Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(4); 668-674

ISSN: 0975-4873

Research Article

Cytotoxic Compounds from *Dysoxylum gaudichaudianum* (A. Juss.) Miq.

Mariquit M. De Los Reyes[1,2*], Glenn G. Oyong[3], Vincent Antonio S. Ng[4], Chien-Chang Shen[5] and Consolacion Y. Ragasa[4,6]

[1]*Biology Department, De La Salle University Science & Technology Complex, Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines*
[2]*Biology Department, De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines*
[3]*Center for Natural Science and Environmental Research, De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines*
[4]*Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines*
[5]*National Research Institute of Chinese Medicine, Ministry of Health and Welfare, 155-1, Li-Nong St., Sec. 2, Taipei 112, Taiwan*
[6]*Chemistry Department, De La Salle University Science & Technology Complex, Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines*

Available Online: 31st March, 2016

ABSTRACT

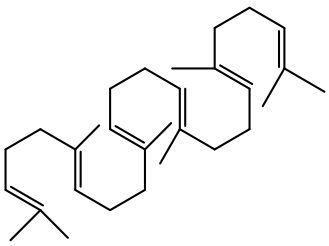
Squalene (**1**), polyprenol (**2**), triglycerides (**3**), and β-sitosterol (**4**), isolated from the dichloromethane extract of the leaves of *Dysoxylum gaudichaudianum*, were evaluated for their anti-proliferative activities against three human cancer cell lines, breast (MCF-7) and colon (HT-29 and HCT-116), and a normal cell line, human dermal fibroblast neonatal (HDFn) using the *in vitro* PrestoBlue® cell viability assay. The HT-29 cell line was most susceptible to the compounds tested. Squalene (**1**) and polyprenol (**2**) were most cytotoxic against HT-29 (IC_{50} of 0.84 and 1.059 µg/mL, respectively), followed by MCF-7 (IC_{50} of 4.404 and 3.786 µg/mL, respectively), and HCT-116 (IC_{50} of 7.859 and 9.258 µg/mL, respectively). Triglycerides (**3**) was highly anti-proliferative against HT-29 cells (IC_{50} of 2.738 µg/mL) and moderately inhibitory against HCT-116 and MCF-7 cells (IC_{50} of 13.05 and 16.12 µg/mL, respectively). β-Sitosterol (**4**) was most cytotoxic against HT-29 and MCF-7 (IC_{50} of 1.41 and 2.277 µg/mL, respectively) and moderately cytotoxic against HCT-116 (IC_{50} of 11.63 µg/mL). Comparing the cytotoxic effects of these compounds against the three cancer cell lines, **1**-**4** were most cytotoxic against HT-29. Compounds **1**, **2** and **4** were less inhibitory to MCF-7 and least inhibitory to HCT-116. Comparing the effects of **1**-**4** on the two colon cancer cell lines, their IC_{50} values against HT-29 were lower than those of HCT-116. Compounds **1**-**4** showed moderate cytotoxicity against HDFn cells (11.18 to 17.72 µg/mL) and were less cytotoxic to the normal cells than the cancer cells.

Keywords: *Dysoxylum gaudichaudianum* (A. Juss.) Miq., squalene, polyprenol, triglycerides, β-sitosterol, cytotoxicity, MCF-7, HCT-116, HT-29, HDFn

