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Terpenoids from *Eucalyptus deglupta*

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ABSTRACT

*Chemical investigations of the dichloromethane extracts of the leaves of Eucalyptus deglupta (Blume) led to the isolation of nerolidol (**1**), ursolic acid (**2**), oleanolic acid (**3**), and squalene (**4**), while the twigs yielded **2**-**4**. The structures of **1**-**4** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature.*

Keywords: *Eucalyptus deglupta*, Myrtaceae, nerolidol, ursolic acid, oleanolic acid, squalene

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INTRODUCTION

Eucalyptus deglupta (Blume) of the family Myrtaceae, commonly known as Mindanao gum or rainbow eucalyptus and locally known as “bagras”, is native to the Philippines and other western Pacific islands, but has already been introduced in other countries, 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].

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]. An antihypertensive food and food additive containing an extract prepared from eucalyptus plants (e.g. dried leaves of *E. deglupta* and *E. citriodora*) was reported [6]. Essential oils from the leaves of *E. deglupta* and other related aromatic plants were reported to inhibit the growth of *Pseudomonas aeruginosa*, resulting to a 10-18 mm diameter of zone inhibition using the diffusion method for antibacterial testing [7]. In a previous study on gout and cataractogenesis prevention in streptozotocin diabetic rats

using nineteen Myrtaceae plant extracts, *E. deglupta* was one of the two plants which gave a high percentage of xanthine oxidase inhibition (IC_{50} of 44.5 μg/mL) and also prevented cataract development [8].

The essential oils obtained from the dried leaves of five species of the genus *Eucalyptus* (*E. camaldulensis*, *E. deglupta*, *E. grandis*, *E. torelliana*, *E. urophylla*) were analyzed by GC and GC/MS. The major components of all these oils are α-pinene, β-pinene, α-phellandrene, limonene, γ-terpinene, p-cymene and β-caryophyllene [9]. Another study reported that the leaf essential oil of *E. deglupta* is mainly composed of sesquiterpenoids (48%), of which E-nerolidol was the major component (34.8%) [10]. Furthermore, the steam-volatile portion of *E. deglupta* yielded 40% terpene fraction which contained (±)-α-pinene, (-)-α-phellandrene, p-cymene, ocimene, isovaleraldehyde, (-)-carvotanacetone, and (+)-nerolidol [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].

We report herein the isolation and identification of nerolidol (**1**), ursolic acid (**2**), oleanolic acid (**3**), and squalene (**4**) (Fig. 1) from the leaves and **2-4** from the twigs of *E. deglupta*. To the best of our knowledge this is the first report on the isolation of **2-4** from *E. deglupta*.

