

Chemical Constituents of *Cycas sancti-lasallei*

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ABSTRACT

Chemical investigation of the dichloromethane extracts of *Cycas sancti-lasallei*, a plant endemic to the Philippines, led to the isolation of squalene (**1**), β -sitosterol (**2a**), stigmasterol (**2b**), and triglycerides (**3**) from the sarcotesta; **2a**, **2b**, **3**, and phytol fatty acid esters (**4**) from the endotesta; **2a**, **2b**, **3**, and β -sitosteryl fatty acid esters (**5**) from the sclerotesta; and **3** and **5** from the bark. The structures of **1-5** were identified by comparison of their ¹H NMR and/or ¹³C NMR data with those reported in the literature.

INTRODUCTION

Cycas, the only currently known genus of the Family Cycadaceae, are considered as fossil plants though they may have evolved only about 12 million years ago (Nagalingum *et al.*, 2011). The cycads resemble palms in morphology and are commonly called sago palm. These are widely distributed in the Tropics, with species found in Asia, Africa, Southeast Asia, Pacific, and Australia (Donaldson, 2013). They also grow on volcanic, limestone, ultramafic, sandy, or even water-logged soils in grassland and forest habitats (Madulid and Agoo, 2009). In the Philippines, there are eleven cycad species namely, *C. aenigma* K. D. Hill & Lindstrom, *C. curranii* (J. Schust.) K. D. Hill, *C. edentata* de Laub., *C. lacrimans* Lindstrom & K. D. Hill, *C. nitida* K. D. Hill & Lindstrom, *C. riuminiana* Porte ex Regel, *C. saxatilis* K. D. Hill & Lindstrom, *C. sancti-lasallei* Agoo & Madulid, *C. wadei* Merr., *C. vespertilio* Lindstrom & K. D. Hill, and *C. zambalensis* Madulid & Agoo (Madulid and Agoo, 2009; Lindstrom *et al.*, 2008; Agoo and Madulid, 2012). All species,

except for *C. edentata*, are endemic to the Philippines (Lindstrom *et al.*, 2008). *C. revoluta*, a widely cultivated species, is an introduced species from Japan and Taiwan. The demand of *Cycas* species for domestic and international horticultural trade, grassland and forest fires of anthropogenic origin, and conversion of their natural habitats to settlements and other land uses have threatened to varying degrees the wild populations of the genus (IUCN 2010). Some of these species which have been assessed and evaluated in the 2010 IUCN Red List of Threatened Species are *C. curranii* (Agoo *et al.*, 2010), *C. wadei* (Hill, 2010) and *C. zambalensis* as Critically Endangered (CR) (Agoo *et al.*, 2010), *C. riuminiana* as Endangered (E) (Agoo *et al.*, 2010), and *C. saxatilis* as Vulnerable (V) (Bosenberg, 2010). *Cycas sancti-lasallei* (Cycadaceae) is a new species from Mindanao, Philippines (Agoo and Madulid, 2012). There are no reported chemical and biological activity studies on *C. sancti-lasallei*. However, some *Cycas* species have been studied for their chemical constituents and biological activities. The most studied *Cycas* are *Cycas revoluta* Thunb. and *C. circinalis* which contain the carcinogenic toxin cycasin (Nishida *et al.*, 1956; Laqueur *et al.*, 1963). The methanolic extract of the leaflets of *C. circinalis* L. and the chloroform extract of *C. revoluta* Thunb. yielded biflavonoids, lignans, flavan-3-ols, flavone-C-glucosides, nor-isoprenoids, and a flavanone. Three of

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the biflavonoids exhibited moderate activity against *S. aureus* and methicillin-resistant *S. aureus* (Moawad *et al.*, 2010). Further studies on the chemical constituents of the leaves of *C. revoluta* Thunb. and *C. circinalis* L. afforded lariciresinol, naringenin and biflavonoids which are derivatives of amentoflavone and hinokiflavone (Ferreira *et al.*, 2009). Studies on other *Cycas* species have also been conducted. The seeds of *C. micronesica* K. D. Hill yielded β -sitosterol β -D-glucoside, stigmasterol β -D-glucoside, β -sitosterol, and stigmasterol (Marler *et al.*, 2006). *C. beddomei* afforded a new biflavonoid, along with pinoresinol, hinokiflavone, and amento flavones (Das *et al.*, 2006, 2005). The leaves of *C. panzhihuaensis* afforded a new flavone, along with