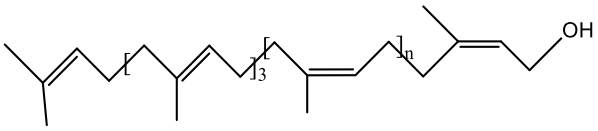
INTRODUCTION

Dysoxylum gaudichaudianum (A. Juss.) Miq. of the family Meliaceae may be found in thickets and primary and secondary lowland forests up to 1,800 m asl in many parts of the Philippines where it is locally known as "igyo", and in other Malesian regions down to Samoa[1]. Commonly known as ivory mahogany, the mature plant may be used as a general purpose timber[2]. In traditional folk medicine, decoction of chopped leaves and bark are used to treat coughs, skin irritations, body aches, lung hemorrhage, and sexually transmitted diseases and are also used to induce vomiting after food poisoning[3,4]. In addition, it was reported that the juice extracted from the leaves may be used as an early abortifacient[5]. In another study, the plant's bark yielded four new compounds, dysoxylins A−D, belonging to the tetranortriterpenoid family, which demonstrated strong bioactivity against anti-respiratory syncytial virus (RSV)[6,7]. Structure elucidation of isolates from hexane and choloroform extracts of the plant's stem bark yielded *p*-hydroxyacetophenone, β-sitosterol, and stigmasterol[8]. A new limonoid, gaudichaudysolin A, isolated from the plant's bark, was evaluated *in vitro* for its cytotoxic effects against five human cancer cell lines: HL60 (human blood premyelocytic leukemia), RPMI8226 (multiple myeloma), NCI-H226 (non-small cell lung carcinoma), HCT116 (human colon cancer), and MCF7 (human breast adenocarcinoma) cells[9]. This study was conducted as part of our research on the chemical constituents of the genus *Dysoxylum* found in the Philippines. We earlier reported the isolation of new glabretal-type triterpenoids along with the known

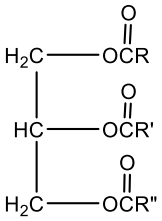
**Author for Correspondence*



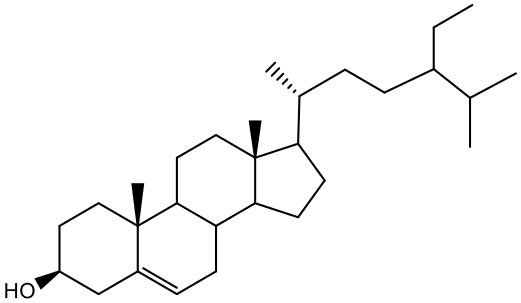
1



2



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



4

Figure 1: Chemical structures of squalene (**1**), polyprenols (**2**), triglycerides (**3**), and β-Sitosterol (**4**) from the leaves of *D. gaudichaudianum.*

