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Secondary Metabolites from *Cycas edentata*

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Abstract

Objective:
The study was undertaken to isolate the chemical constituents of the dichloromethane extracts of the sarcotesta, endotesta, leaflets and male cone of *Cycas edentata.*

Methods:
The compounds were isolated by silica gel chromatography and identified by nuclear magnetic resonance spectroscopy.

Results:
The dichloromethane extracts of *C. edentata* afforded β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**) from the sarcotesta; β-sitosteryl fatty acid ester (**2**), unsaturated fatty acid methyl esters (**3**), and a mixture of β-sitosterol (**4a**) and stigmasterol (**4b**) in about 5:1 ratio from the endotesta; chlorophyll a (**5**) from the leaflets; and triacylglycerols (**6**) from the male cone.The structures of **1–6** were identified by comparison of their NMR data with those reported in the literature.

Conclusion:
The different parts of *C. edentata* yielded as a major constituent β-sitosterol which was also found in other *Cycas* species. β-Sitosteryl fatty acid ester isolated from the endotesta was also reported from the bark and sclerotesta.

Keywords: *Cycas edentata*, Cycadaceae, β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate, β-sitosteryl fatty acid ester, β-sitosterol

INTRODUCTION

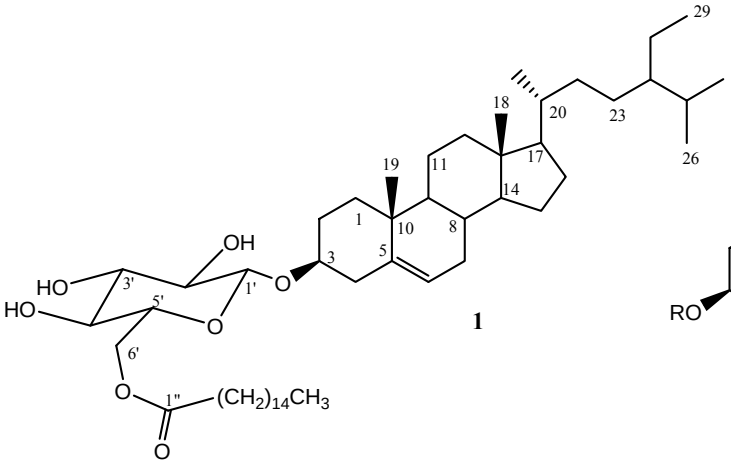
Cycas edentata of the Cycadaceae family is classified as near threatened by the IUCN [1]. This species which accurs along shorelines is declining due to loss of coastal habitat. *C. edentata* is native to Indonesia, Malaysia, Myanmar, Philippines, Thailand, and Vietnam [1]. Recently, we reported the isolation of **2** together with a mixture of **4a** and **4b** from the bark and sclerotesta of *C. edentata*. The bark also yielded 9αH-isopimara-7,15-diene (**7**) [2].

This study was conducted as part of our research on the chemical constituents of *Cycas* species that are endemic and native to the Philippines. We recently reported the isolation of **4a**, **4b**, **6**, and squalene (**8**) from the sarcotesta; **4a**, **4b**, **6** and phytyl fatty acid esters (**9**) from the endotesta; **2**, **4a**, **4b**, and **6** from the sclerotesta; and **2** from the bark of *Cycas sancti-lasallei* [3]. Another *Cycas* species, *C. lacrimans* yielded isopimaran-19-ol (**10**) from the megasporophyll lamina; **6** and **7** from the bark; **6**, oleic acid (**11**), and 1,2-dioleylglycerol (**12**) from the leaflets; **4a**, **4b** and **6** from the petiole and rachis; **4a** from the roots; and **4a** and **6** from the endotesta and sclerotesta [4]. Chemical investigation of *C. vespertilio* afforded **6**, pinoresinol (**13**), sesamin (**14**), and paulownin (**15**), and a mixture of **4a** and **4b** from the cone base; **4a**, **4b**, **6**, **13**, **15**, and lariciresinol (**16**) from the cataphylls; **4a** from the megasporophyll lamina; **4a** and a mixture of trans-4-hydroxycinnamate fatty acid esters (**17**) and cis-4-hydroxycinnamate fatty acid esters (**18**) from the unripe sarcotesta; and **4a** and **6** from the ripe sarcotesta [5]. Furthermore, *C. vespertilio* male cone yielded **6**, **13**, **16**, mixtures of **4a** and **4b** in a 2:1 ratio, and α-amyrin acetate (**19**) and lupeol acetate (**20**) in a 2.5:1 ratio, and fatty alcohols (**21**) [6]. Another study on *C. vespertilio* led to the isolation of **4a**, **4b**, **7**, and **8** from the bark; **4a**, **8**, and **9** from the petiole and rachis; **4a**, **4b**, **6** and **9** from the endotesta; **4a**, **6**, **7**, **8**, and adianenone (**22**) from the roots; **4a**, **4b**, **5** and **6** from the leaflets; and **4a** and **6** from the sclerotesta [7]. Another endemic Philippine *Cycas* is *C. aenigma* which yielded a rare neolignan, 2-[2-hydroxy-5-(3-hydroxypropyl)-3-methoxyphenyl]-1-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol (**23**) together with **13** and **21** from the leaflets; and **6** and a mixture of **4a** and **4b** from the petiole and rachis [8]. On the other hand, *C. zambalensis* afforded dihydrodehydrodiconiferyl alcohol (**24**), **8**, β-carotene (**25**), **5** and lutein (**26**) from the leaflets; **4a**, **8**, **26**, and balanophonin (**27**) from the petiole and rachis; **4a**, **10**, and 3-oxoisopimara-7,15-diene (**28**) from the bark; **4a**, **4b**, **8**, and dihydrodehydrodiconiferyl alcohol (**28**), from the roots; **4a**, **8**, **25**, and **26** from the sarcotesta; and **4a** from the endotesta [9].