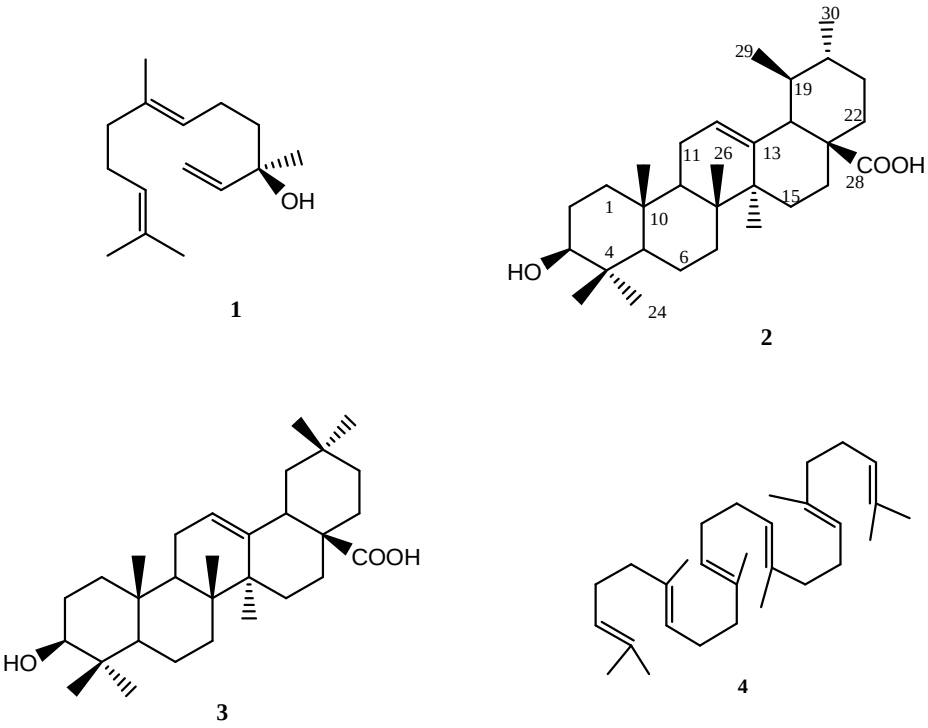

Fig. 1. Chemical constituents from *E. deglupta*: nerolidol (1), ursolic acid (2), oleanolic acid (3), and squalene (4)

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.

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 *Rf* 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 leaves of *E. deglupta* (373 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 (28 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using 1% EtOAc in petroleum ether to afford **4** (5 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to afford **1** (25 mg). The 80% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to afford **3** (7 mg) after trituration with petroleum ether. The 90% acetone in CH_2Cl_2 fraction was rechromatographed (5 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to afford **2** (10 mg) after trituration with petroleum ether.

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 CH_2Cl_2 fraction was rechromatographed (3 ×) using 1% EtOAc in petroleum ether to afford **4** (3 mg). The 70% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to afford a mixture of **2** and **3** (5 mg) after trituration with petroleum ether. The 80% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to afford **2** (4 mg) after trituration with petroleum ether.

Nerolidol (**1**): Colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.90 (1H, dd, *J* = 10.8, 17.4 Hz, H-2), 5.19 (1H, dd, *J* = 1.2, 17.4 Hz, H-1), 5.04 (1H, dd, *J* = 1.2, 10.8 Hz, H-1), 5.06, 5.12 (2H, m, H-6, H-10), 2.05-1.94 (6H, m, H-5, H-8, H-9), 1.66 (3H, d, *J* = 1.2 Hz, Me-12), 1.58 (6H, br s; Me-13, Me-14), 1.579-1.53 (2H, m, H-4), 1.26 (3H, s, Me-15). ^{13}C NMR (150 MHz, $CDCl_3$): δ 145.05 (C-2), 135.59 (C-7), 131.45 (C-11), 124.22, 124.19 (C-6, C-10), 111.66 (C-1), 73.51 (C-3), 42.03 (C-4), 39.69 (C-8), 27.89 (Me-15), 26.63 (C-9), 25.69 (Me-13), 22.71 (C-5), 17.68 (Me-12), 16.01 (Me-14).

Ursolic Acid (**2**): Colorless solid. ^{13}C NMR (150 MHz, $CDCl_3$): δ 36.98 (C-1), 28.12 (C-2), 79.04 (C-3), 38.59 (C-4), 55.20 (C-5), 18.28 (C-6), 32.95 (C-7), 39.47 (C-8), 47.52 (C-9), 38.74 (C-10), 23.27 (C-11), 125.87 (C-12), 137.92 (C-13), 42.00 (C-14), 27.22 (C-15), 24.17 (C-16), 47.88 (C-17), 52.69 (C-18), 39.05 (C-19), 38.82 (C-20), 30.60 (C-21), 36.68 (C-22), 28.00 (C-23), 15.46 (C-24), 15.59 (C-25), 17.08 (C-26), 23.55 (C-27), 180.86 (C-28), 16.97 (C-29), 21.16 (C-30).

Oleanolic acid (**3**): Colorless solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.20 (dd, *J* = 4.2, 11.4 Hz, H-3α), 5.27 (t, *J* = 3.6 Hz, H-12), 2.80 (dd, *J* = 4.2, 13.2 Hz, H-18), 0.97 (s, Me-23), 0.74 (s, Me-24), 0.89 (s, Me-25), 0.76 (s, Me-26), 1.11 (s, Me-27), 0.91 (s, Me-29), 0.88 (s, Me-30).

Squalene (**4**): Colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.07-5.13 (6H, =CH), 1.58 (18H, allylic Me, *cis*), 1.66 (6H, allylic Me, *trans*), 1.94-2.06 (20H, allylic CH_2).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the leaves of *Eucalyptus deglupta* (Blume) led to the isolation of nerolidol (**1**) [14], ursolic acid (**2**) [15], oleanolic acid (**3**) [16], and squalene (**4**) [16], while the twigs

yielded **2**-**4**. Triterpene **3** from the twigs of *E. deglupta* was obtained as a mixture with **2** in a 1:2 ratio as deduced from the integrations of the ^{1}H NMR resonances for the olefinic protons of **2** at δ 5.24 (t, *J* = 3.6 Hz) and **3** at δ 5.28 (t, *J* = 3.6 Hz). The structures of **1-4** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature [14-16].

