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Chemical constituents of *Strongylodon macrobotrys*

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ABSTRACT

*Chemical investigation of the dichloromethane extracts of Strongylodon macrobotrys led to the isolation of taraxerone (**1**), stigmasterol (**2**), β-sitosterol (**3**), and triglycerides (**4**) from the stems; **2**, **3**, and β-*stigmasteryl *3-O-*β-D-glucopyranoside *(**5**) from the flowers; and polyprenol (**6**), lutein (**7**), squalene (**8**) and chlorophyll a (**9**) from the leaves. The structures of these compounds were identified by comparison of their ^{13}C NMR data with those reported in the literature.*

Keywords: *Strongylodon macrobotrys*, Leguminosae, taraxerone, stigmasterol, β-sitosterol, β-stigmasteryl *3-O*-β-D-glucopyranoside, polyprenol, lutein, squalene, chlorophyll a

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INTRODUCTION

Strongylodon macrobotrys A. Gray, of the family Leguminosae, commonly known as jade vine or emerald creeper and locally known as "tayabak", is a leguminous perennial woody vine that can reach up to 18 m in length. It is native to the Philippines, thriving best in tropical forests, along streams or in ravines from 700 to 1,000 m asl [1, 2]. It is considered as one of the most beautiful of all tropical climbers because of its elegant and striking turquoise flowers that dangle in mid-air when in full bloom. In the Philippines, jade vine is cultivated as an ornamental plant. Naturally pollinated by bats, the destruction of rainforests in the Philippines threatens *S. macrobotrys* in the wild resulting in the plant being listed as vulnerable in the IUCN list of threatened species [3].

There are few studies on the chemical constituents of *S. macrobotrys*. An earlier study reported that the major visible pigment in the jade vine flower is the anthocyanin, malvidin 3,5-di-*O*-glucoside, accompanied with *C*-glycosylflavones, isovitexin 7-*O*-glucoside and isovitexin [4]. Recently, the flower color of *S. macrobotrys* which is luminous blue green was attributed to a mixture of an anthocyanin, malvin, and a flavone, saponarin, in approximately 1:9 molar ratio [5]. The isolation and identification of two saponins, the dimethyl esters of pseudoginsenoside-RP1 and zingibroside-R1 from the seeds of *S. macrobotrys* were also reported [6]. There is no reported study on the biological activity of jade vine.

This study is part of our research on the chemical constituents of endemic and native Philippine ornamental plants. Our chemical investigation of *Hoya mindorensis*, an endemic ornamental plant of the Philippines, led to the isolation of lupenone and lupeol from the roots; lupeol, squalene and β-sitosterol from the leaves; and betulin from the stems of the plant. Except for lupenone, all the isolated secondary metabolites from *H. mindorensis* are known anticancer compounds [7].

We report herein the isolation and identification of taraxerone (**1**), a mixture of stigmasterol (**2**) and β-sitosterol (**3**) in a 2:1 ratio, and triglycerides (**4**) from the stems; a mixture of **2** and **3** in a 3:1 ratio and stigmasterol-β-D-glucoside (**5**) from the flowers; and polyprenol (**6**), lutein (**7**), squalene (**8**) and chlorophyll a (**9**) from the leaves (Fig. 1) of *S. macrobotrys*. To the best of our knowledge this is the first report on the isolation of these compounds from the plant.