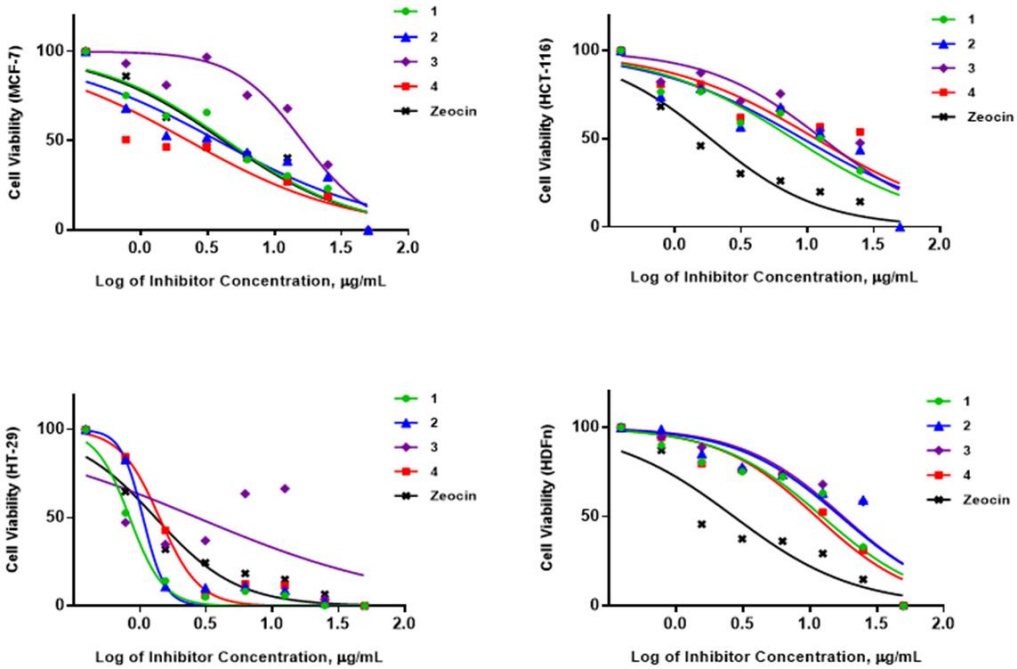


Figure 2: Dose-response curves showing the cytotoxic activities of **1-4** and Zeocin on the cell viability of MCF-7, HCT-116, HT-29 and HDFn. Each plot shows the effect of **1-4** and Zeocin against each cell line. Data shown are means of triplicates. GraphPad Prism 6.07 was used to perform extra sum-of-squares F test to (A) evaluate if the best-fit parameter (half maximal inhibitory concentration) differs among data sets (treatments), and (B) determine the differences among the dose-response curve fits. The results are: MCF-7 (A) F (DFn, DFd) = F (5, 124) = 3.864, p = 0.0027 and (B) F (10, 124) = 5.384, p < 0.0001; HCT-116 (A) F (5, 116) = 9.586, p < 0.0001 and (B) F (10, 116) = 5.080, p < 0.0001; HT-29 (A) F (5, 124) = 5.644, p = 0.0001 and (B) F (10, 124) = 6.194, p < 0.0001; HDFn (A) F (5, 124) = 11.35, p < 0.0001 and (B) F (10, 124) = 6.025, p < 0.0001.

compounds, 24,25-epoxy-3β,23-dihydroxy-7-tirucallene, squalene, polyprenol, linoleic acid and lutein from the leaves of *Dysoxylum mollissimum* Blume[10]. Recently, we reported that the crude dicloromethane leaf extract of *Dysoxylum gaudichaudianum* (A. Juss.) Miq. exhibited IC_{50} values of 7.35 and 13.19 µg/mL against breast cancer (MCF-7) and colon cancer (HT-29) cells, respectively. Chemical investigation of the dichloromethane leaf extract led to the isolation of squalene (**1**), polyprenols (**2**), triglycerides (**3**), and β-sitosterol (**4**)[11]. We report herein

