Chemical Constituents of *Champereia manillana* (Blume) Merrill

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ABSTRACT

Chemical investigation of the dichloromethane extract of the air-dried leaves of *Champereia manillana* (Blume) Merrill led to the isolation of squalene (1), lutein (2), β-carotene (3), phytol (4), chlorophyll a (5), and 1,2-dilinoleoyl-3-linolenoylglycerol (6). The structures of 1-6 were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Champereia manillana*, Opiliaceae, squalene, lutein, β-carotene, phytol, chlorophyll a, 1,2-dilinoleoyl-3-linolenoylglycerol

INTRODUCTION

*Champereia manillana* (Blume) Merrill, commonly known as false olive or chemperai and locally known as apeng or pannalayapen belongs to the family Opiliaceae [1]. It grows in forests, hills, valleys, and thickets in Guangxi, Taiwan, Yunnan, India, Indonesia, Malaysia, Myanmar, New Guinea, Philippines, Thailand, Vietnam [2]. The leaves and young fruits are eaten as vegetables. The boiled root is used for rheumatism [3]. The leaves and roots are pounded to make a poultice for ulcers, while the leaves are pounded and applied for headache and stomach ache [3]. A study reported that among five Malaysian vegetables tested, *C. manillana* gave the highest calcium content (565 mg kg⁻¹ edible fresh sample). The vegetable extracts significantly prevented the oxidation of haemoglobin and low-density lipoprotein, resulting in a reduced production of malondialdehyde [4]. *C. manillana* oil contains oleic acid 78-86, linoleic acid 7-10, linolenic acid 0.2-0.8, palmitic acid 8-10, and stearic acid 1.5-3.5 by wt.%. The oil can eliminate bruising, relieve itching and pain, prevent colds, dizziness, headache, treat diaper rash, and eczema [5].

We report herein the isolation of squalene (1), lutein (2), β-carotene (3), phytol (4), chlorophyll a (5), 1,2-dilinoleoyl-3-linolenoylglycerol (6) (Fig. 1) from the leaves of *C. manillana*. To the best of our knowledge, this is the first report on the isolation of these compounds from *C. manillana*. 
Fig. 1. Chemical structures of squalene (1), lutein (2), β-carotene (3), phytol (4), chlorophyll a (5), and 1,2-dilinoleoyl-3-linolenoylglycerol (6) from C. manillana
MATERIALS AND METHODS

General Experimental Procedure

$^1$H (500 MHz) NMR spectra were acquired in CDCl$_3$ on a 500 MHz Agilent DD2 NMR spectrometer with referencing to solvent signal ($\delta$ 7.26 CHCl$_3$). Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed using plastic backed plates coated with silica gel F254 and the plates were visualized by spraying with vanillin/H$_2$SO$_4$ 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

Champereia manillana (Blume) Merrill was bought from Batac Public Market in Batac, Ilocos Norte, Philippines in March 2015. The sample was authenticated by Flordeliz Rapon Estira of Mariano Marcos State University.

Isolation of chemical constituents from the leaves

The air dried leaves of C. manillana (201 g) were ground in a blender, soaked in CH$_2$Cl$_2$ for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (5.0 g) which was chromatographed using increasing proportions of acetone in CH$_2$Cl$_2$ at 10% increment by volume. The CH$_2$Cl$_2$ fraction was rechromatographed using petroleum ether. The less polar fractions were combined and rechromatographed using petroleum ether (2 ×) to afford 1 (6 mg). The more polar fractions were combined and rechromatographed using 2.5% EtOAc in petroleum ether (3 ×) to afford 3 (2 mg) after washing with petroleum ether. The 20% acetone in CH$_2$Cl$_2$ fraction was rechromatographed (4 ×) using 7.5% EtOAc in petroleum ether to afford 6 (5 mg). The 30% acetone in CH$_2$Cl$_2$ fraction was rechromatographed using CH$_2$Cl$_2$. The less polar fractions were combined and rechromatographed (3 ×) in CH$_2$Cl$_2$ to afford 4 (7 mg). The more polar fractions were combined and rechromatographed (2 ×) in CH$_2$Cl$_2$ to yield 5 (4 mg) after washing with petroleum ether, followed by Et$_2$O. The 50% acetone in CH$_2$Cl$_2$ fraction was rechromatographed (3 ×) in CH$_3$CN:Et$_2$O:CH$_2$Cl$_2$ (1:1:8, v/v) to afford 2 (4 mg) after washing with petroleum ether, followed by Et$_2$O.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the leaves of C. manillana afforded 1-6. The structures of 1-6 were identified by comparison of their NMR data with those reported in the literature for squalene (1) [6], lutein (2) [7], β-carotene (3) [8], phytol (4) [9], chlorophyll a (5) [10], and 1,2-dilinoleoyl-3-linolenoylglycerol (6) [6]. The presence linoleic acid in 6 was deduced from the methyl triplet at $\delta$ 0.86 ($J$ = 6.5 Hz), the double allinic methylene at $\delta$ 2.8 and the olefinic protons at $\delta$ 5.34 (m) [11, 12]. The presence of α-linolenic acid in 6 was deduced from the methyl triplet at $\delta$ 0.96 ($J$ = 7.5 Hz), the double allylic methylenes at $\delta$ 2.78 and the olefinic protons at $\delta$ 5.34 (m) [11, 13]. Based on integrations of the triacylglycerol methyls at $\delta$ 0.86 (t, $J$ = 6.5 Hz) and $\delta$ 0.96 (t, $J$ = 7.5 Hz), the ratio of linoleic acid and linolenic acid in 6 is 2:1.

