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Chemical constituents of *Cayratia trifolia*

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ABSTRACT

*The wide-ranging medicinal values of Cayratia trifolia (L.) Domin. are well-documented both in folk medicine and pharmacological studies. This study was conducted to investigate the chemical constituents of the dichloromethane extract of the air-dried leaves of C. trifolia. This led to the isolation of a mixture of β-sitosterol (**1**) and stigmasterol (**2**) in about 5:1 ratio, squalene (**3**) and lutein (**4**). The structures of **1**-**4** were identified by comparison of their ^{13}C NMR data with those reported in the literature.*

Keywords: *Cayratia trifolia*, Vitaceae, β-sitosterol, stigmasterol, squalene, lutein

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INTRODUCTION

The three-leaf cayratia *(Cayratia trifolia* (L.) Domin., Family Vitaceae), locally known in the Philippines as *kalit-kalit*, is a weak herbaceous climber in thickets and open forests at low altitudes [1]. It is widely distributed in tropical and subtropical Asia, Africa, India, Australia and Pacific islands where its wide-ranging medicinal values are well-documented both in folk medicine and pharmacological studies [1]. Leaf decoction or the juice of the fresh leaves is used to cure scurvy in the Philippines, to prevent head itch and dandruff in Java, to relieve inflammation and high fever in Thailand and Peninsular Malaysia while the young leaves are eaten as vegetable in Moluccas [1]. The root is used as an antidote against snake bite [2], while the stem is reportedly with aphrodisiac property [3]. The petroleum ether extract of *C. trifolia* leaves exhibited a potent anti-implantation activity [4]. Another study reported that the ethanol extract of the root of *C. trifolia* has the potential for the treatment of diabetes mellitus caused in the consequences of resistance to stimulatory effect of insulin on GLUT-4 protein [5]. Furthermore, the ethyl acetate extract of the root of *C. trifolia* was reported to possess potent antidiabetic property [6]. A recent study reported that 75% and 100% *C. trifolia* alcoholic leaf extract treatments possess a comparable antibacterial property as an alternative remedy for boils caused primarily by *Staphylococcus aureus* [7]. The aqueous extract of *C. trifolia* leaf was reported to be a cost effective and potent larvicidal agent against *Culex quinquefasciatus* [8]. The ethanol extract of *C. trifolia* contains antioxidants and exhibits a good free radical scavenging activity. Phytochemical screening and HPTLC analysis confirmed the presence of alkaloids and flavonoids in the ethanolic extract of this plant, which support its free radical scavenging activity [9]. The methanolic extract of *C. trifolia* leaves possesses anti-ulcerogenic and ulcer healing properties which migh be attributed to its antisecretory activity [10]. A review on

chemical and biological properties of *Cayratia trifolia* Linn. (Vitaceae) has been provided [11]. The plant was reported to exhibit antibacterial, antifungal, antiprotozoal, antiviral, hypoglycemic, anticancer, antioxidant, anti-inflammatory and diuretic properties. The aerial parts of the plant contain kaempferol, myricetin, quercetin, triterpenes and epifriedelanol; the leaves also contain stilbenes such as piceid, resveratrol, viniferin and ampelopsin; and the seeds and fruits contain cyanogenic compounds [11].

We report herein the isolation and identification of a mixture of β-sitosterol (**1**) and stigmasterol (**2**) in about 5:1 ratio, squalene (**3**), and lutein (**4**) (Fig. 1) from the dichloromethane extract of the leaves of *C. trifolia*.