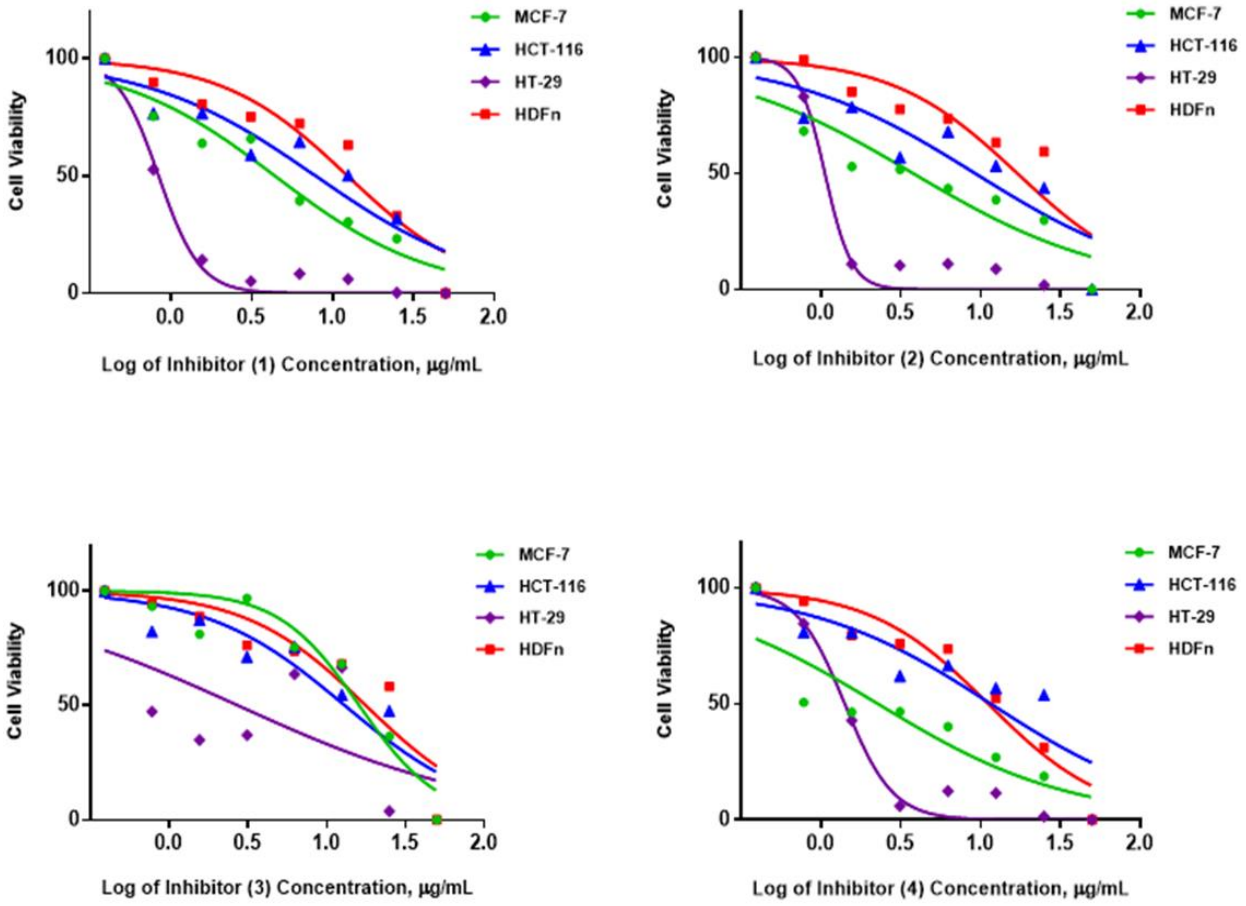


Figure 3: Dose-response curves showing the cytotoxic activities of **1-4** and Zeocin on the cell viability of MCF-7, HCT-116, HT-29 and HDFn. Each plot shows the effect of a compound against all cell lines tested. Data shown are means of triplicates. GraphPad Prism 6.07 was used to perform extra sum-of-squares F test to (A) evaluate if the best-fit parameter (half maximal inhibitory concentration) differs among data sets (treatments), and (B) determine the differences among the dose-response curve fits. The results are: compound **1** (A) F (DFn, DFd) = F (3, 88) = 39.06, p < 0.0001 and (B) F (6, 88) = 19.64, p < 0.0001; compound **2** (A) F (3, 80) = 30.83, p < 0.0001 and (B) F (6, 80) = 15.61, p < 0.0001; compound **3** (A) F (3, 88) = 3.711, p = 0.0145 and (B) F (6, 88) = 3.797, p = 0.0021; compound **4** (A) F (3, 88) = 10.27, p < 0.0001 and (B) F (6, 88) = 5.419, p < 0.0001.

the cytotoxicity studies on **1-4** (Figure 1) isolated from the leaves of *D. gaudichaudianum*. To the best of our knowledge this is the first report on the IC_{50} values of **2** and **3** against breast and colon cancers.

MATERIALS AND METHODS

Sample Collection

Samples of leaves and twigs of *Dysoxylum gaudichaudianum* (A. Juss.) Miq. were collected from the De La Salle University – Science and Technology Complex (DLSU-STC) riparian forest in February 2014. The samples were authenticated and deposited at the De La Salle University Herbarium with voucher specimen #920.

Isolation and Structure Elucidation

The isolation and structure elucidation of **1-4** from the leaves of *D. gaudichaudianum* were reported previously[11].

Preparation of Compounds for Cytotoxicity Tests

The compounds (**1-4**) from *D. gaudichaudianum* were dissolved in dimethyl sulfoxide (DMSO) to make a 4 mg/mL stock solution. Working solutions were prepared in complete growth medium to a final non-toxic DMSO concentration of 0.1%.

Maintenance and Preparation of Cell Lines for Cytotoxicity Tests

The cytotoxicity of **1-4** from the dichloromethane (CH_2Cl_2) extracts from *D. gaudichaudianum* was tested on the following human cell lines: breast cancer (MCF-7), colon cancer (HCT-116 and HT-29; ATCC, Manassas, Virginia, U.S.A.), and human dermal fibroblast, neonatal (HDFn; Invitrogen Life Technologies, U.S.A.), which are routinely maintained at the Cell and Tissue Culture Laboratory, Molecular Science Unit, Center for Natural Science and Environmental Research, De La Salle University, Manila, Philippines. Following standard procedures[12,13], cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Gibco®, USA) containing 10% fetal bovine serum (FBS, Gibco®, USA) and 1x antibiotic-antimycotic (Gibco®, USA) and kept in an incubator (37°C, 5% CO_2, 98% humidity). At 80% confluence, the monolayers were washed with phosphate-buffered saline (PBS, pH 7.4, Gibco®, USA), trypsinized with 0.05%

Table 1: Cytotoxic activities (IC_{50}) of **1-4** and Zeocin against MCF-7, HCT-116, HT-29 and HDFn.

