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A Flavone from *Eucalyptus deglupta.*

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ABSTRACT

Chemical investigations of the dichloromethane extract of the twigs of *Eucalyptus deglupta* (Blume) led to the isolation of 5-hydroxy-7,4'-dimethoxy-6,8-dimethylflavone (**1**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy.

Keywords: *Eucalyptus deglupta*, Myrtaceae, 5-hydroxy-7,4'-dimethoxy-6,8-dimethylflavone

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INTRODUCTION

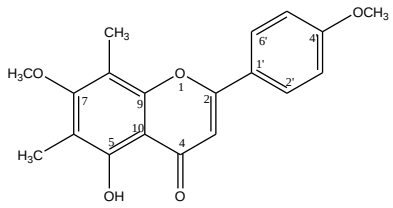
Eucalyptus deglupta (Blume) of the family Myrtaceae, commonly known as Mindanao gum or rainbow eucalyptus, is native to the Philippines and other western Pacific islands, adapting well in lowland humid tropical conditions and reaching up to 100 ft. in height [1]. It is cultivated throughout the world as an ornamental tree, noted for its spectrum of colors developed by the peeling of its smooth bark in a fatigue-inspired pattern, revealing multi-colored streaks of yellow, red, blue, orange, green, purple, brown, and gray shades [1, 2]. The genus *Eucalyptus* has been a traditional source of timber, cellulose-related products and essential oils from the leaves [3, 4].

An antihypertensive food and food additive containing an extract prepared from eucalyptus plants was reported [5]. Essential oils from the leaves of *E. deglupta* and other related aromatic plants were reported to inhibit the growth of *Pseudomonas aeruginosa* [6]. *E. deglupta* was reported to exhibit a high percentage of xanthine oxidase inhibition (IC_{50} of 44.5 µg/mL) and also prevented cataract development in streptozotocin diabetic rats [7]. A review on the chemical constituents and biological activities of the genus *Eucalyptus* has been provided [5]. More than two hundred nonvolatile compounds from this genus have been characterized for their chemical structures and biological activities [5]. Phloroglucinol derivatives from *Eucalyptus* have growth regulatory, antigranulation, antiinflammatory and antimalarial activities, while rutin and other flavonoids, terpenoids and tannins from *Eucalyptus* are of great pharmaceutical importance [5].

The essential oils from the dried leaves of five species of the genus *Eucalyptus* (*E. camaldulensis*, *E. deglupta*, *E. grandis*, *E. torelliana*, *E. urophylla*) were found to contain as major components the following terpenes: α-pinene, β-pinene, α-phellandrene, limonene, γ-terpinene, p-cymene and β-caryophyllene [9]. Another study reported that the steam-volatile portion of *E. deglupta* yielded 40% terpene fraction which contained (±)-α-pinene, (-)-α-phellandrene, p-cymene, ocimene, isovaleraldehyde, (-)-carvotanacetone, and (+)-nerolidol [10]. Moreover, the leaf essential oil of *E. deglupta* is mainly composed of sesquiterpenoids (48%), of which E-nerolidol was the major component (34.8%) [11].

Another study reported the isolation of tritriacontane-16,18-dione, 8-methoxyellagic acid-2-rhamnoside, ellagic acid, and ellagitannins from the extracts of the wood and bark of *E. deglupta*. The extracts also afforded a low molecular weight Si containing compound which is effective in inhibiting HIV-1 virus [12]. The Si containing substance is manufactured by hot water extraction of *E. deglupta* followed by gel filtration [13]. The isolation of ursolic acid, oleanolic acid, and squalene from the leaves and twigs and nerolidol from the leaves of *E. deglupta* have been reported [14].

We report herein the isolation and structure elucidation of 5-hydroxy-7,4'-dimethoxy-6,8-dimethylflavone (**1**) from the twigs of *E. deglupta*. To the best of our knowledge this is the first report on the isolation of **1** from *E. deglupta.*



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MATERIALS AND METHODS

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.

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Sample Collection

Samples of leaves and twigs of *Eucalyptus deglupta* (Blume) were a generous gift collected from the Center for Ecozoic Living and Learning (CELL), Silang, Cavite in May 2014. The samples were authenticated at the Botany Division of the National Museum, Manila, Philippines and deposited with voucher # 268-2014.

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 R_f 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.

Isolation

The air-dried twigs of *E. deglupta* (173 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 (18 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) in 20% EtOAc in petroleum ether to afford **1** (7 mg) after washing with petroleum ether, followed by Et_2O.