Although no biological activity tests were conducted on the isolated compounds, a literature search of 1-5 revealed that these have diverse bioactivities.

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 [14]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [15]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells [16]. The preventive and therapeutic potential of 1 on tumor promotion and regression have been reported [17]. A recent review on the bioactivities of 1 has been provided [18].
Dietary lutein (2), especially at 0.002%, inhibited tumor growth by selectively modulating apoptosis, and by inhibiting angiogenesis [19]. Another study reported that the chemopreventive properties of all-trans retinoic acid and 2 may be attributed to their differential effects on apoptosis pathways in normal versus transformed mammary cells [20]. Moreover, very low amounts of dietary 2 (0.002%) can efficiently decrease mammary tumor development and growth in mice [21]. Another study reported that 2 and zeaxanthine reduces the risk of age related macular degeneration [22].

β-Carotene (3) and 2 inhibit hydrogen peroxide-induced activation of NF-κB and IL-8 expression in gastric epithelial AGS cells [23]. Another study reported that the redox regulation of NF-κB induced by 4 is involved in the growth-inhibitory and proapoptotic effects of the carotenoid in human leukemia and colon adenocarcinoma cells [24]. Furthermore, 4 possesses anti-inflammatory activity by functioning as a potential inhibitor for redox-based NF-κB activation, probably due to its antioxidant activity [25].

Phytol (4) was isolated and identified as Acyl-CoA cholesterol acyltransferase (ACAT) inhibiting compound from the leaves of Lactuca sativa [26]. It was also reported as a skin brightening agent by inhibiting melanin synthesis [27]. Another study reported that 2 exhibited pronounced antinociceptive effects through its central and peripheral actions and it also showed antioxidant properties [28]. The antimicrobial activity of 4 was evaluated against eight bacterial and eight fungal strains and was proven to be active against all tested bacteria and fungi with MIC values of 0.003-0.038 mg/mL and MBC values of 0.013-0.052 mg/mL for bacteria and MIC values of 0.008-0.016 mg/mL and MFC values of 0.090-0.520 mg/mL for fungi [29]. It reduced the production of all tested radicals: CO$_2$(-), CH$_2$OH and ·DPPH radicals (56%, 50% and 48%, respectively) and NO, ·O$_2$(-) and ·OH radicals (38%, 23% and 15%, respectively) [29]. Another study reported that 4 showed high antinociceptive activity, high stability, and low toxicity [30]. Diterpene 2 was reported to exhibited promising antischistosomal properties in vitro and in a mouse model of Schistosomiasis mansoni [31]. Furthermore, 2 was exhibited anti-inflammatory and antiallergic effects [32] and antimicrobial activity against Mycobacterium tuberculosis [33, 34] and Staphylococcus aureus [35]. Diterpene 2 was also reported as an immunostimulant [36] and with no cumulative inflammatory or toxic effects even in immuno-compromised mice [37].

Chlorophyll and its various derivatives are used in traditional medicine and for therapeutic purposes [38]. Natural chlorophyll and its derivatives have been studied for wound healing [39], anti-inflammatory properties [40], control of calcium oxalate crystals [41], utilization as effective agents in photodynamic cancer therapy [42-44], and chemopreventive effects in humans [45, 46]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided [47].

**CONCLUSION**

The leaves of *C. manillana* are pounded and applied for headache and stomach ache, while the leaves and roots are pounded to make a poultice for ulcers. The compounds (1-5) isolated from the leaves of the plant were reported to possess diverse biological activities. The medicinal properties of the plant may be partly attributed to 4 which was reported to exhibit antinociceptive, antimicrobial and immunostimulant properties.

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**REFERENCES**

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