Compound	IC_{50}*(µg/mL)			
	MCF-7	HCT-116	HT-29	HDFn
1	4.404	7.859	0.84	12.4
2	3.786	9.258	1.059	17.01
3	16.12	13.05	2.738	17.72
4	2.277	11.63	1.41	11.18
Zeocin	4.168	1.856	1.318	2.713

*IC_{50} values were extrapolated from dose-response curves generated from nonlinear regression analysis done using GraphPad Prism 6.05. For each cell line, one-way ANOVA was conducted to determine differences among data sets (treatments). The results are: MCF-7, $F(5, 118) = 35.74$, $p < 0.0001$; HCT-116, $F(5, 110) = 182.4$, $p < 0.0001$; HT-29, $F(5, 118) = 107.1$, $p < 0.0001$; HDFn, $F(5, 118) = 275.3$, $p < 0.0001$. Results of the Tukey's multiple comparison post hoc test are discussed in this section.

Trypsin-EDTA (Gibco®, USA) and resuspended with fresh complete media. Cells were counted following standard trypan blue exclusion method using 0.4% Trypan Blue Solution (Gibco®, USA). Cells were seeded in 100-µL aliquots into 96-well microtiter plates (Falcon™, USA) using a final inoculation density of 1 x 10^4 cells/well. The plates were further incubated overnight (37°C, 5% CO_2, 98% humidity) until complete cell attachment was reached. These cells were used for the cytotoxicity studies as described below.

Cell Viability Assay

The cytotoxicity of the *D. gaudichaudianum* compounds was determined in a cell viability assay using PrestoBlue® (Molecular Probes®, Invitrogen, USA). This test is based on the presence of mitochondrial reductase in viable cells which then reduces the resazurin dye (blue and nonfluorescent) in the reagent to resorufin (red and highly fluorescent). The conversion is proportional to the number of metabolically active cells and is determined quantitatively using absorbance measurements. To the monolayers in the microtiter plate, 100 µL of filter-sterilized **1-4** were added to corresponding wells at two-fold serial dilutions to make final screening concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 µg/mL. The treated cells were further incubated (37°C, 5% CO_2, 98% humidity) for 4 days. Ten microliters of PrestoBlue® was added to each well. The cells were incubated (37°C, 5% CO_2, 98% humidity) for 2 hr. Wells with no compound added served as negative controls while wells with Zeocin™ (Gibco®, USA) served as positive controls. Wells containing cell culture media only were used to correct for background absorbance. Absorbance measurements were carried out using BioTek ELx800 Absorbance Microplate Reader (BioTek® Instruments, Inc., U.S.A.) at 570 nm and normalized to 600 nm values (reference wavelength). Absorbance readings were used to calculate for the cell viability for each sample concentration following the equation below.

$$Cell\ Viability\ (\%) = \frac{(Absorbance\ of\ Treated\ Sample - Absorbance\ of\ Blank)}{(Absorbance\ of\ Negative\ Control - Absorbance\ of\ Blank)}\ x\ 100$$

Nonlinear regression and statistical analyses were done using GraphPad Prism 6.07 (GraphPad Software, Inc.) to extrapolate the half maximal inhibitory concentration, IC_{50} (the concentration of the compound which resulted in a 50% reduction in cell viability). The cytotoxicity of **1-4** was expressed as IC_{50} values. All tests were performed in triplicates and data were shown as means. The extra sum-of-squares F test was used to evaluate the differences in the best-fit parameter (half maximal inhibitory concentration) among data sets (treatments) and to determine the differences among dose-response curve fits according to the software's recommended approach. One-way ANOVA ($p < 0.05$) was also used to determine significant differences among treatments, followed by Tukey's multiple comparison post hoc test ($p < 0.05$), to compare different pairs of data sets. Results were considered significant at $p < 0.05$.