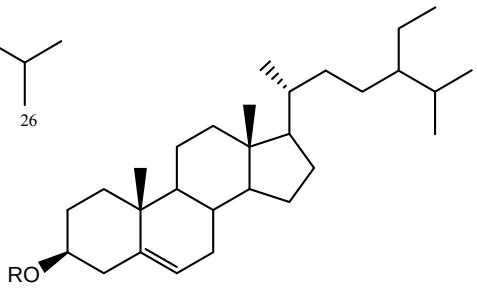
Other *Cycas* species have been studied for their chemical constituents and biological activities. The most studied *Cycas* are *Cycas revoluta* Thunb., also known as Japanese sago palm or king sago and *C. circinalis*. They contain the toxin cycasin, an azoxyglucoside which is carcinogenic [10-11]. The methanolic extract of the leaflets of *C. circinalis* L. afforded (2S,2"S)-2,3,2",3"-tetrahydro-4',4"'-di-*O*-methylamentoflavone (tetrahydroisoginkgetin). This extract and the chloroform extract of *C. revoluta* Thunb. yielded biflavonoids, lignans, flavan-3-ols, flavone-C-glucosides, nor-isoprenoids, and a flavanone. Three of the bioflavonoids exhibited moderate activity against *S. aureus* and methicillin-resistant *S. aureus* [12]. 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. The antimicrobial, antimalarial, and antileishmanial activities of these compounds were tested [13]. Another *Cycas* species that was investigated chemically is *C. beddomei* which afforded a new biflavonoid, 2",3"-dihydrohinokiflavone, along with pinoresinol, hinokiflavone, and amentoflavones [14]. Furthermore, the cones of *C. beddomei* yielded a new biflavonoid, 2,3-dihydro-4"'-O-methyl amentoflavone, along with hinokiflavone and amentoflavones [15]. The leaves of another *Cycas* species, *C. panzhihuaensis* afforded a new flavone, panhihuacycaside along with 2,3-dihydrohinokiflavone, a biflavone, vanillic acid, sitosterol and daucosterol [16]. Moreover, 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 [17]. Furthermore, the seeds of *C. micronesica* K. D. Hill yielded β-sitosterol β-D-glucoside, stigmasterol β-D-glucoside, β-sitosterol, and stigmasterol [18].

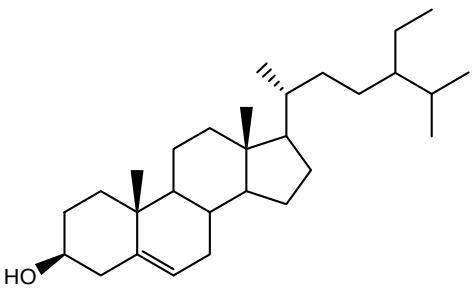
We report herein the isolation of β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**) from the sarcotesta; β-sitosteryl fatty acid ester (**2**),unsaturated fatty acid methyl esters (**3**), a mixture of β-sitosterol (**4a**) and stigmasterol (**4b**) in about 1:5 ratio from the endotesta; chlorophyll a (**5**) from the leaflets; and triacylglycerols (**6**) from the male cone of *C. edentata*. The chemical structures (**1**-**6**) are presented in Fig. 1.



