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Chemical Constituents of *Talinum triangulare*.

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ABSTRACT

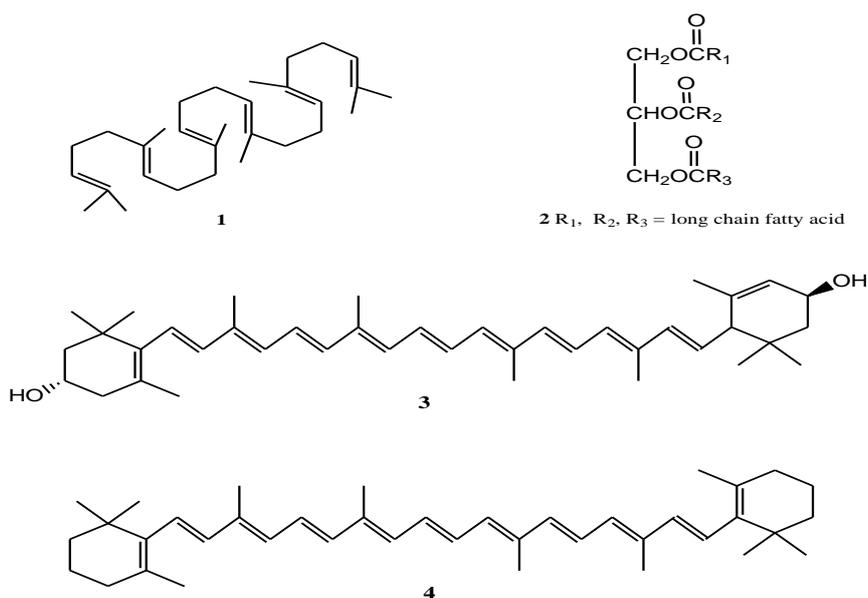
Chemical investigation of the dichloromethane extract of the leaves of *Talinum triangulare* led to the isolation of squalene (**1**), triglyceride (**2**), lutein (**3**) and β -carotene (**4**). The structures of **1-4** were identified by comparison of their ¹³C NMR data with those reported in the literature.

Keywords: *Talinum triangulare*, Portulacaceae, squalene, triglyceride, lutein, β -carotene

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INTRODUCTION

Talinum triangulare (Jacq.) Willd. commonly known as waterleaf and locally known as talinum and Philippine spinach is usually consumed as vegetable and also used as an ornamental plant. The crushed plant is applied as a poultice on contusion, inflammation and tumor. Decoctions are used for painful eyes and to aid recovery from blows and falls [1]. A recent study reported that the hydromethanolic extract of *T. triangulare* exhibited powerful antioxidant activity and inhibited the activity of tyrosinase enzyme [2]. Moreover, *T. triangulare* polysaccharides (TTP) possess significant hypoglycemic effect, but no significant hypolipidemic effect [3]. Another *in vivo* study showed that TTP at 200 mg per kg bw significantly inhibited the growth of tumor by 49.07% in H22-bearing Kunming mice [4]. TTP also exhibited a potent antitumor activity *in vitro* as indicated by their significant inhibition on the proliferation of HepG2 cells and reduction of their survival in a concentration-and time-dependent manner [5]. Pretreatment with TTP in an *in vivo* assay had significantly decreased the levels of AST, ALT and MDA against CCl₄ injures, and restored the activities of defense antioxidant substances SOD and GSH towards normalization and demonstrated a relatively strong antioxidant activity [6]. Another study reported that *T. triangulare* leaf could be used for the treatment of anemia and is used by pregnant women and growing children to boost their blood level [7]. The leaves were also reported to be effective in reducing total plasma cholesterol and plasma LDL-cholesterol, and increasing plasma HDL-cholesterol and blood hematocrit in hypercholesterolemic humans; and for preventing or treating coronary heart disease [8]. Chemical investigation of *T. triangulare* yielded 3-N-(acryloyl, N-pentadecanoyl) propanoic acid, ficuschlorin D, talichorin A, and phaeophytin b peroxy lactone [9]. Furthermore, the methanolic extracts of *T. triangulare* exhibited moderate antimicrobial activity. Purification of the extract afforded β -sitosterol, oleanolic acid, oleanolic acid glycoside, oleanolic acid rhamno glucose and β -sitosterol-3- β -D-glucoside [10]. Two saponins, chikusetsusaponin and β -D-glucopyranosyl methyl spergulagenate 3-O- β -D-glucuronopyranoside were isolated from roots of *T. triangulare* [11].



We report herein the isolation of squalene (1), triglyceride (2), lutein (3) and β -carotene (4) from the leaves of *Talinum triangulare*.

EXPERIMENTAL

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in CDCl₃ at 600 MHz for ¹H NMR and 150 MHz for ¹³C 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 F₂₅₄ and the plates were visualized by spraying with vanillin/H₂SO₄ solution followed by warming.

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.

Sample Collection

Samples of the leaves (1.05 kg) of *Talinum triangulare* (Jacq.) Willd. were collected from the Department of Science and Technology Compound, Bicutan, Taguig City, Philippines in April 2012. The samples were authenticated by Josephine T. Garcia of the Bureau of Plant Industry, San Andres, Manila, Philippines.