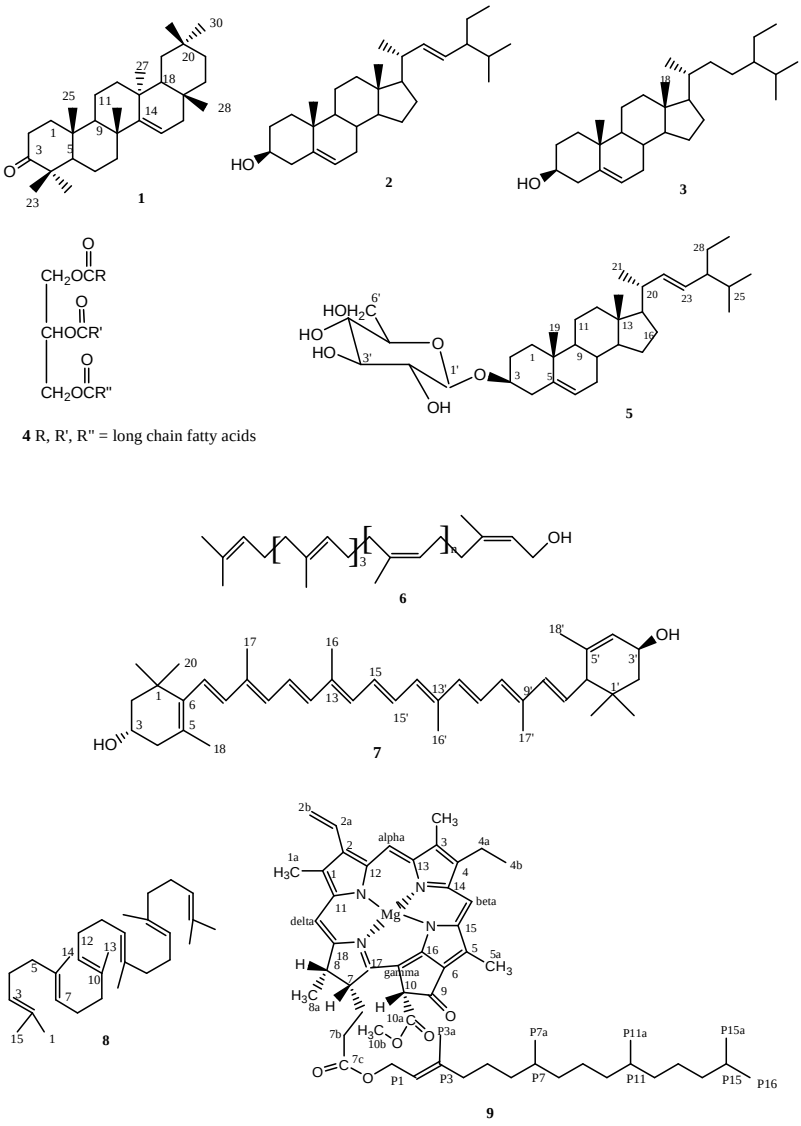

Fig. 1. Chemical constituents of *Strongylodon macrobotrys*: taraxerone (1), stigmasterol (2), β-sitosterol (3), triglycerides (4), β-stigmasteryl *3-O*-β-D-glucopyranoside (5), polyprenol (6), lutein (7), squalene (8) and chlorophyll a (9).

MATERIALS AND METHODS

Sample Collection

Samples of leaves, twigs and flowers of *Strongylodon macrobotrys* A. Gray 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 and deposited with voucher # 268-2014.

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.

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 stems of *S. macrobotrys* (40.8 g) was 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 (1.5 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 5% EtOAc in petroleum ether to afford **1** (5 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) in 5% EtOAc using petroleum ether to afford **4** (7 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to afford a mixture of **2** and **3** (9 mg) after washing with petroleum ether.

The air-dried flowers of *S. macrobotrys* (28.5 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 (0.15 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to afford a mixture of **2** and **3** (5 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2 was rechromatographed (5 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6, v/v) to afford **5** (4 mg) after trituration with petroleum ether.

The air-dried leaves of *S. macrobotrys* (69.2 g) was 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 (3.0 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (2 ×) using 1% EtOAc in petroleum to afford **8** (5 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 12.5 % EtOAc in petroleum ether to afford **6** (6 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using 20% EtOAc in petroleum ether to afford **9** (8 mg) after washing with petroleum ether, followed by Et_2O. The 60% acetone in CH_2Cl_2 fraction was rechromatographed (5 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to afford **7** (9 mg) after washing with petroleum ether, followed by Et_2O.

Taraxerone (**1**): ^1H NMR (600MHz, $CDCl_3$) δ: 5.54 (dd, *J* = 3.6, 8.4 Hz, H-15), 1.06 (s, H_3-23), 1.05 (s, H_3-24), 1.07 (H_3-25), 0.888, 0.894 (s, H_3-26, H_3-30), 1.12 (s, H_3-27), 0.81 (s, H_3-28), 0.93 (s, H_3-29); ^{13}C NMR ($CDCl_3$) δ: 38.33 (C-1), 34.14 (C-2), 217.60 (C-3), 47.58 (C-4), 55.76 (C-5), 19.94 (C-6), 35.08 (C-7), 38.86 (C-8), 48.68 (C-9), 35.77 (C-10), 17.43 (C-11), 37.73, 37.67 (C-12, C-13), 157.58 (C-14), 117.18 (C-15), 36.65 (C-16), 37.52 (C-17), 48.76 (C-18), 40.61 (C-19), 28.79 (C-20), 33.55 (C-21), 33.06 (C-22), 26.08 (C-23), 21.33 (C-24), 14.80 (C-25), 29.84 (C-26), 25.56 (C-27), 29.91 (C-28), 33.34 (C-29), 21.48 (C-30).