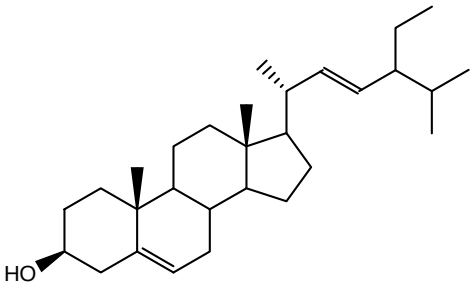
1



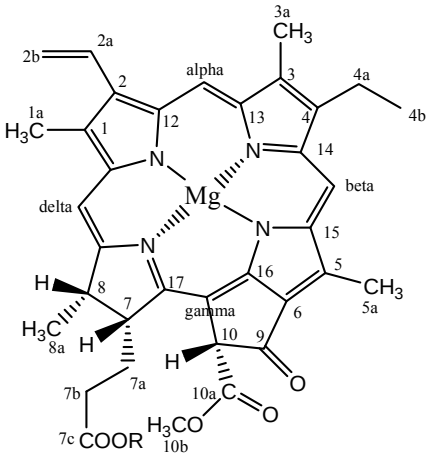
2 R = long chain fatty acids



4a



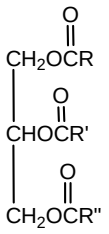
4b



5 R = phytyl

$CH_3(CH_2)nCOOCH_3$

3 R = long chain fatty acids



6 R, R', R" = long chain fatty acids

Fig. 1. Chemical structures of β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**), β-sitosteryl fatty acid ester (**2**), fatty acid methyl esters (**3**), β-sitosterol (**4a**), stigmasterol (**4b**), chlorophyll a (**5**), and triacylglycerols (**6**) from *C. edentata*.

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.

Plant material

Cycas edenta bark and sclerotesta were collected in November 2014 and authenticated by one of the authors (EMGA). Voucher specimens were deposited in the De La Salle University-Manila Herbarium (DLSUH 3114).

General isolation procedure

A glass column 18 inches in height and 1.0 inch internal diameter was used for the chromatography of the crude extracts. Twenty 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 of smaller fractions from the first column. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the chemical constituent of the sarcotesta

The air-dried *C. edentata* sarcotesta (178 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 (0.3 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 80% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) in CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5.0 by volume) to yield a mixture of **1** (2 mg) after trituration with petroleum ether.

Isolation of the chemical constituents of the endotesta

The air-dried endotesta of *C. edentata* (85 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 (0.8 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed in 5% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed (2 ×) in 5%EtOAc in petroleum ether to yield **2** (1 mg) after washing with petroleum ether. The more polar fractions were rechromatographed (3 ×) in 7.5% EtOAc in petroleum ether to afford **3** (2 mg). The 60% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) in 20% EtOAc in petroleum ether to yield a mixture of **4a** and **4b** (3 mg) after washing with petroleum ether.

Isolation of the chemical constituent of the leaflets

The air-dried *C. edentata* leaflets (174 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 (5 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) in 20% EtOAc in petroleum ether to yield **5** (6 mg) after washing with petroleum ether, followed by Et_2O.

Isolation of the chemical constituent of the male cone

The air-dried *C. edentata* male cone (105 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 (0.8 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) in 7.5% EtOAc in petroleum ether to yield **6** (5 mg).

β-Sitosteryl-3β-glucopyranoside-6'-O-palmitate (**1**)
^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 29.71 (C-2), 79.53 (C-3), 38.88 (C-4), 140.26 (C-5), 122.19 (C-6), 31.92 (C-7), 31.92 (C-8), 50.15 (C-9), 36.72 (C-10), 21.05 (C-11), 39.74 (C-12), 42.31 (C-13), 56.74 (C-14), 24.28 (C-15), 28.23 (C-16), 56.05 (C-17), 11.84 (C-18), 19.34 (C-19),36.13 (C-20), 18.77 (C-21), 33.92 (C-22), 26.05 (C-23), 45.82 (C-24), 29.12 (C-25), 19.01 (C-26), 19.81 (C-27), 23.05 (C-28), 11.97 (C-29), 101.18 (C-1'), 73.68 (C-2'), 75.89 (C-3'), 69.98 (C-4'),73.97 (C-5'), 63.09 (C-6'), 174.79 (C-1"), 34.21 (C-2"), 24.94 (C-3"), 29.31 (C-4"), 29.51 (C-5"), 29.71 (C-6"), 29.66 (C-7"-C-12"), 29.36 (C-13"), 31.85 (C-14"), 22.69 (C-15"), 14.12 (C-16").