Isolation

Leaf samples of *T. triangulare* were air-dried for about one week. The air-dried leaves (210 g) were ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (9.6 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment. The CH₂Cl₂ fraction was rechromatographed using petroleum ether. The less polar fractions were rechromatographed in 1% EtOAc in petroleum ether to afford **1** (28 mg). The more polar fractions were rechromatographed (2 ×) in 2.5% EtOAc in petroleum ether to yield **4** (12 mg) after washing with petroleum ether. The 10% to 30% acetone in CH₂Cl₂ were combined and rechromatographed (4 ×) using 7.5% EtOAc in petroleum ether to yield **2** (15 mg). The 40% acetone in CH₂Cl₂ fraction was rechromatographed using CH₃CN:Et₂O:CH₂Cl₂ (0.5:0.5:9) by volume ratio to afford **3** (55 mg) after washing with petroleum ether, followed by Et₂O.

Squalene (1): ¹³C NMR (150 MHz, CDCl₃): δ 25.69 (C-1), 131.26 (C-2), 124.39 (C-3), 26.65 (C-4), 39.7 (C-5), 134.89 (C-6), 124.26 (C-7), 26.76 (C-8), 39.72 (C-9), 135.10 (C-10), 124.29 (C-11), 28.27 (C-12), 17.67 (C-2'), 15.99 (C-6'), 16.03 (C-10').

Triglyceride (2): ¹³C NMR (150 MHz, CDCl₃): δ 62.09 (glyceryl CH₂), 68.86 (glyceryl CH), 173.26 (C=O α), 172.84 (C=O β), 34.01 (C-2α), 34.18 (C-2β), 24.82 (C-3α), 24.86 (C-3β), 29.08 (C-4α), 29.04 (C-4β), 29.19 (C-5α), 29.27 (C-5β), 29.11 (C-6α), 29.17 (C-6β), 29.60 (C-7α), 29.70 (C-7β), 29.19 (both C-8), 130.01 (C-9α), 129.9 (C-9β), 128.06 (C-10α), 128.07 (C-10β), 25.60 (both C-11), 127.88 (C-12α), 127.74 (C-12β), 130.20 (both C-13), 27.20 (both C-14), 29.36 (both C-15), 31.52 (both C-16), 22.57 (both C-17), 14.27, 14.11, 14.07 (C-18).

Lutein (3): ¹³C NMR (150 MHz, CDCl₃): 12.81, 13.10, 21.62, 22.86, 24.26, 28.72, 29.49, 30.25, 34.03, 37.12, 42.54, 44.63, 48.41, 54.95, 65.09, 65.92, 124.46, 124.80, 124.93, 125.58, 126.16, 128.72, 130.03, 130.08, 130.80, 131.29, 132.57, 135.06, 135.69, 136.49, 137.56, 137.72, 138.00, 138.49.

β-Carotene (4): ¹³C NMR (150 MHz, CDCl₃): δ 34.27 (C-1, 1'), 39.64 (C-2, 2'), 19.26 (C-3, 3'), 33.11 (C-4, 4'), 129.38 (C-5, 5'), 137.91 (C-6, 6'), 126.65 (C-7, 7'), 137.76 (C-8, 8'), 136.02 (C-9, 9'), 132.41 (C-10, 10'), 129.98 (C-11, 11'), 137.23 (C-12, 12'), 136.47 (C-13, 13'), 130.83 (C-14, 14'), 125.03 (C-15, 15'), 29.69 (C-16, 16'), 28.97 (C-17, 17'), 21.76 (C-18, 18'), 12.82 (C-19, 19'), 12.76 (C-20, 20').

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the leaves of *Talinum triangulare* afforded squalene (**1**) [12], triglyceride (**2**) [13], lutein (**3**) [14] and β-carotene (**3**) [14]. The structures of **1-4** were identified by comparison of their ¹³C NMR data with those reported in the literature [12-14].

Although bioassays were not conducted on the isolated compounds, there were previous studies that reported on the biological activities of **1**, **3** and **4** 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 [15]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [16]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells [17]. The preventive and therapeutic potential of squalene containing compounds on tumor promotion and regression have been reported [18]. A recent review on the bioactivities of squalene has been provided [19].

Dietary lutein (**3**), especially at 0.002%, inhibited tumor growth by selectively modulating apoptosis, and by inhibiting angiogenesis [20]. 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 [21]. Moreover, very low amounts of dietary lutein (0.002%) can efficiently decrease mammary tumor development and growth in mice [22].

β -Carotene (**4**) and lutein inhibit hydrogen peroxide-induced activation of NF- κ B and IL-8 expression in gastric epithelial AGS cells [23]. Another study reported that the redox regulation of NF- κ B induced by β -carotene is involved in the growth-inhibitory and proapoptotic effects of the carotenoid in human leukemia and colon adenocarcinoma cells [24]. Furthermore, β -carotene possesses anti-inflammatory activity by functioning as a potential inhibitor for redox-based NF- κ B activation, probably due to its antioxidant activity [25].

CONCLUSION

Talinum triangulare afforded squalene (**1**), triglyceride (**2**), lutein (**3**) and β -carotene (**4**) which were reported to exhibit diverse biological activities. Compounds **1**, **3** and **4** are known antioxidant and anticancer compounds. Carotenoid **4** also showed anti-inflammatory activity. The leaves were reported to be effective in reducing total plasma cholesterol and preventing or treating coronary heart disease. This maybe attributed in part to the presence of squalene (**1**) as a major constituent of the leaves which was reported to exhibit cardioprotective and hypolipidemic effects.

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