2,3-dihydrohinokiflavone, a biflavone, vanillic acid, sitosterol and daucosterol (Zhou *et al.*, 2002). The methanolic extracts of the stems, flowers and seeds of *C. panzhihuaensis* L. yielded chavicol β -rutinoside, amentoflavone, podocarpusflavone A, a biflavone, β -sitosterol, daucosterol and palmitic acid (Zhou *et al.*, 2009).

We report herein the isolation and identification of squalene (**1**), β -sitosterol (**2a**), stigmasterol (**2b**), and triglycerides (**3**) from the sarcotesta; **2a**, **2b**, **3**, and phytol fatty acid esters (**4**) from the endotesta; **2a**, **2b**, **3**, and β -sitosteryl fatty acid esters (**5**) from the sclerotesta; and **3** and **5** from the bark (Fig. 1) of *C. sancti-lasallei*. To the best of our knowledge this is the first report on the isolation of these compounds from *C. sancti-lasallei*.

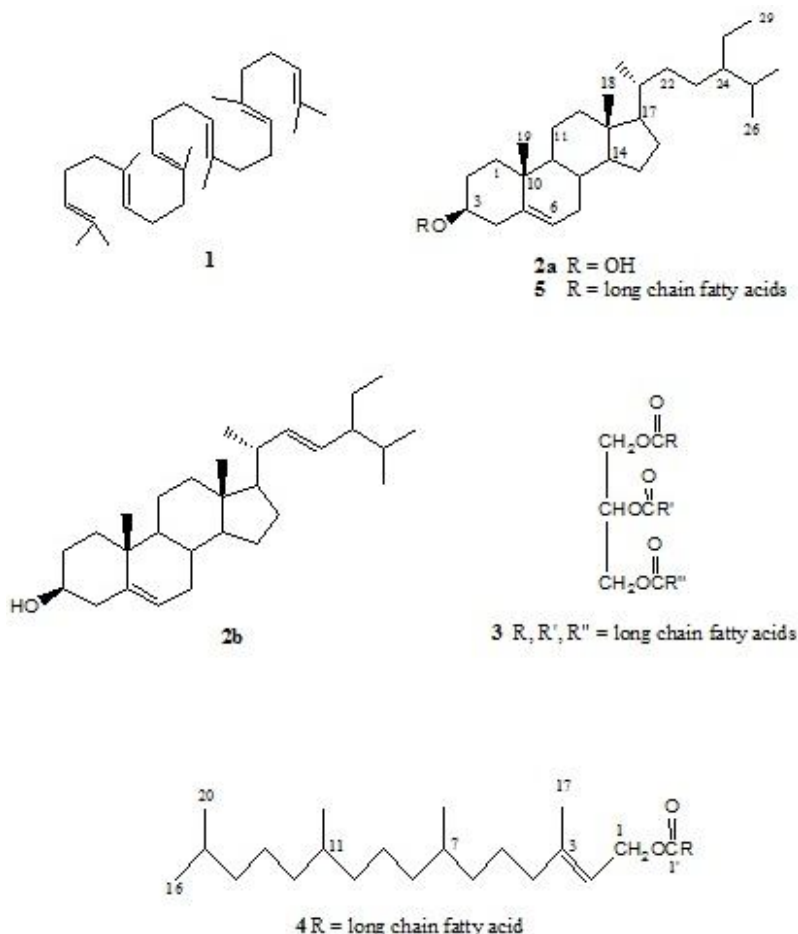


Fig. Chemical constituents of *cycas sancti-lasallei*: squalene (**1**), β -sitosterol (**2a**), stigmasterol (**2b**), triglycerides (**3**), phytol fatty acid esters (**4**), and β -sitosteryl fatty acid esters (**5**).