β-Sitosteryl Fatty Acid Esters (**2**):^{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), 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), 24.28 (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), 33.92 (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 (C-2'), 29.76, 29.70, 29.65, 29.58, 29.52, 29.47, 29.44, 29.35, 29.34, 29.32, 29.27, 29.25, 29.16, 29.15, 29.13, 29.10, 29.08, 28.24, 27.80, 27.21, 27.19, 27.18, 27.16, 26.04, 25.62, 25.06, 25.04, 24.96, 24.28, 23.05, 22.71, 22.68, 22.57 (CH_2), 130.21, 130.06, 129.98, 129.76 (CH=), 14.12, 14.07 (terminal CH_3).

Fatty Acid Methyl Esters (**3**):^{13}C NMR (150 MHz, $CDCl_3$): δ14.06, 14.11, 22.56, 22.68, 24.94, 25.00, 25.62, 27.15, 27.18, 27.19, 27.21, 29.08, 29.11, 29.14, 29.25, 29.31, 29.34, 29.35, 29.44, 29.51, 29.58, 29.64, 29.67, 29.69, 29.76, 31.52, 31.90, 31.91, 32.35, 34.10, 34.12, 51.44, 127.90, 128.03, 129.74, 130.00, 130.05, 130.21, 174.34, 174.37.

β-Sitosterol (**4a**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.81 (C-3), 42.31 (C-4), 140.74 (C-5),

121.72 (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).

Stigmasterol (**4b**): ^{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), 121.72 (C-6), 31.89 (C-7, C-8), 50.11 (C-9), 36.49 (C-10), 21.07 (C-11), 39.67 (C-12), 42.20 (C-13), 56.85 (C-14), 24.35 (C-15), 28.91 (C-16), 55.93 (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).

Chlorophyll a (**5**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 131.97 (C-1), 12.11 (C-1a), 136.59 (C-2),129.06 (C-2a), 122.90 (C-2b), 136.59 (C-3), 11.25 (C-3a), 145.15 (C-4), 19.48 (C-4a), 17.38 (C-4b), 137.95 (C-5), 12.11 (C-5a), 129.06 (C-6), 51.17 (C-7), 29.79 (C-7a), 31.18 (C-7b), 172.93 (C-7c), 50.14 (C-8), 23.07 (C-8a), 189.61 (C-9), 64.70 (C-10), 169.56 (C-10a), 52.86 (C-10b), 142.85 (C-11), 136.59 (C-12), 155.66 (C-13), 150.00 (C-14), 129.06 (C-15), 150.00 (C-16), 161.24 (C-17), 172.93 (C-18), 97.53 (C-α), 104.45 (C-β), 105.22 (C-γ), 97.53 (C-δ), 61.46 (P-1), 117.69 (P-2), 142.10 (P-3), 16.27 (P-3a), 39.78 (P-4), 24.97 (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).

Triacylglycerols (**6**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 62.07 (glyceryl CH_2), 68.85 (glyceryl CH), 173.28, 173.23 (C=O α), 172.83, 172.82 (C=O β), 34.00, 34.03, 34.17 (C-2), 24.81, 24.84 (C-3), 29.06 29.03, 29.18, 29.16, 29.10, 29.47 (C4–C-6), 22.56 (C-8), 130.20, 129.98 (C-9), 127.87, 128.06 (C-10), 25.61, 25.50 (C-11), 127.87, 128.06 (C-12), 130.20, 128.06 (C-13), 27.18, 25.61 (C-14), 29.35 (C-15), 127.09 (C-15), 31.52 (C-16), 131.93 (C-16), 22.56, 22.68 (C-17), 14.05, 14.10, 14.26 (C-18).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *C. edentata* yielded **1** from the sarcotesta;**2**–**4b** from the endotesta; **5** from the leaflets; and **6** from the male cone.Compounds **1**–**6** were identified by comparison of their NMR data with those reported in the literature for β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**) [19], β-sitosteryl fatty acid ester (**2**) [4], unsaturated fatty acid methyl esters (**3**) [20], β-sitosterol (**4a**) [21], stigmasterol (**4b**) [21], chlorophyll a (**5**) [22], and triacylglycerols (**6**) [22]. The mixture of **4a** and **4b** in about 5:1 ratio was deduced from the ^{1}H NMR resonances for the olefinic protons of **4a** at δ 5.33 (dd, *J*=1.8, 5.4 Hz, H-6) and **4b** 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).

These results indicate that *C. edentata* shares similar chemical characteristics with other *Cycas* species which contain **4a**, **4b** and **6**. To our knowledge, this is the first report on the occurrence of **1** in the genus *Cycas* and the family Cycadaceae. Thus, **1** may become a chemotaxonomic marker for *Cycas edentata* and could be used to distinguish among *Cycas* species.