Stigmasterol (**2**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.81 (C-3), 42.29 (C-4), 140.74 (C-5), 121.71 (C-6), 31.89 (C-7), 31.89 (C-8), 50.14 (C-9), 36.51 (C-10), 21.08 (C-11), 39.67 (C-12), 42.20 (C-13), 56.75

(C-14), 24.35 (C-15), 28.91 (C-16), 55.94 (C-17), 12.04 (C-18), 19.39 (C-19), 40.49 (C-20), 21.08 (C-21), 138.31 (C-22), 129.26 (C-23), 51.23 (C-24), 31.89 (C-25), 21.20 (C-26), 18.97 (C-27), 25.40 (C-28), 12.25 (C-29).

β-Sitosterol (**3**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.82 (C-3), 42.29 (C-4), 140.74 (C-5), 121.71 (C-6), 31.89, 31.90 (C-7, C-8), 50.14 (C-9), 36.49 (C-10), 21.07 (C-11), 39.76 (C-12), 42.20 (C-13), 56.75 (C-14), 24.35 (C-15), 28.24 (C-16), 56.04 (C-17), 11.97 (C-18), 19.39 (C-19), 36.14 (C-20), 18.77 (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), 11.85 (C-29).

Triglyceride (**4**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 62.09 (glyceryl CH_2), 68.87 (glyceryl CH), 173.25 (C=O α), 172.84 (C=O β), 34.01, 34.05, 34.18 (C-2), 24.83, 24.86, 24.87 (C-3), 29.08 29.04 (C-4), 29.17, 29.19, 29.27 (C-5), 29.48 (C-6), 22.19 (C-8), 130.00, 129.98 (C-9), 127.87, 128.05 (C-10), 25.62, 25.52 (C-11), 127.88. 128.07 (C-12), 130.22, 128.07 (C-13), 27.19, 25.62 (C-14), 29.35 (C-15), 127.10 (C-15), 31.52 (C-16), 132.00 (C-16), 22.57, 22.69 (C-17), 14.07, 14.12 (C-18).

β-Stigmasteryl* 3-O-*β-D-glucopyranoside (**5**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.84 (C-2), 79.53 (C-3), 42.20 (C-4), 140.25 (C-5), 122.17 (C-6), 31.84, 31.87 (C-7, C-8), 50.15 (C-9), 36.72 (C-10), 19.81 (C-11), 38.88 (C-12), 42.30 (C-13), 56.83 (C-14), 24.34 (C-15), 28.91 (C-16), 55.94 (C-17), 11.84 (C-18), 18.77 (C-19), 39.64 (C-20), 20.04 (C-21), 138.28 (C-22), 129.29 (C-23), 51.23 (C-24), 31.84 (C-25), 19.34 (C-26), 18.97 (C-27), 24.98 (C-28), 12.26 (C-29), 101.18 (C-1'), 73.99 (C-2'), 76.79 (C-3'), 69.98 (C-4'), 76.79 (C-5'), 63.10 (C-6').

Polyprenol (**6**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 59.01 (CH_2OH), 139.90, 136.08, 135.37, 135.28, 135.24, 135.20, 134.93, 134.86, 131.25, 125.01, 124.99, 124.99, 124.92, 124.39, 124.31, 124.24, 124.21, 124.11, 39.71, 32.21, 32.19, 31.97, 26.75, 26.66, 26.62, 26.39, 26.32, 25.68, 23.45, 23.42, 23.37, 17.66, 15.99, 15.98.

Lutein (**7**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.12 (C-1), 48.42 (C-2), 65.10 (C-3), 42.55 (C-4), 126.15 (C-5), 138.01 (C-6), 125.57 (C-7), 138.49 (C-8), 135.69 (C-9), 131.29 (C-10), 124.93 (C-11), 137.56 (C-12), 136.41 (C-13), 132.57 (C-14), 130.08 (C-15), 28.72 (C-16), 30.26 (C-17), 21.62 (C-18), 12.81, 12.75 (C-19, C-20), 34.02 (C-1'), 44.22 (C-2'), 65.93 (C-3'), 124.45 (C-4'), 137.72 (C-5'), 54.96 (C-6'), 128.72 (C-7'), 130.80 (C-8'), 135.07 (C-9'), 137.56 (C-10'), 124.80 (C-11'), 137.72 (C-12'), 136.49 (C-13'), 132.57 (C-14'), 130.08 (C-15'), 28.28 (C-16'), 29.49 (C-17'), 22.87 (C-18'), 12.81, 3.11 (C-19'), 12.81 (C-20').

