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Chemical Constituents of *Exoecaria agallocha* Linn.

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Chemical investigation of the dichloromethane extracts of the mangrove, *Exoecaria agallocha* Linn. has led to the isolation of alkyl ferulates (**1**), lupeol (**2**), a mixture of 3-epi-α-amyrin (**3**) and 3-epi-β-amyrin (**4**) in about 2:3 ratio, and agallochaol K (**5**) from the bark; taraxerol (**6**), epi-taraxerol (**7**) and chlorophyll a from the leaves; and a mixture of compounds **3** and **4** in about 1:2 ratio, squalene, olean-12-en-3-one (**8**) and fully epoxidized linoleic acid from the twigs. The structures of compounds **5** and **7** were elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of their NMR data with those reported in the literature. The structures of the other compounds were identified by comparison of their NMR data with literature data.

Keywords: *Exoecaria agallocha* Linn., Euphorbiaceae, Alkyl ferulates, lupeol, Amyrin, Agallochaol K, Taraxerol, epi-Taraxerol.

INTRODUCTION

Exoecaria agallocha Linn. of the family Euphorbiaceae, also known as milky mangrove and locally known as buta-buta, grows along the seashore throughout the Philippines [1]. A number of studies were conducted on the chemical constituents of *E. agallocha* which afforded triterpenes [2-5] and diterpenes [6-11] as major constituents. Previous studies reported the isolation of the following triterpenes and sterols: 4-taraxeren-3-one, dibutyl phthalate, β-amyrin, 18-oleanen-3-ol, 18-oleanen-3-one, phaeophytin A, betulin, β-rosasterol, β-sitosterol, betulinic acid, oleanolic acid and ursolic acid [2]; stigmast-5-en-3β-yl(6′-*O*-heptadecanoyl-β-D-glucopyranoside), taraxer-14-ene-3-one and taraxer-14-ene-3α-ol, taraxer-14-ene-3β-ol [3]; 3β-[(2*E*,4*E*)-6-oxo-decadienoyloxy]-olean-12-ene, β-amyrin acetate, taraxerone, 3-epitaraxerol, 3-epilupeol and taraxerol [4] and taraxerone, β-amyrin acetate, 3β-[(2*E*,4*E*)-6-oxo-decadienoyloxy]olean-12-ene, taraxerol, acetylaleuritolic acid, cycloart-22-ene-3β,25-diol, β-sitostenone, (24*R*)-24-ethylcholesta-4,22-dien-3-one and β-sitosterol [5] from *E. agallocha*. Furthermore, the stems and twigs of *E. agallocha* afforded agallochaols K-Q [14] which is of relevance to our present report.

This study is part of our research on the chemical constituents of Philippine mangroves. We report herein the isolation of alkyl ferulates (**1**), lupeol (**2**), 3-epi-α-amyrin (**3**), 3-epi-β-amyrin (**4**) and agallochaol K (**5**) from the bark; taraxerol (**6**), epi-taraxerol (**7**) and chlorophyll a from leaves; and a mixture of compounds **3** and **4**, squalene, olean-12-en-3-one (**8**) and fully epoxidized linoleic acid from the twigs of *E. agallocha*. The chemical structures of compounds **1-8** are presented in Fig. 1. To the best of our knowledge, this is the first report on the isolation of compounds **1**, **3**, **4**, **8**, squalene and epoxidized linoleic acid from *E. agallocha*.