MATERIALS AND METHODS

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.

Sample Collection

Cycas sancti-lasallei endotesta, sarcotesta, and bark were collected from Malasag, Cugman, Cagayan de Oro, Misamis Oriental, Philippines on April 25, 2014. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH 3116).

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH₂Cl₂ (10% increment) as eluents. Fifty 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. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation

The freeze-dried sarcotesta of *C. sancti-lasallei* (218 g) was ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (0.7 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment. The CH₂Cl₂ fraction was rechromatographed (3 ×) using 1% EtOAc in petroleum ether to afford **1** (2 mg). The 20% acetone in CH₂Cl₂ fraction was rechromatographed (4 ×) in 7.5% EtOAc using petroleum ether to afford **3** (7 mg). The 40% acetone in CH₂Cl₂ fraction was rechromatographed (2 ×) using 20% EtOAc in petroleum ether to afford a mixture of **2a** and **2b** after washing with petroleum ether (5 mg).

The freeze-dried endotesta of *C. sancti-lasallei* (273.5 g) was ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (11.3 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment. The CH₂Cl₂ fraction was rechromatographed (4 ×) using 2.5% EtOAc in petroleum ether to afford **4** (2mg). The 20% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to afford **3** (9mg). The 40% acetone in CH₂Cl₂

fraction was rechromatographed (2 ×) using 20% EtOAc in petroleum ether to afford a mixture of **2a** and **2b** after washing with petroleum ether (6 mg).

The air-dried sclerotesta of *C. sancti-lasallei* (166 g) was ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (0.2 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment. The CH₂Cl₂ fraction was rechromatographed (3 ×) using 5% EtOAc in petroleum ether to afford **5** (4 mg). The 20% acetone in CH₂Cl₂ fraction was rechromatographed (4 ×) using 7.5% EtOAc in petroleum ether to afford **3** (7 mg). The 40% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) using 20% EtOAc in petroleum ether to afford a mixture of **2a** and **2b** (6 mg) after washing with petroleum ether.

The air-dried bark of *C. sancti-lasallei* (98 g) was ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (1.0 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment. The CH₂Cl₂ fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to afford **5** (5 mg). The 20% acetone in CH₂Cl₂ fraction was rechromatographed (4 ×) using 7.5% EtOAc in petroleum ether to afford **3** (8 mg).

Squalene (**1**)

colorless oil. ¹H NMR (600 MHz, CDCl₃): δ 5.08-5.13 (6H, =CH), 1.58 (18H, allylic CH₃, *cis*), 1.66 (6H, allylic CH₃, *trans*), 1.94-2.08 (20H, allylic CH₂).

β-Sitosterol (**2a**)

colorless solid. ¹³C NMR (150 MHz, CDCl₃): δ 37.24 (C-1), 31.66 (C-2), 71.81 (C-3), 42.31 (C-4), 140.75 (C-5), 121.72 (C-6), 31.90, 31.89 (C-7, C-8), 50.12 (C-9), 36.14 (C-10), 21.07 (C-11), 39.76 (C-12), 42.31 (C-13), 56.75 (C-14), 24.30 (C-15), 28.24 (C-16), 56.04 (C-17), 11.85 (C-18), 19.39 (C-19), 36.49 (C-20), 19.02 (C-21), 33.93 (C-22), 29.13 (C-23), 45.82 (C-24), 26.04 (C-25), 18.77 (C-26), 19.81 (C-27), 23.05 (C-28), 11.97 (C-29).

Stigmasterol (**2b**)

colorless solid. ¹³C NMR (150 MHz, CDCl₃): δ 37.24 (C-1), 31.66 (C-2), 71.81 (C-3), 42.30 (C-4), 140.75 (C-5), 121.72 (C-6), 31.89, 31.90 (C-7, C-8), 50.12 (C-9), 36.49 (C-10), 21.07 (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), 21.07 (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).