RESULTS AND DISCUSION

This work presents studies on the cytotoxic activities of squalene (**1**), polyprenol (**2**), triglycerides (**3**), and β-sitosterol (**4**), previously isolated from the dichloromethane extracts of *D. gaudichaudianum* leaves[11], against three human cancer cell lines, breast (MCF-7) and colon (HT-29 and HCT-116), and a human normal cell line, human dermal fibroblast, neonatal (HDFn). A known anti-cancer drug, Zeocin, was used as positive control. The % cell viability as a function of the logarithmic values of compound concentration is shown in Figures 2 and 3. Most plots nearly follow the typical sigmoidal curve which is characteristic of an inhibitory dose-response relationship between treatments and cell viability. Figure 2 compares the anti-proliferative effects per cell line, while Figure 3 compares the effects per compound. The corresponding IC_{50} values are summarized in Table 1. The breast cancer cell line (MCF-7) is most susceptible to **4**, **2**, and **1** with IC_{50} values of 2.277, 3.786, and 4.404 µg/mL, respectively, and least susceptible to **3** with an IC_{50} value of 16.12 µg/mL. One-way ANOVA showed that all treatments are statistically different ($p < 0.0001$), but Tukey's multiple comparison post hoc test revealed that there are no pairwise differences between **1** and **2**, **1** and Zeocin, **2** and **4**, and **2** and Zeocin ($p > 0.05$). The colon cancer cell line (HCT-116) is most susceptible to **1** and **2** with IC_{50} values of 7.859 and 9.258 µg/mL, respectively, followed by **4** and **3** with IC_{50} values of 11.63, and 13.05 µg/mL, respectively. One-way ANOVA showed that all treatments are statistically different ($p < 0.0001$), but Tukey's multiple comparison post hoc test showed pairwise differences only between **1** and **4** ($p < 0.0001$), and **2** and **3** ($p < 0.01$). The growth of