EXPERIMENTAL

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

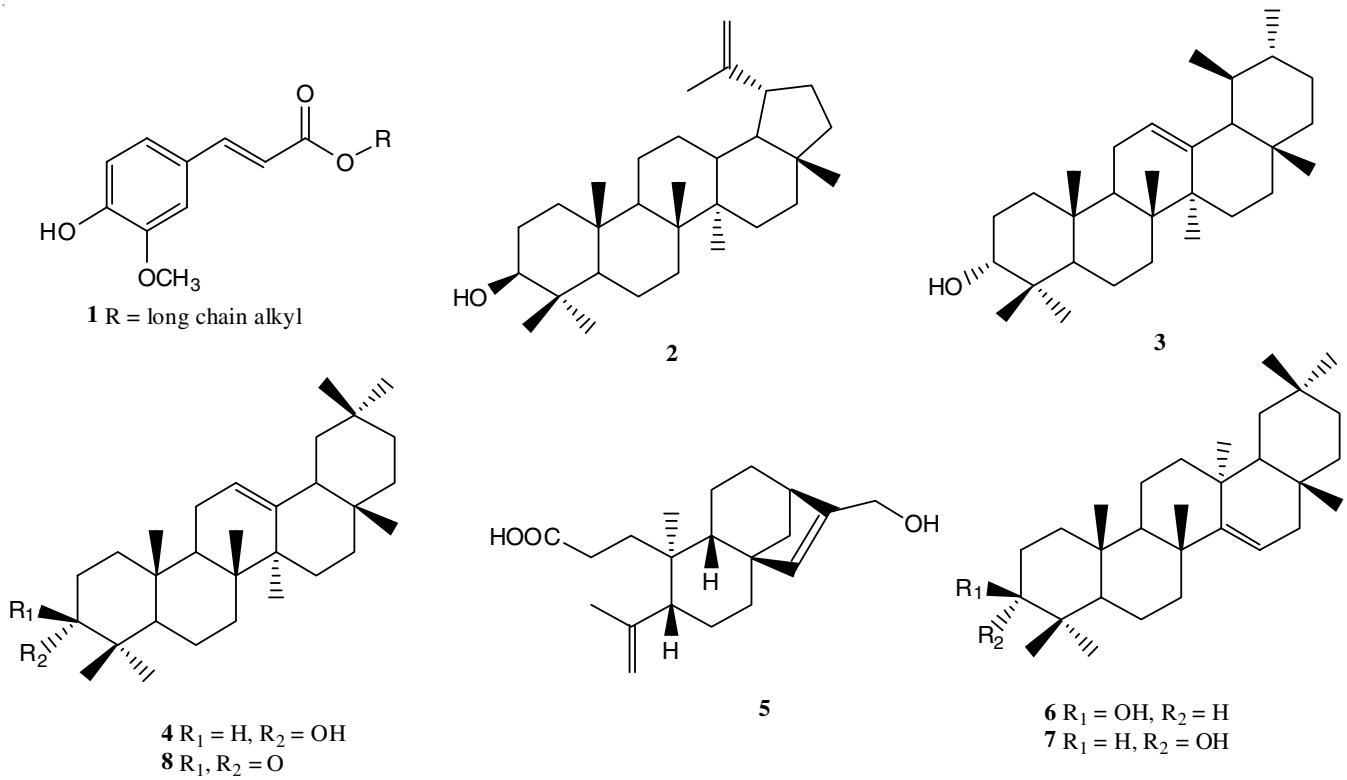

Fig. 1. Chemical structures of alkyl ferulates (**1**), lupeol (**2**), 3-epi-α-amyrin (**3**), 3-epi-β-amyrin (**4**), agallochaol K (**5**), taraxerol (**6**), epi-taraxerol (**7**), and olean-12-en-3-one (**8**) from *E. agallocha*

F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

Sample collection: Samples of the bark, leaves and twigs of *Exoecaria agallocha* Linn. were collected from the mangrove forest of Caramoan, Camarines Sur Philippines in September 2016. The samples were authenticated at the Botany Division, Philippine National Museum.

General isolation procedure: A glass column 12 inches in height with 0.5 inch internal diameter was used for the fractionation of the crude extract. Fractions of 10 mL volumes were collected and monitored by thin layer chromatography. Fractions containing spots with similar R_f values were combined and rechromatographed using the appropriate solvent. Final purification was carried out using Pasteur pipette as the column, collecting 1 mL fractions. TLC-pure isolates were combined and after evaporation of the solvent were subjected to NMR analysis.