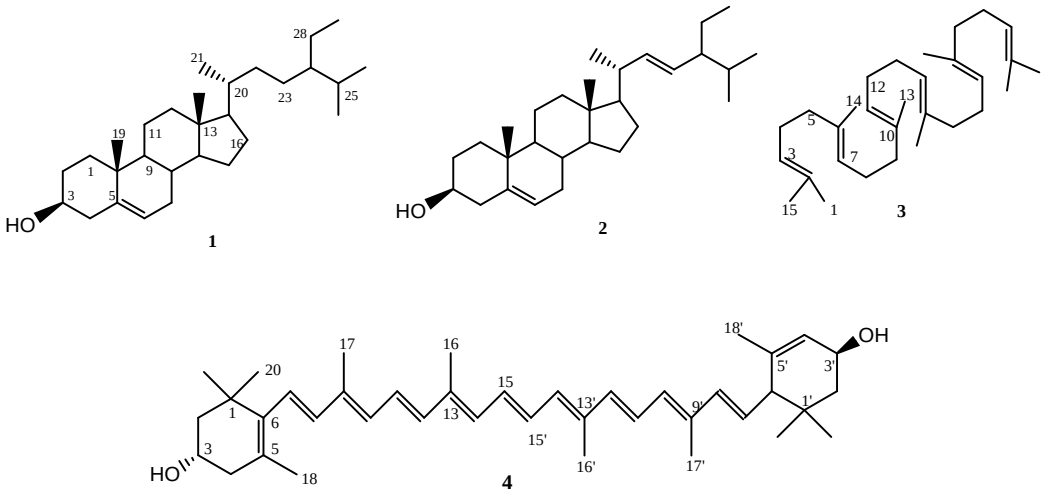

Fig.1. Chemical constituents of the leaves of *C. trifolia*: β-sitosterol (1), stigmasterol (2), squalene (3) and lutein (4)

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.

Collection of Leaf Samples

Leaf samples were collected from a private land in Socorro, Oriental Mindoro in July 2011. Voucher specimens were collected and identified by one of the authors (EHM) with collection #895 and deposited at the herbarium of De La Salle University-Manila.

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 *C. trifolia* were air-dried for about one week. The air-dried leaves (82.4 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 (9 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 **3** (3 mg). The 40% and 50%

acetone in CH_2Cl_2 fractions were combined and rechromatographed using 15% EtOAc in petroleum ether, followed by 20% EtOAc in petroleum ether. The fractions eluted with 15% EtOAc in petroleum ether were combined and rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford a mixture of **1** and **2** (4 mg) after washing with petroleum ether. The fractions eluted with 20% EtOAc in petroleum ether were combined and rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) afford **4** (6 mg) after washing with petroleum ether, followed by Et_2O.

β-Sitosterol (**1**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.80 (C-3), 42.28 (C-4), 140.74 (C-5), 121.71 (C-6), 31.88, 31.90 (C-7, C-8), 50.14 (C-9), 36.49 (C-10), 21.07 (C-11), 39.75 (C-12), 42.28 (C-13), 56.75 (C-14), 24.29 (C-15), 28.24 (C-16), 56.03 (C-17), 11.97 (C-18), 19.38 (C-19), 36.13 (C-20), 18.76 (C-21), 33.93 (C-22), 26.04 (C-23), 45.82 (C-24), 29.13 (C-25), 19.02 (C-26), 19.81 (C-27), 23.05 (C-28), 11.84 (C-29).

Stigmasterol (**2**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.80 (C-3), 42.29 (C-4), 140.74 (C-5), 121.71 (C-6), 31.89 (C-7), 31.89 (C-8), 50.15 (C-9), 36.49 (C-10), 21.07 (C-11), 39.67 (C-12), 42.20 (C-13), 56.76 (C-14), 24.35 (C-15), 28.91 (C-16), 55.94 (C-17), 12.03 (C-18), 19.39 (C-19), 40.48 (C-20), 21.07 (C-21), 138.31 (C-22), 129.26 (C-23), 51.23 (C-24), 31.90 (C-25), 21.20 (C-26), 18.97 (C-27), 25.40 (C-28), 12.24 (C-29).

Squalene (**3**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.69 (C-1, C-1'), 131.26 (C-2, 2'), 124.30 (C-3, C-3'), 26.65 (C-4, C-4'), 39.72 (C-5, C-5'), 134.90 (C-6, C-6'), 124.40 (C-7, C-7'), 26.76 (C-8, C-8'), 39.75 (C-9, C-9'), 135.11 (C-10, C-10'), 124.30 (C-11, C-11'), 28.27 (C-12, C-12'), 17.67 (C-13, C-13'), 16.04 (C-14, C-14'), 15.99 (C-15, C-15').