CONCLUSION

The different parts of *C. edentata* yielded as a major constituent β-sitosterol (**4a**) which was also found in other *Cycas* species. Other common constituents of *Cycas* species are triacylglycerols (**6**) which are found in the different parts of *C. sancti-lasallei, C. lacrimans, C. aenigma*, and *C.vespetilio.* β-Sitosteryl fatty acid ester (**2**) isolated from the endotesta was also reported from the bark and sclerotesta of *C. edentata* and the bark of *C. sancti-lasallei.*

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REFERENCES

[1] Osborne, R., Hill KD, Nguyen HT, Phan KL. 2010. *Cycas edentata*. The IUCN Red List of Threatened Species. Version 2015.1. <www.iucnredlist.org>. Downloaded on 04 June 2015.
[2] Ragasa, C. Y., Ng, V. A. S., Agoo, E. M., Shen, C,-C., *Int. J. Pharm. Sci. Rev. Res.* 2015, *33*(2).
[3] Ng, V. A. S., Agoo, E. M., Shen, C.-C., Ragasa, C.Y., *J. Appl. Pharm. Sci.* 2015; *5*, 12–17.
[4] Ng, V. A. S., Agoo, E. M., Shen, C.-C., Ragasa, C. Y., *Int. J. Pharmacog. Phytochem. Res.* 2015, *7*, 616-620.
[5] Ragasa, C. Y., Ng, V. A. S., Agoo. E. M., Shen, C.-C., *Braz. J. Pharmacog.* 2015, *25*.
[6] Ng, V. A. S., Agoo, E. M., Shen, C.-C., Ragasa, C. Y., *Int. J. Pharmacog. Phytochem. Res.* 2015, *7*,
[7] Ragasa, C. Y., Ng, V. A. S., Agoo, E. M., Shen, C.-C., *Chem. Nat. Compd.* (*in-press*).
[8] Ng, V. A. S., Agoo, E. M., Shen, C.-C., Ragasa, C. Y., *J. Appl. Pharm. Sci.* 2015, 5,
[9] Ragasa, C. Y., Ng, V. A. S., Agoo, E. M., Shen, C.-C., *Chem. Nat. Compd.* (*in-press*).
[10] K. Nishida, A. Kobayashi, T. Nagahama, *Bull. Agric. Chem. Soc. Japan.* 1956, *20*, 74–76.
[11] Laqueur, G. L., Mickelsen, O., Whiting, M. G., Kurlad, L. T., *J. Natl. Cancer Inst. 31*, 919–951 (1963).
[12] Moawad, A., Hetta, M., Zjawiony, J. K., Jacob, M. R., Hifnawy, M., Marais, J. P., Ferreira, D., *Planta Med*. 2010, *76*, 796–802.
[13] Ferreira, D., Zjawiony, J. K., Moawad, A., Hifnawy, M., Hetta, M. *Planta Med*. 2009, *75*, 53.
[14] Das, B., Mahender, G., Rao, Y. K., Thirupathi, P. *Indian J. Chem.–Sec. B. 45B*, 1933–1935 (2006).
[15] Das, B., Mahender, G., Rao, Y. K., Prabhakar, A., Jagadeesh, B. *Chem. Pharm. Bull.,53*, 135–136 (2005).
[16] Zhou, Y., Peng, S.-L., Li, C.-L., Wang, M.-K., Ding, L.-S. *Acta Botanica Sinica.* 2002, *44*, 101–103.
[17] Zhou, Y., Zhang, X., Jiang, S., Li, C., Peng, S. *Chin. J. Appl. Environ. Biol. 5*, 367–370 (1999).
[18] Marler, T. A., Lee, V., Chung, J., Shaw, C. A. *Functional Plant Biol*. 2006, *33*, 857–862.
[19] Tsai, P.-W., de Castro-Cruz, K., Shen, C.-C., Ragasa, C. Y. *Pharm. Chem. J.* 2012, *46*, 225-227.
[20] Basumatary, S., Deka. D. C., *Der Chemica Sinica.* 2012, *3*, 1384-1393.
[21] Ragasa, C. Y., Caro, J .L., Lirio, L. G., Shen, C.-C. *Res. J. Pharm. Biol. Chem. Sci.* 2014, *5*, 344-348.
[22] Ragasa, C. Y., Ebajo, Jr. V., Ng, V. A. S., De Los Reyes, M. M., Shen, C.-C. *Der Pharma Chemica.* 2014, *6*, 366-373.