

February 2015. The samples were authenticated at the Philippine National Museum.

General Isolation Procedure

A glass column 12 inches in height and 0.5 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 (10% by volume increment) as eluents. Five milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f value 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 the chemical constituents of the leaves of M. multiglandulosa

The air-dried leaves of *M. multiglandulosa* (285.1 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 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The CH_2Cl_2 extract was rechromatographed using petroleum ether to afford **7** (5 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed using petroleum ether, followed by 1% EtOAc in petroleum ether. The fractions eluted with petroleum ether were combined and rechromatographed using petroleum ether to yield **3** (1 mg) and **4** (2 mg). The fractions eluted with 1% EtOAc in petroleum ether were combined and rechromatographed using 1% EtOAc in petroleum ether to afford **2** (3 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to yield **1** (4 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fractions was rechromatographed using 10% EtOAc in petroleum ether, followed by 15% EtOAc in petroleum ether. The fractions eluted with 10% EtOAc in petroleum ether were rechromatographed using 10% EtOAc in petroleum ether to yield a mixture of **5a** and **5b** (4 mg) after washing with petroleum ether. The fractions eluted with 15% EtOAc in petroleum ether were rechromatographed using 10% EtOAc in petroleum ether to yield **6** (3 mg).

Isolation of the chemical constituents of the twigs of M. multiglandulosa

The air-dried twigs of *M. multiglandulosa* (87.3 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 (0.6 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The 10% acetone in CH_2Cl_2 fraction was rechromatographed using petroleum ether to yield **7** (3 mg) and **9** (4 mg). The 20% acetone in CH_2Cl_2 fraction

was rechromatographed using 1% EtOAc in petroleum ether, followed by 2.5% EtOAc in petroleum ether. The fractions eluted with 2.5% EtOAc in petroleum ether were combined and rechromatographed using 2.5% EtOAc in petroleum ether to yield **8** (5 mg).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the different parts of *M. multiglandulosa* yielded **1**–**5**. The NMR spectra of **1** are in accordance with data reported in the literature for taraxeryl fatty acid esters[4]; **2** for squalene[5], **3** for (*E*)-3-alkenoic acid[6]; **4** for β-carotene[7], **5a** for β-sitosterol[5], **5b** for stigmasterol[5]; **6** for long-chain fatty alcohols[8]; **7** for long-chain hydrocarbons[9]; **8** for triacylglycerols[10]; and **9** for long-chain saturated fatty acid esters[11].

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