Triacylglycerols (**3**)

colorless oil. ¹H NMR (600 MHz, CDCl₃): δ 4.27 (dd, *J* = 4.2, 12.0 Hz, glyceryl CH₂O), 4.12 (dd, *J* = 6.0, 12.0 Hz, glyceryl CH₂O), 5.24 (m, glyceryl CHO), 2.29 (t, *J* = 7.2 Hz, α-CH₂), 5.35 (m, olefinic H), 2.75 (t, *J* = 6.6 Hz, double allylic CH₂), 1.97-2.04 (allylic, CH₂), 1.56-1.60 (β-CH₂), 1.23-1.35 (CH₂), 0.96

(t, $J = 7.2$ Hz, CH_3), 0.86 (t, $J = 6.6$ Hz, CH_3), 0.87 (t, $J = 6.6$ Hz, CH_3); ^{13}C NMR (150 MHz, CDCl_3): δ 62.08 (glyceryl CH_2), 68.85 (glyceryl CH), 173.30, 173.26 ($\text{C}=\text{O}$ α), 172.85 ($\text{C}=\text{O}$ β), 34.01, 34.04, 34.18 (C-2), 19.20, 19.27, 22.56, 22.67, 24.83, 24.85, 24.87, 25.61, 27.16, 27.19, 27.21, 29.04, 29.08, 29.11, 29.12, 29.17, 29.19, 29.27, 29.31, 29.34, 29.35, 29.47, 29.52, 29.60, 29.62, 29.65, 29.70, 29.76, 31.51, 31.90, 31.91 (CH_2), 130.23, 130.02, 130.01, 129.98, 129.71, 129.68, 128.07, 128.06, 127.89, 129.88 ($\text{CH}=\text{CH}$), 14.07, 14.11 (terminal CH_3).

Phytol fatty acid ester (4)

colorless oil. δ 4.57 (d, $J = 6.6$ Hz, H_2 -1), 5.34 (H-2), 2.00 (H_2 -4), 0.87 (d, $J = 6.6$ Hz, CH_3 -16), 1.67 (br s, CH_3 -17), 0.85 (d, $J = 6.6$ Hz, CH_3 -18), 0.83 (d, $J = 6.6$ Hz, CH_3 -19), 0.87 (d, $J = 6.6$ Hz, CH_3 -20), 2.27 (t, $J = 7.8$ Hz, H_2 -2'), 1.60 (H_3 -3'), 1.23-1.36 (CH_2)_n, 0.87 (t, $J = 6.6$ Hz, CH_3 -terminal).

β -Sitosteryl fatty acid esters (5)

colorless solid. ^{13}C NMR (150 MHz, CDCl_3): δ 36.99 (C-1), 31.52 (C-2), 73.68 (C-3), 42.30 (C-4), 139.71 (C-5), 122.58 (C-6), 32.19, 31.92 (C-7, C-8), 50.01 (C-9), 36.15 (C-10), 21.02 (C-11), 39.71 (C-12), 42.30 (C-13), 56.68 (C-14), 24.29 (C-15), 28.24 (C-16), 56.01 (C-17), 11.84 (C-18), 19.32 (C-19), 36.59 (C-20), 19.02 (C-21), 34.05 (C-22), 29.13 (C-23), 45.82 (C-24), 26.04 (C-25), 18.76 (C-26), 19.81 (C-27), 23.05 (C-28), 11.97 (C-29), 173.30 (C-1'), 34.70, 34.05 (C-2'), 29.76, 29.70, 29.65, 29.59, 29.52, 29.48, 29.36, 29.34, 29.32, 29.27, 29.16, 29.11, 29.08, 27.80, 27.21, 27.193, 27.186, 25.62, 25.04, 22.69, 22.57 (CH_2), 130.21 ($\text{CH}=\text{CH}$), 130.06 ($\text{CH}=\text{CH}$), 14.12, 14.07 (terminal CH_3).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *Cycas sancti-lasallei* led to the isolation of squalene (1) (Ragasa *et al.*, 2014a), β -sitosterol (2a) (Tsai *et al.*, 2012), stigmasterol (2b) (Ragasa *et al.*, 2014b), and triglycerides (3) (Ragasa *et al.*, 2014c) from the sarcotesta; 2a, 2b, 3, and phytol fatty acid esters (4) (Ragasa *et al.*, 2014c) from the endotesta; 2a, 2b, 3, and β -sitosteryl fatty acid esters (5) (Julien-Davidet *et al.*, 2008) from the sclerotesta; and 3 and 5 from the bark. The structures of 1-5 were identified by comparison of their ^1H NMR and/or ^{13}C NMR data with those reported in the literature. The ratios of the mixture of 2a and 2b were deduced from the integrations of the ^1H NMR resonances for the olefinic protons of 2a at δ 5.33 (dd, $J = 1.8, 4.8$ Hz, H-6) and 2b 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). Based on these integrations, the ratios of 2a and 2b in the sarcotesta, endotesta, and sclerotesta are 3:1, 5:1, and 2:1, respectively.