Air-dried bark (175 g), leaves (103.5 g) and twigs (192.3 g) were ground in a blender and then soaked in CH_2Cl_2 for 3 days and filtered. The filtrates were concentrated *in vacuo* to afford crude 4.84 g of extract from the bark, 1.23 g from the leaves, and 1.75 g from the twigs which were chromatographed by gradient elution using increasing proportions of acetone in CH_2Cl_2 at 10 % increment by volume.

Isolation of chemical constituents of *E. agallocha* bark: The 20 % acetone in CH_2Cl_2 fraction from the chromatography of the crude bark extract was rechromatographed using 7.5 % ethyl acetate in petroleum ether to afford compound **1** (1.2 mg). The fraction eluted with 50 % acetone in CH_2Cl_2 was rechromatographed (2×) using 7.5 % ethyl acetate in petroleum ether to yield a mixture of compounds **3** and **4** (3.1 mg) after washing with petroleum ether. The fraction eluted with 70 % acetone in CH_2Cl_2 was rechromatographed using 15 % ethyl acetate in petroleum ether to provide compound **2** (1.5 mg) after washing with petroleum ether. The fraction eluted with acetone was rechromatographed (3×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to obtain compound **5** (3 mg) after washing with petroleum ether.

Isolation of chemical constituents of the leaves of *E. agallocha*: The 10 % acetone in CH_2Cl_2 fraction from the chromatography of the crude leaf extract was rechromatographed using 5 % ethyl acetate in petroleum ether to afford compound **7** (2.5 mg) after washing with petroleum ether. The fractions eluted with 20 and 30 % acetone in CH_2Cl_2 were combined and rechromatographed using 10 % ethyl acetate in petroleum ether to yield compound **6** (6 mg) after washing with petroleum ether. The fraction eluted with 70 % acetone in CH_2Cl_2 was rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5, v/v) to provide chlorophyll a (5.4 mg) after washing with petroleum ether, followed by diethyl ether.

Isolation of chemical constituents of twigs of *E. agallocha*: The CH_2Cl_2 fraction from the chromatography of crude twigs extract was rechromatographed using petroleum ether to afford squalene (1 mg). The fraction eluted with 10 % acetone in CH_2Cl_2 was rechromatographed using 2.5 % ethyl acetate in petroleum ether to yield compound **8** (25 mg) after washing with petroleum ether. The fraction eluted with 20 and 30 % acetone in CH_2Cl_2 were combined and rechromatographed using 5 % ethyl acetate in petroleum ether to provide a mixture of compounds **3** and **4** (5 mg) after washing with petroleum ether. The fraction eluted with 50 % acetone in CH_2Cl_2 was rechromatographed (2×) using 20 % ethyl acetate in petroleum ether to yield fully epoxidized linoleic acid (35 mg).

RESULTS AND DISCUSSION

Silica gel chromatography of CH_2Cl_2 extracts of *E. agallocha* yielded alkyl ferulates (**1**), lupeol (**2**), a mixture of 3-epi-α-amyrin (**3**) and 3-epi-β-amyrin (**4**) in about 2:3 ratio, and agallochaol K (**5**) from the bark; taraxerol (**6**), epi-taraxerol (**7**), and chlorophyll a from the leaves; and a mixture of compounds **3** and **4** in about 1:2 ratio, olean-12-en-3-one (**8**), squalene, and fully epoxidized linoleic acid from the twigs.

The ratios of compounds **3** and **4** in the bark and twigs of *E. agallocha* were deduced from the relative intensities and integrations of ^{1}H NMR resonances of the olefinic protons at δ 5.11 (t, J = 3.6 Hz) [15] for compound **3** and δ 5.16 (t, J = 3.6 Hz) [15] for compound **4**.

The NMR data of compound **1** are in accordance with the data reported in the literature for alkyl ferulates [16]; compound **2** for lupeol [17]; compound **3** for 3-epi-α-amyrin [15]; compound **4** for 3-epi-β-amyrin [15]; compound **5** for agallochaol K [14]; compound **6** for taraxerol [18]; compound **7** for epi-taraxerol [19]; compound **8** for olean-12-en-3-one [20]; chlorophyll a [21]; squalene [22]; and fully epoxidized linoleic acid [23].

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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