Squalene (**8**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.69 (C-1, C-1'), 131.24 (C-2, 2'), 124.30 (C-3, C-3'), 26.66 (C-4, C-4'), 39.73 (C-5, C-5'), 134.89 (C-6, C-6'), 124.40 (C-7, C-7'), 26.76 (C-8, C-8'), 39.75 (C-9, C-9'), 135.10 (C-10, C-10'), 124.30 (C-11, C-11'), 28.27 (C-12, C-12'), 17.67 (C-13, C-13'), 16.03 (C-14, C-14'), 15.99 (C-15, C-15').

Chlorophyll a (**9**): dark green crystals. ^{13}C NMR (150 MHz, $CDCl_3$): δ 131.85 (C-1), 12.12 (C-1a), 136.51 (C-2), 129.06 (C-2a), 122.80 (C-2b), 136.18 (C-3), 11.24 (C-3a), 145.24 (C-4), 19.47 (C-4a), 17.43 (C-4b), 137.93 (C-5), 12.12 (C-5a), 129.06 (C-6), 51.11 (C-7), 29.79 (C-7a), 31.16 (C-7b), 172.94 (C-7c), 50.10 (C-8), 23.06 (C-8a), 189.65 (C-9), 64.68 (C-10), 169.60 (C-10a), 52.84 (C-10b), 142.84 (C-11), 136.18 (C-12), 155.66 (C-13), 150.99 (C-14), 129.10 (C-15), 149.65 (C-16), 161.24 (C-17), 172.94 (C-18), 97.55 (C-α), 104.44 (C-β), 105.22 (C-γ), 93.12 (C-δ), 61.45 (P-1), 117.69 (P-2), 142.02 (P-3), 16.27 (P-3a), 39.77 (P-4), 24.96 (P-5), 36.62 (P-6), 32.60 (P-7), 19.64 (P-7a), 37.30 (P-8), 24.40 (P-9), 37.37 (P-10), 32.74 (P-11), 19.70 (P-11a), 37.24 (P-12), 24.76 (P-13), 39.33 (P-14), 27.95 (P-15), 22.60 (P-15a), 22.70 (P-16).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *S. macrobotrys* afforded taraxerone (1) [8], a mixture of stigmasterol (**2**) [9] and β-sitosterol (**3**) [10] in a 2:1 ratio, and triglycerides (**4**) [11] from the stems; a mixture of **2** and **3** in a 3:1 ratio and stigmasterol-β-D-glucoside (**5**) [12] from the flowers; and polyprenol (**6**) [13], lutein (**7**) [14], squalene (**8**) [15] and chlorophyll a (**9**) [16] from the leaves. The ratios of the mixture of **2** and **3** were deduced from the integrations of the ^{1}H NMR resonances for the olefinic protons of **2** at δ 5.33 (dd, *J* = 1.8, 4.8 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) and **3** at δ 5.33 (dd, *J* = 1.8, 4.8 Hz, H-6). The fatty acids esterified to the glycerol in the triglycerides are linolenic acid, linoleic acid and saturated fatty acid. These were deduced from the integration of the resonances at δ 0.95 (t, *J* = 7.8 Hz, CH_3) and 2.76 (2 double allylic CH_2) for the linolenic acid; δ 0.88 (t, *J* = 6.6 Hz, CH_3) and 2.74 (double allylic CH_2) for the linoleic acid; and δ 0.86

(t, J = 7.2 Hz, CH_3) for the saturated fatty acid. The structures of **1**-**9** were identified by comparison of their ^{13}C NMR data with those reported in the literature [8-16].

Although no biological activity tests were conducted on the isolated compounds (**1**-**9**), literature search revealed that these have diverse bioactivities as follows.

Taraxerone (**1**) was reported to exhibit antioxidant activity with IC_{50} values of 102.34±1.53 μM and 1,763.81±12.63 μM/mL by the DPPH and ferric reducing ability of plasma assays, respectively [17]. Another study reported that **1** exhibited the highest giardicidal activity (IC_{50}= 11.33 μg/mL) against *Giardia lamblia* trophozoites among the samples tested [18]. Moreover, **1** enhances alcohol oxidation via increases of alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) activities and gene expressions [19]. It also showed *in vitro* anti-leishmanial activity against promastigotes of *Leishmania donovani* (strain AG 83) and anti-tumour activity on K562 leukemic cell line [20]. It was also reported to exhibit weak antiviral activity against herpes simplex virus (type I and II) [21].