The fatty acids esterified to the glycerol in the triglycerides of endotesta are oleic acid, linoleic acid and saturated fatty acid. These were deduced from the integrations of the resonances at δ 0.87 (t, $J = 6.6$ Hz, CH_3), 1.97-2.06 (allylic CH_2), 2.74 (double allylic CH_2), and 5.30-5.37 ($\text{CH}=\text{CH}$) for the linoleic acid; δ 0.87 (t, $J = 6.6$ Hz, CH_3), 1.97-2.06 (allylic CH_2), and 5.30-

5.37 ($\text{CH}=\text{CH}$) for the oleic acid; and δ 0.87 (t, $J = 6.6$ Hz, CH_3) for the saturated fatty acid. A small amount of linolenic acid was detected from the low intensity resonance at δ 0.95 (t, $J = 7.2$ Hz) for the terminal methyl and 2.76 (double allylic CH_2 's) of this fatty acid (Human Metabolome, 2013). For the sarcotesta and sclerotesta triglycerides, the same fatty acids esterified to the glycerol were deduced from the integrations in the ^1H NMR spectra. However, larger amounts of linolenic acid were detected from the more intense resonances of the terminal methyl at δ 0.95 (t, $J = 7.2$ Hz) and the double allylic methylenes at δ 2.76.

Although bioassays were not conducted on the isolated compounds (1-3), there were previous studies that reported on their biological activities.

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 (Rao *et al.*, 1998). It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties (Farvin *et al.*, 2006). A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells (Loganathan *et al.*, 2013). The preventive and therapeutic potential of squalene containing compounds on tumor promotion and regression have been reported (Desai *et al.*, 1996). A recent review on the bioactivities of squalene has been provided (Ronco and De Stéfani, 2013).

β -Sitosterol (2a) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells (Awad *et al.*, 2007). It was shown to be effective for the treatment of benign prostatic hyperplasia (Jayaprakasha *et al.*, 2007). It was also reported to attenuate β -catenin and PCNA expression, as well as quench radical *in vitro*, making it a potential anticancer drug for colon carcinogenesis (Baskar *et al.*, 2010). It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake (Jesch *et al.*, 2009). It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells (Moon *et al.*, 2007). Stigmasterol (2b) shows therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions (Ghosh *et al.*, 2011). It lowers plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Wistar as well as WKY rats (Batta *et al.*, 2006). Other studies reported that stigmasterol showed cytostatic activity against Hep-2 and McCoy cells (Gómez *et al.*, 2001), markedly inhibited tumour promotion in two stage carcinogenesis experiments (Kasahara *et al.*, 1994), exhibited antimutagenic (Lim *et al.*, 2005), topical anti-inflammatory (Garcia *et al.*, 1999), anti-osteoarthritic (Gabay *et al.*, 2010) and antioxidant (Panda *et al.*, 2009) activities. Triacylglycerides (3) exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes* (Ragasa *et al.*, 2013). Another study reported that triglycerides showed a direct relationship between

toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation (Ferruzzi and Blakeslee, 2007). The fatty acids esterified to the triglycerides were deduced to be oleic acid, linoleic acid and linolenic acid. Oleic acid has been reported to be responsible for the reduction of blood pressure induced by olive oil (Teres *et al.*, 2008). It may hinder the progression of adrenoleukodystrophy, a fatal disease that affects the brain and adrenal glands (Rizzo *et al.*, 1986). Oleic acid inhibited cancer cell growth and survival in low metastatic carcinoma cells, such as gastric carcinoma SGC7901 and breast carcinoma MCF-7 cell lines (Li *et al.*, 2014). Linoleic acid 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 (Chan *et al.*, 2002) and lowers cardiovascular disease risk and inflammations (Whelan, 2008). Linolenic acid belongs to omega-3 fatty acid. A previous study reported that α -linolenic acid (ALA) inhibited the human renal cell carcinoma (RCC) cell proliferation (Yang *et al.*, 2013). 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 (Vecchini *et al.*, 2004). γ -Linolenic acid (GLA) and α -linolenic acid (ALA) exhibited greater than 90% cytotoxicity between 500 μ M and 1 mM against all but two malignant microorganism cultures tested in 5-10% serum. GLA and ALA killed tumor at concentrations of 2 mM and above in tests using 30-40% serum (Scheim, 2009).

CONCLUSION

The dichloromethane extracts of *Cycas sancti-lasallei*, a plant endemic to the Philippines, afforded squalene (1), β -sitosterol (2a), stigmasterol (2b), triglycerides (3), phytol fatty acid esters (4), and β -sitosteryl fatty acid esters (5). Compounds 1-3 were reported to exhibit diverse biological activities, such as anticancer properties.

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REFERENCES

- Agoo EMG, Madulid DA. *Cycas sancti-lasallei* (Cycadaceae), a new species from the Philippines. *Blumea* 2012; 57:131–133.
- Agoo EMG, Madulid DA, Linis VC, Sambale E, In: IUCN 2010. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 16 December 2013.
- Awad AB, Chinnman M, Fink CS, Bradford PG. β -Sitosterol activates Fas signaling in human breast cancer cells. *Phytomed* 2007;14:747–754.
- Baskar AA, Ignacimuthu S, Paulraj G, Numair K. Chemopreventive potential of β -sitosterol in experimental colon cancer model-an *in vitro* and *in vivo* study. *BMC Comp Alt Med* 2010; 10:24.