Although no biological activity tests were conducted on the isolated compounds (**1**-**4**), literature search revealed that these compounds have diverse biological activities as follows.

A study reported that nerolidol (**1**) exhibited a pronounced ovicidal activity at 1 % concentration with the failure of 50 % of the *Pediculus capitis* (head lice) eggs to hatch after 4 days [17]. Furthermore, a 1:2 ratio of tea tree oil (0.5 %) and **1** (1 %) produced both the death of all head lice at 30 min and after 5 days the abortive effect of louse eggs [17]. Another study reported that the (*E*)-nerolidol rich essential oil from the leaves of *Piper claussenianum* showed efficient growth inhibition of promastigote forms of *Leishmania amazonensis* and its arginase activity [18]. Furthermore, the essential oil of *Piper gaudichaudianum* and **1** induced significant cytotoxic effects in *Saccharomyces cerevisiae* that are related to the generation of reactive oxygen species and the formation of single-strand breaks [19]. Natural and synthetic **1** at < 5 μM exhibited strong antitumor effects, while synthetic **1** reduced the number of HeLa and Jurkat cells to 50% (CC_{50}). Inhibition of viral activity was also exhibited by synthetic **1** at CC_{50} 3.2±1.5 μM and natural **1** at CC_{50} 1.2±0.4 μM [20]. Nerolidol was also reported to inhibit the growth of *Streptococcus mutans* [21]. Moreover, the hexane extract of *Myroxylon balsamum* afforded **1** which exhibited larvicidal activity on third instar *Aedes aegypti* larvae [22].

Ursolic acid (**2**) was found to induce apoptosis in tumor cells by activation of caspases and modulation of other pathways involved in cell proliferation and migration. It decreased proliferation of cells and induced apoptosis, thereby inhibiting growth of tumor cells both *in vitro* and *in vivo* [23]. An earlier study reported that it exhibited anti-tumor activity against human colon carcinoma cell line HCT15 [24]. Moreover, it inhibited the growth of colon cancer-initiating cells by targeting STAT3 [25]. Furthermore, it has potential therapeutic use in prostate cancer through its antiproliferative and apoptotic effects [26]. A recent study reported that it inhibited cell growth and proliferation of Jurkat leukemic T-cells, as well as suppressed PMA/PHA induced IL-2 and TNF-α production in a concentration and time-dependent manner [27]. Another study reported that ursolic acid-activated autophagy induced cytotoxicity and reduced tumor growth of cervical cancer cells TC-1 in a concentration-dependent manner [28].

Oleanolic acid (**3**) exhibited anti-inflammatory effects by inhibiting hyperpermeability, the expression of CAMs, and the adhesion and migration of leukocytes [29]. It showed anti-inflammatory activities through the inhibition of the HMGB1 signaling pathway [30]. It exhibited anti-inflammatory, hepatoprotective, gastroprotective, immunoregulatory and anti-ulcer activities [31], and gastroprotective effect on experimentally induced gastric lesions in rats and mice [32]. It was also reported to inhibit mouse skin tumor [33], protect against hepatotoxicants and treat hepatitis [34], and showed significant antitumor activity on human colon carcinoma cell line HCT 15 [35].

Squalene (**4**) was reported to significantly suppress colonic ACF formation and crypt multiplicity which strengthened the hypothesis that it possesses chemopreventive activity against colon carcinogenesis [36]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [37]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells [38]. The preventive and therapeutic potential of squalene containing compounds on tumor promotion and regression have been reported [39]. A recent review on the bioactivities of squalene has been provided [40].

CONCLUSION

The dichloromethane extracts of the leaves of *E. deglupta* yielded a sesquiterpene, nerolidol which is mainly responsible for the woody aroma of the leaves. The leaves and twigs of *E. deglupta* also afforded the triterpenes, ursolic acid, oleanolic acid and squalene which were reported to exhibit anticancer, antitumor and cytotoxic properties.

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