5-Hydroxy-7,4'-dimethoxy-6,8-dimethylflavone (**1**): Yellowish solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.61 (1H, s, H-3), 12.89 (1H, 5-OH), 2.20 (3H, s, Me-6), 3.78 (3H, s, 7-OMe), 2.38 (3H, s, Me-8), 7.86 (2H, d, *J* = 9 Hz, H-2', H-6'), 7.02 (2H, d, *J* = 9 Hz, H-3', H-5'), 3.88 (3H, s, 4'-OMe); ^{13}C NMR (150 MHz, $CDCl_3$): δ 163.88 (C-2), 104.09 (C-3), 183.27 (C-4), 157.29 (C-5), 114.10 (C-6), 162.58 (C-7), 108.78 (C-8), 153.00 (C-9), 107.40 (C-10), 123.90 (C-1'), 128.00 (C-2', C-6'), 114.56 (C-3', C-5'), 162.58 (C-4'), 8.27 (Me-6), 60.51 (7-OMe), 8.57 (Me-8), 55.53 (4'-OMe).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the twigs of *Eucalyptus deglupta* (Blume) led to the isolation of 5-hydroxy-7,4'-dimethoxy-6,8-dimethylflavone (**1**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy as follows.

The ^{1}H NMR spectrum of **1** indicated resonances for two aromatic methyl singlets at δ 2.20 and 2.38; methoxy groups at δ 3.78 and 3.88; a deshielded olefinic proton singlet at δ 6.61; aromatic proton doublets at δ 7.02 (2H) and 7.86 (2H) othro coupled to each other by 9 Hz which was confirmed by COSY; and a chelated OH at δ 12.89.

The ^{13}C NMR spectrum of **1** gave resonances for a conjugated carbonyl carbon at δ 183.27; oxygenated aromatic carbons at δ 163.88, 162.58 (2C), 157.29 and 153.00; protonated aromatic carbons at δ 128.00 (2C), 114.56 (2C) and 104.09; four non-protonated aromatic carbons at δ 123,9, 114.10, 108.78 and 107.40; methoxy carbons at δ 60.51 and 55.53; and methyl carbons at δ 8.57 and 8.27. These resonances indicated a flavone with a hydroxyl, two methoxy and two methyl subtituents.

The protons attached to carbon atoms were assigned from HSQC 2D NMR data (see experimental), and the structure of **1** was elucidated by analysis of the HMBC 2D NMR data. Key HMBC correlations are shown in Fig. 1. The ortho coupled protons could only be in ring C of the flavone skeleton at C-2', C-3', C-5' and C-6', while the chelated hydroxyl could only be attached to C-5. One of the methoxy groups was attached to C-4' on the basis of long-range correlations between this methoxy, the ortho aromatic protons and C-4'. The position of the hydroxyl proton was confirmed by correlations with C-5, C-6 and C-10. One of the methyl groups was attached to C-6 due to long-range correlations between this methyl and C-6, C-5 and C-7. The second methoxy group was attached to C-7 based on long-range correlations between this methoxy and C-7. The second methyl group was attached to C-8 since long-range correlations were observed between this

methyl and C-8, C-7 and C-9. The carbonyl was assigned to C-4 on the basis of long-range correlations between the H-3 olefinic proton singlet and this carbon. All long-range correlations observed were consistent with the structure of **1**. The structure of **1** was confirmed by positive ESI-MS which gave an $[M+H]^+$ = 327.18 corresponding to the molecular formula of $C_{19}H_{19}O_5$.

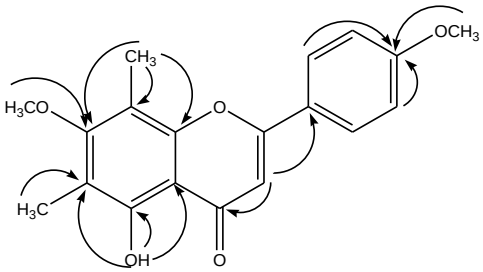

Figure 1: HMBC correlations of 1

The positions of the methyl and methoxy groups were confirmed by NOESY spectrum (Fig. 2). One of the methoxy groups was close in space to two methyl groups in ring A. The olefinic proton singlet in ring C was close to the more deshielded aromatic proton doublet in ring B, which was in turn close to the relatively more shielded aromatic proton doublet, which was finally close to the second methoxy group. These confirm the positions of the methyl and methoxy groups in **1**.

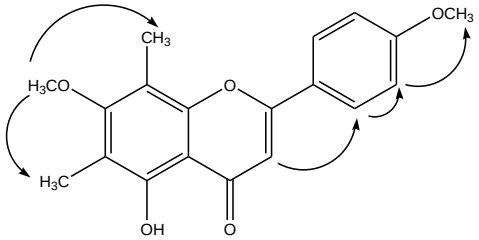

Figure 2: NOESY correlations of 1.

Literature search revealed that 5-hydroxy-7,4'-dimethoxy-6,8-dimethylflavone (**1**) has been previously isolated from the leaves of *Callistemon lanceolatus* [15]. The structure of this compound was determined by MS and ^{13}C NMR spectroscopy. However, the ^{13}C NMR resonances in the literature do not match with our ^{13}C NMR data and assignments which were based on extensive 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC, HMBC and NOESY) NMR spectroscopy.

ACKNOWLEDGEMENT

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