- Batta AK, Xu G, Honda A, Miyazaki T, Salen G. Stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the rat. *Metabolism* 2006; 55(3):292–299.
- Bosenberg JD. 2010. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 16 December 2013.
- Chan P, Thomas GN, Tomlinson B. Protective effects of trilinolein extracted from *Panax notoginseng* against cardiovascular disease. *Acta Pharmacol Sin* 2002; 23(12):1157–1162.
- Das B, Mahender G, Rao YK, Prabhakar A, Jagadeesh B. Biflavonoids from *Cycas beddomei*. *Chem Pharm Bull* 2005; 53:135–136.
- Das B, Mahender G, Rao YK, Thirupathi P. A new biflavonoid from *Cycas beddomei*. *Indian J Chem Sec B* 2006; 45B:1933–1935.
- Desai KN, Wei H, Lamartiniere CA. The preventive and therapeutic potential of the squalene-containing compound, Roindex, on tumor promotion and regression. *Cancer Lett* 1996; 101:93–96.
- Donaldson JS. Cycads. Status survey and conservation action plan. IUCN Gland, Switzerland and Cambridge, U.K.; 2003.
- Farvin KHS, Anandan R, Hari S, Kumar S, Shing KS, Mathew S, Sankar TV, Nair PGV. Cardioprotective effect of squalene on lipid profile in isoprenaline-induced myocardial infarction in rats. *J Med Food* 2006; 9(4):531–536.
- Ferreira D, Zjawiony JK, Moawad A, Hifnawy M, Hetta M. Chemical investigation of two species of the family Cycadaceae. *Planta Med* 2009; 75:P-53.
- Ferruzzi MG, Blakeslee J. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutr Res* 2007; 27:1–12.
- Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G. Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. *Osteoarthritis Cartilage* 2010; 18(1):106–116.
- García MD, Sáenz MT, Gómez MA, Fernández MA. Topical anti-inflammatory activity of phytosterols isolated from *Eryngium foetidum* on chronic and acute inflammation models. *Phytother Res* 1999; 13(1):78–80.
- Ghosh T, Maity TK, Singh J. Evaluation of antitumor activity of stigmasterol, a constituent isolated from *Bacopa monnieri* Linn aerial parts against ehrlich ascites carcinoma in mice. *Orient Pharm Exp Med* 2011; 11:41–49.
- Gómez MA, García MD, Sáenz MT. Cytostatic activity of *Achillea ageratum* L. *Phytother Res* 2001; 15(7):633–634.
- Hill KD. 2010. *Cycas wadei*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 26 December 2013.
- Human Metabolome Database. Linolenic acid. Downloaded from http://www.hmdb.ca/spectra/nmr_one_d/1471 on Nov. 27, 2013.
- Jayaprakasha GK, Mandadi KK, Poulse SM, Jadegoud Y, Gowda GA, Patil BS. Inhibition of colon cancer growth and antioxidant activity of bioactive compounds from *Poncirus trifoliata* (L.) Raf. *Bioorg Med Chem* 2007; 15:4923–4932.
- Jesch ED, Seo JM, Carr TP, Lee JY. Sitosterol reduces messenger RNA and protein expression levels of Niemann-Pick C1-like 1 in FHs 74 Int cells. *Nutr Res* 2009; 29(12):859–66.
- Julien-David D, Geoffroy P, Marchioni E, Raul F, Aoud'e-Werner D, Miesch M. Synthesis of highly pure oxyphytosterols and (oxy)phytosterol esters Part II. (Oxy)-sitosterol esters derived from oleic acid and from 9,10-dihydroxystearic acid. *Steroids* 2008; 73:1098–1109.
- Kasahara Y, Kumaki K, Katagiri S, Yasukawa K, Yamanouchi S, Takido M. Carthami flos extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis. *Phytother Res* 1994; 8(6):327–331.
- Laqueur GL, Mickelsen O, Whiting MG, Kurlat LT. Carcinogenic properties of nuts from *Cycas Circinalis* L. indigenous to Guam. *J Natl Cancer Inst* 1963; 31:919–951.
- Li S, Zhou T, Li C, Dai Z, Che D, Yao Y, Li L, Ma J, Yang X, Gao G. High metastatic gastric and breast cancer cells consume oleic acid in an AMPK dependent manner. *PLoS ONE* 2014; 9(5):e97330.

