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Chemical constituents of *Terminalia microcarpa* Decne

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ABSTRACT

*Chemical investigation of the dichloromethane extract of the air-dried leaves of Terminalia microcarpa led to the isolation of squalene (**1**), lutein (**2**) and fatty alcohols. The structures of these compounds were identified by comparison of their ^{1}H NMR data with those reported in the literature.*

Keywords: *Terminalia microcarpa*, Combretaceae, squalene, lutein, fatty alcohols

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INTRODUCTION

Terminalia microcarpa Decne. of the family Combretaceae and locally known as kalumpit is a Malesian widespread tall tree occurring in both evergreen and seasonal deciduous forests up to 800 m (1, 2). This tree is a popular rainforestation species in the Philippines exhibiting good growth performance and producing plum-like sweet-sour fruits relished by wild animals. The fruits are usually sold in local markets and either eaten raw or made into preserves. The fruits may also be used in lotions for the eye and skin [1], or in eyewashes in the same manner as the fruits of aroma (*Acacia farnesiana*), while the bark contains up to 42% tannin [3].

A recent study reported the α-glucosidase inhibitor activity of *Terminalia* species. *T. kaerbachii* exhibited the highest α-glucosidase inhibitor activity with an IC_{50} value of 0.27±0.17 μg mL^{-1}; *T. arjuna*, *T. ballerica*, *T. chebula* and *T. catappa* gave IC_{50} values of approximately 5 μg mL^{-1}; and *T. microcarpa* gave an IC_{50} value of 25.15±0.04 μg mL^{-1} [4]. There is no reported study on the chemical constituents of *Terminalia microcarpa.*

We report herein the isolation of squalene (**1**), lutein (**2**) (Fig. 1) and fatty alcohols from the leaves of *T. microcarpa.* To the best of our knowledge this is the first report on the isolation of these compounds from *T. microcarpa.*

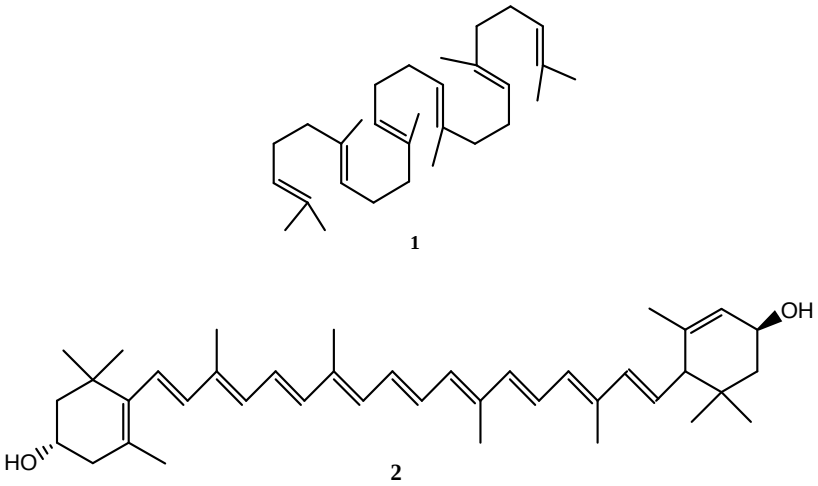

Fig. 1. Chemical constituents of *Terminalia microcarpa:* squalene (1) and lutein (2)

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

Fresh leaves were collected from three-year old saplings from a private farm in Socorro, Oriental Mindoro in May 2013. The plant's taxonomic identity was verified by one of the authors (EHM) and voucher specimen thereof is deposited at De La Salle University-Manila with voucher # 906.

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 leaves of *T. microcarpa* were air-dried for about one week. The air-dried leaves (125 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (7 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (3 ×) in 1% EtOAc in petroleum ether to afford **1** (9 mg). The 20% and 30% acetone in CH_2Cl_2 fractions were combined and rechromatographed (3 ×) in 10% EtOAc in petroleum ether to afford fatty alcohols (6 mg) after washing with petroleum ether. The 40% and 50% acetone in CH_2Cl_2 fractions were combined and rechromatographed (4 ×) using 15% EtOAc in petroleum ether to afford **2** (12 mg) after washing with petroleum ether.

Squalene (**1**): colorless oil. ^1H NMR (600 MHz, $CDCl_3$): δ 5.08-5.13 (6H, =CH), 1.58 (18H, allylic CH_3, *cis*), 1.66 (6H, allylic CH_3, *trans*), 1.94-2.07 (20H, allylic CH_2).

Lutein (**2**): orange crystals. ^1H NMR (600 MHz, $CDCl_3$): δ 1.05 (s, 2 ring A CH_3), 0.83 (s, ring B CH_3), 0.98 (s, ring B CH_3), 1.60 (allylic CH_3), 1.72 (allylic CH_3), 1.89 (allylic CH_3), 1.952 (allylic CH_3), 1.945 (2 allylic CH_3), 1.45,

1.75 (CH_2), 1.35, 1.85 (CH_2), 2.35, 2.00 (allylic CH_2), 2.38 (allylic CH), 4.23 (br s, CHOH), 3.98 (m, CHOH), 5.52 (br s, =CH), 5.41 (dd, *J* = 9.5, 15.5 Hz, =CH).

Fatty Alcohols: colorless solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.62 (t, *J* = 7.2 Hz, terminal CH_2OH), 1.56 (m, α-CH_2), 1.23-1.34 (br s, CH_2), 0.85 (t, *J* = 5.4 terminal CH_3).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the air-dried leaves of *T. microcarpa* afforded squalene (**1**) [5], lutein (**2**) [6] and fatty alcohols [7]. The structures of **1**, **2** and fatty alcohols were identified by comparison of their ^{1}H NMR data with those reported in the literature [5-7].

Although no biological activity tests were conducted on the isolated compounds, literature search revealed that these have diverse biological activities as follows.

Squalene (**1**) was reported to significantly suppress colonic ACF formation and crypt multiplicity which strengthened the hypothesis that it possesses chemopreventive activity against colon carcinogenesis [8]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [9]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells [10]. The preventive and therapeutic potential of squalene containing compounds on tumor promotion and regression have been reported [11]. A recent review on the bioactivities of squalene has been provided [12].

Dietary lutein (**3**), especially at 0.002%, inhibited tumor growth by selectively modulating apoptosis, and by inhibiting angiogenesis [13]. Another study reported that the chemopreventive properties of all-*trans* retinoic acid and lutein may be attributed to their differential effects on apoptosis pathways in normal *versus* transformed mammary cells [14]. Moreover, very low amounts of dietary lutein (0.002%) can efficiently decrease mammary tumor development and growth in mice [15]. Another study reported that lutein and zeaxanthine reduces the risk of age related macular degeneration [16].

A study reported that long chain fatty alcohols from pomace olive oil may have a protective effect on some mediators involved in the inflammatory damage development [17].

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