Stigmasterol (**2**) shows therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions [22]. 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 [23]. Other studies reported that stigmasterol showed cytostatic activity against Hep-2 and McCoy cells [24], markedly inhibited tumour promotion in two stage carcinogenesis experiments [25], exhibited antimutagenic [26], topical anti-inflammatory [27], anti-osteoarthritic [28] and antioxidant [29] activities.

β-Sitosterol (**3**) was reported to exhibit growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [30]. It was shown to be effective for the treatment of benign prostatic hyperplasia [31]. It attenuated β-catenin and PCNA expression, as welll as quenched radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis [32]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [33]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [34].

Triglycerides (**4**) exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes* [35]. Another study reported that triglycerides showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation [36]. Linoleic acid which is one of the fatty acids esterified to **4** belongs to the omega-6 fatty acids. It was reported to be a strong anticarcinogen in a number of animal models. It reduces risk of colon and breast cancer [37] and lowers cardiovascular disease risk and inflammations [38]. Linolenic acid which is another fatty acid esterified to **4** belongs to omega-3 fatty acid. A previous study reported that α-linolenic acid (ALA) inhibited the human renal cell carcinoma (RCC) cell proliferation [39]. Another study reported that apoptosis of hepatoma cells was induced by the α-linolenic acid enriched diet which correlated with a decrease in arachidonate content in hepatoma cells and decreased cyclooxygenase-2 expression [40]. γ-Linolenic acid (GLA) and α-linolenic acid (ALA) exhibited greater than 90% cytotoxicity between 500 μM and 1 mM against all but two malignant micro-organ cultures tested in 5-10% serum. GLA and ALA killed tumor at concentrations of 2 mM and above in tests using 30-40% serum [41].

β-Stigmasteryl *3-O*-β-D-glucopyranoside (**5**) was reported as an auxin synergist which promoted markedly the elongation of *Avena* coleoptile segments induced by indole acetic acid (IAA) [42]. β-Sitosteryl and stigmasteryl glucosides were reported as selective DNA polymerase β lyase inhibitors and also potentiators of bleomycin cytotoxicity in the A549 human lung cancer cell line [43]. The antiproliferative test showed growth inhibition of human leukemic cell lines (NB4, HT93A, Kasumi and K562) by a fraction containing β-sitosteryl and stigmasteryl glucosides as major compounds, with IC_{50} values of 22.68, 31.54, 28.88 and 47.72 μg/mL, respectively [44]. Saponin **5** revealed only marginal antifungal activity against R. solani (25%) and P. ultimum (50%) at dose 10 mg /disk [45].

Polyprenols (**6**) act as co-enzymes of membrane active transport systems for polysaccharides, peptidoglycans and carbohydrate containing biopolymers [46]. Polyprenols from *Ginkgo biloba* L showed hepatoprotective effects against CCl_4-induced hepatotoxicity in rats [47] and exhibited antitumor activity [48]. The antidyslipidemic activity of polyprenols from *Coccinia grandis* in high-fat diet-fed hamster model was also reported [49].

Dietary lutein (**7**), especially at 0.002%, inhibited tumor growth by selectively modulating apoptosis, and by inhibiting angiogenesis [50]. 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 [51]. Moreover, very low amounts of dietary lutein (0.002%) can efficiently decrease mammary tumor development and growth in mice [52]. Another study reported that lutein and zeaxanthine reduces the risk of age related macular degeneration [53].

Squalene (**8**) was reported to significantly suppress colonic ACF formation and crypt multiplicity which strengthened the hypothesis that it possesses chemopreventive activity against colon carcinogenesis [54]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [55]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells [56]. The preventive and therapeutic potential of squalene containing compounds on tumour promotion and regression have been reported [57]. A recent review on the bioactivities of squalene has been provided [58].

Chlorophyll a (**9**) and its various derivatives are used in traditional medicine and for therapeutic purposes [59]. Natural chlorophyll and its derivatives have been studied for wound healing [60], anti-inflammatory properties [61], control of calcium oxalate crystals [62], utilization as effective agents in photodynamic cancer therapy [63-65], and chemopreventive effects in humans [66, 67]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided [68].

CONCLUSION

S. macrobotrys is a native Philippine ornamental plant with no reported biological activity. Our chemical investigation of the stems, flowers and leaves of the plant yielded compounds with diverse biological activities. All the compounds (**1**-**9**) isolated from the different parts of the plant were reported to exhibit anticancer properties.

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