- Lim J-C, Park JH, Budesinsky M, Kasal A, Han Y-H, Koo B-S, Lee S-I, Lee D-U. Antimutagenic constituents from the thorns of *Gleditsia sinensis*. *Chem Pharm Bull* 2005; 53(5):561–564.
- Lindstrom AJ, Hill KD, Stanberg LC. The genus *Cycas* (Cycadaceae) in the Philippines. *Telopea* 2008; 12:119–145.
- Loganathan R, Selvaduray KR, Nesaretnam K, Radhakrishnan A. Differential and antagonistic effects of palm tocotrienols and other phytonutrients (carotenoids, squalene and coenzyme Q10) on breast cancer cells *in vitro*. *J Oil Palm Res* 2013; 25:208–215.
- Madulid DA, Agoos EMG. Taxonomy and conservation of Philippine cycads. *Blumea* 2009; 54:99–102.
- Marler TA, Lee V, Chung J, Shaw CA. Steryl glucoside concentration declines with *Cycas micronesica* seed age. *Funct Plant Biol* 2006; 33:857–862.
- Moawad A, Hetta M, Zjawiony JK, Jacob MR, Hifnawy M, Marais JP, Ferreira D. Phytochemical investigation of *Cycas circinalis* and *Cycas revoluta* leaflets: moderately active antibacterial biflavonoids. *Planta Med* 2010; 76:796–802.
- Moon DO, Kyeong Jun L, Yung HC, Gi-Young K. *Int Immunopharmacol* 2007; 7:1044–1053.
- Nagalingum NS, Marshal CR, Quental TB, Tai HS, Little DP, Matthews S. Recent synchronous radiation of a living fossil. *Science* 2011; 334:796–799.
- Nishida K, Kobayashi A, Nagahama T. Cycasin, a new toxic glycoside of *Cycas revoluta* Thunb. I. Isolation and structure of cycasin. *Bull Agric Chem Soc Japan* 1955; 19:77–84.
- Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, anti-oxidative and hypoglycemic effects of stigmaterol, isolated from *Butea monosperma*. *Fitoter* 2009; 80(2):123–126.
- Ragasa CY, Lorena GS, Mandia EH, Raga DD, Shen C-C. Chemical constituents of *Abrus precatorius*. *Amer J Essent Oils Nat Prod* 2013; 1(2):7–10.
- Ragasa CY, Ng VAS, Ebajo Jr V, Fortin D, De Los Reyes MM, Shen C-C. Triterpenes from *Shorea negrosensis*. *Der Pharmacia Lettre* 2014a; 6(6):453–458.
- Ragasa CY, Ebajo Jr V, Ng VAS, De Los Reyes MM, Shen C-C. Chemical Constituents of *Strongylodon macrobotrys*. *Der Pharma Chemica* 2014b; 6(6):366–373.
- Ragasa CY, Caro JL, Lirio LG, Shen C-C. Chemical constituents of *Coix lacryma-jobi*. *Res J Pharm Biol Chem Sci* 2014c; 5(6):344–348.
- Rao CV, Mark HLN, Reddy RS. Chemopreventive effect of squalene on colon cancer. *Carcinogenesis* 1998; 19:287–290.
- Rizzo WB, Watkins PA, Phillips MW, Cranin D, Campbell B, Avigan J. Adrenoleukodystrophy: oleic acid lowers fibroblast saturated C22-26 fatty acids. *Neurology* 1986; 36(3):357–61.
- Ronco AL, De Stéfani E. Squalene: a multi-task link in the crossroads of cancer and aging. *Functional Foods in Health and Disease* 2013; 3:462–476.
- Schein DE. Cytotoxicity of unsaturated fatty acids in fresh human tumor explants: concentration thresholds and implications for clinical efficacy. *Lipids in Health and Disease* 2009; 8:54.
- Teres S, Barcelo-Coblijn G, Benet M, Alvarez R, Bressani R, Halver JE, Escriba PV. Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. *Proc Natl Acad Sci* 2008; 105(37):13811–13816.
- Tsai P-W, de Castro-Cruz K, Shen C-C, Ragasa CY. Chemical constituents of *Broussonetia luzonensis*. *Phcog J* 2012; 4(31):1–4.
- Vecchini A, Ceccarelli V, Susta F, Caligiana P, Orvietani P, Binaglia L, Nocentini G, Riccardi C, Calviello G, Palozza P, Maggiano N, Di Nardo P. Dietary α -linolenic acid reduces COX-2 expression and induces apoptosis of hepatoma cells. *J Lipid Res* 2004; 45:308–316.
- Whelan J. The health implications of changing linoleic acid intakes. *Prostaglandins, Leukot Essent Fatty Acids* 2008; 79(3-5):165–167.
- Yang L, Yuan J, Liu L, Shi C, Wang L, Tian F, Liu F, Wang H, Shao C, Zhang Q, Chen Z, Qin W, Wen W. α -Linolenic acid inhibits human renal cell carcinoma cell proliferation through PPAR- γ activation and COX-2 inhibition. *Oncol Lett* 2013; 197–202.
- Zhou Y, Peng S-L, Li C-L, Wang M-K, Ding L-S. A new C-glucosylflavone from the leaves of *Cycas panzhihuaensis*. *Acta Bot Sin* 2002; 44:101–103.
- Zhou Y, Zhang X, Jiang S, Li C, Peng S. Chemical constituents of *Cycas panzhihuaensis*. *Chin J Appl Environ Biol* 2009; 5:367–370.

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