Lutein (**4**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.12 (C-1), 48.42 (C-2), 65.10 (C-3), 42.54 (C-4), 126.15 (C-5), 138.00 (C-6), 125.58 (C-7), 138.49 (C-8), 135.69 (C-9), 131.29 (C-10), 124.93 (C-11), 137.55 (C-12), 136.41 (C-13), 132.57 (C-14), 130.08 (C-15), 28.72 (C-16), 30.25 (C-17), 21.61 (C-18), 12.81, 12.75 (C-19, C-20), 34.02 (C-1'), 44.63 (C-2'), 65.93 (C-3'), 124.46 (C-4'), 137.72 (C-5'), 54.96 (C-6'), 128.72 (C-7'), 130.80 (C-8'), 135.06 (C-9'), 137.55 (C-10'), 124.80 (C-11'), 137.72 (C-12'), 136.41 (C-13'), 132.57 (C-14'), 130.08 (C-15'), 24.27 (C-16'), 29.49 (C-17'), 22.87 (C-18'), 13.10 (C-19'), 12.81 (C-20').

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the air-dried leaves of *T. microcarpa* afforded a mixture of β-sitosterol (**1**) [12] and stigmasterol (**2**) [13] in a 5:2 ratio, squalene (**3**) [14] and lutein (**4**) [15]. The mixture of **1** and **2** in about 5:1 ratio was deduced from the ^{1}H NMR resonances for the olefinic protons of **1** at δ 5.33 (dd, *J* = 1.8, 5.4 Hz, H-6) and **2** at δ 5.33 (dd, *J* = 1.8, 5.4 Hz, H-6), 5.13 (dd, *J* = 9.0, 15.0 Hz, H-22) and 5.00 (dd, *J* = 9.0, 15.0 Hz, H-23). The structures of **1**-**4** were identified by comparison of their ^{13}C NMR data with those reported in the literature [12-15].

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

β-Sitosterol (**1**) was reported to exhibit growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [16]. It was shown to be effective for the treatment of benign prostatic hyperplasia [17]. It attenuated β-catenin and PCNA expression, as well as quenched radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis [18]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [19]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [20].

Stigmasterol (**2**) shows therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions [21]. It lowers plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Winstar as well as WKY rats [22]. Other studies reported that stigmasterol showed cytostatic activity against Hep-2 and McCoy cells [23], markedly inhibited tumour promotion in two stage carcinogenesis experiments [24], exhibited antimutagenic [25], topical anti-inflammatory [26], anti-osteoarthritic [27] and antioxidant [28] activities.

Squalene (**3**) was reported to significantly suppress colonic ACF formation and crypt multiplicity which strengthened the hypothesis that it possesses chemopreventive activity against colon carcinogenesis [29]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [30]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells [31]. The preventive and therapeutic potential of squalene containing compounds on tumor promotion and regression have been reported [32]. A recent review on the bioactivities of squalene has been provided [33].

Dietary lutein (**4**), especially at 0.002%, inhibited tumor growth by selectively modulating apoptosis and by inhibiting angiogenesis [34]. 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 [35]. Moreover, very low amounts of dietary lutein (0.002%) can efficiently decrease mammary tumor development and growth in mice [36]. Another study reported that lutein and zeaxanthine reduces the risk of age related macular degeneration [37].

CONCLUSION

Cayratia trifolia (L.) Domin. was reported to exhibit wide-ranging medicinal values, among them is anticancer property. The compounds isolated from the dichloromethane extract of the leaves of the plant are β-sitosterol, stigmasterol, squalene and lutein which were reported to exhibit anticancer properties. Thus, the anticancer activities of the plant may be attributed to the synergistic effects of these compounds, among others which are found in the extract.

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