the other colon cancer cell line (HT-29) exhibited the strongest inhibition at the lowest concentrations of the compounds, with IC_{50} values of 0.84, 1.059, 1.41, 2.738 µg/mL for **1**, **2**, **4** and **3**, respectively. Tukey's multiple comparison post hoc test showed statistical differences between all treatments except for **4** and Zeocin ($p > 0.05$). The normal cell line, HDFn, responded moderately to all the compounds, with IC_{50} values of 11.18, 12.4, 17.01, and 17.72 µg/mL for **4**, **1**, **2**, and **3**, respectively. The pairs of compounds, **1** and **4**, and **2** and **3** are not statistically different ($p > 0.05$). Data analysis showed statistical differences in the best-fit parameter (half maximal inhibitory concentration) among treatments and among the dose-response curve fits (Figures 2 and 3). Overall, comparing the three human cancer cell lines, HT-29 showed the most cytotoxic response to the compounds tested (IC_{50} value of 0.84 µg/mL for squalene (**1**)). This was followed by MCF-7 cells (IC_{50} values of 2.77 and 3.786 µg/mL for β-sitosterol (**4**) and polyprenol (**2**), respectively). In a preliminary study on the cytotoxic activity of the dichloromethane extract of *D. gaudichaudianum* leaves, the crude extract exhibited cytotoxic activities with IC_{50} values of 13.19 and 7.35 µg/mL for HT-29 and MCF-7 cancer cells, respectively[11]. In the current study, the isolated compounds from the extract exhibited high anti-proliferative activities against the cell lines used, indicating that these compounds may be responsible for the cytotoxicity of the extract. Among the cancer cell lines tested, HCT-116 showed the least response to the compounds. On the other hand, the normal cell line, HDFn, exhibited moderate susceptibility only. The most cytotoxic effect was seen in HT-29 cells using squalene (**1**), while the least anti-proliferative effect was observed in MCF-7 cells treated with triglycerides (**3**). Overall, **1**-**4** showed varying, but promising cytotoxic properties. The US National Cancer Institute has defined the active cytotoxic limits of natural products as 20 µg/mL or less for crude extracts and 4 µg/mL or less for pure compounds[14]. Pure compounds that exhibit active cytotoxicity may have some potential for drug development[13]. The results showed that **1**-**4** from the dichloromethane extracts of *D. gaudichaudianum* leaves can be further developed for the treatment of human colorectal type and human breast cancers. The study also showed that the cytotoxic activity of **1**-**4** was a function of the specific type of cancer cells targeted. When the two colon cancer cell lines are compared, the IC_{50} values of **1**-**4** for HT-29 were lower than HCT-116, implying that the former could be more responsive to anti-cancer treatments using the compounds tested. It was reported that variances in the expression profiles of several genes associated with drug sensitivity between HCT-116 and HT-29 could influence the cells' response to different inhibitory compounds[15]. A related study using four human colon cancer cell lines (HCT-116, HT-29, HCT-15, and KM-12) revealed that gene expression profiling after inhibition of signal transduction by 17-allylamino-17-demethoxygeldanamycin, a known inhibitor of the hsp90 molecular chaperone, could explain how cells respond under different treatment conditions[16]. Previous studies revealed that squalene, polyprenols, triglycerides, and β-sitosterol exhibited cytotoxic and anticancer properties. Squalene (**1**) was reported to exhibit antitumor activities against colon cancer in rodents[17]. It was also found to remarkably control colonic aberrant crypt foci (ACF) formation and crypt multiplicity in laboratory rats which supported the hypothesis that it possesses chemopreventive activity against colon carcinogenesis[18]. In a study evaluating the anti-proliferative effects of tocotrienols, carotenoids, squalene, and coenzyme Q10 from palm oil against two human breast cancer cell lines, MDA-MB-231 and MCF-7, it was found that there was a suppression of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) protein in the breast cancer cells exposed briefly to tumor necrosis factor-alpha (TNF-α)[19,20]. The protective and therapeutic effects of squalene-containing compounds on the promotion of tumor cells have been reported[21]. Relevant reviews on the bioactive properties of squalene have been provided[22,23]. Thus, **1** was reported to exhibit cytotoxic properties against colon and breast cancer cells which corroborate our findings that **1** showed high cytotoxicity against colon cancer cells, HT-29 and HCT-116, and breast cancer cells, MCF-7, with IC_{50} values of 0.84, 7.859 and 4.404 µg/mL, respectively. Polyprenols (**2**) from *Ginkgo biloba* L. showed hepatoprotective effects against CCl_4-stimulated hepatotoxicity in rats[24] and exhibited antitumor activity[25]. Polyprenols inhibited Heps, S (180) and EC transplanted tumors by at least 50%[25]. Polyprenol, with concentrations between 10^{-3}-10^{-4} M, induced apoptosis in multidrug-resistant MCF-7 cells by reducing the resistance marker, P-glycoprotein (Pgp), in the plasma membrane[26]. In a related study, MCF-7 cells treated with 10^{-4} M of polyprenol resulted in the downregulation of DPAGT1 overexpression which led to the regulation of E-cadherin expression, an important factor in the prevention of tumors and metastasis[27]. To the best of our knowledge, this is the first report on the cytotoxicity of **2** against colon cancer cells HT-29 and HCT116 and breast cancer cells MCF-7, with IC_{50} values of 1.059, 9.258 and 3.786 µg/mL, respectively. Triacylglycerols (**3**) from Tuna (1000 mg/kg) was reported to significantly inhibit the tumor growth in the spleen of mice with intrasplenically-implanted Lewis lung carcinoma[28]. Triacylglycerols, which account for 12-20% of the total seed weight of pomegranate (*Punica granatum*) seed oil (PSO) extract, was found to significantly decrease cell viability in two breast cancer cell lines, MDA-MB-231 and MCF-7[29]. To our knowledge, this is the first report on the cytotoxicity of **3** against colon cancer cells, HT-29 and HCT116, and breast cancer cells, MCF-7, with IC_{50} values of 2.738, 13.05, and 16.12 µg/mL, respectively. β-Sitosterol (**4**) was found to affect the programmed cell death pathway in human breast cancer cells (MCF-7) and human adenocarcinoma cells (MDA-MB-231), hindering tumor growth by promoting apoptosis[30]. It also inhibited cell proliferation of human colon cancer cells (HT-29)[31]. β-Sitosterol (**4**) inhibited the growth of HT116 human colon cancer cells by different mechanisms including the activation of caspase-3 and caspase-9, accompanied by proteolytic cleavage of

poly(ADP-ribose)-polymerase and the reduction of the expression of the anti-apoptotic Bcl-2 protein and mRNA and a subsequent increase of the pro-apoptotic Bax protein and mRNA[32]. *In vitro* studies showed that **4** inhibited the proliferation of human colon cancer cells (COLO 320 DM) with an IC_{50} of 266.2 µM, inducing apoptosis by scavenging oxidants and attenuating β-catenin and PCNA expression while *in vivo* studies showed that **4** reduced the number of aberrant crypt and crypt multiplicity in DMH-initiated rats in a dose-dependent fashion[33]. β-Sitosterol (**4**) significantly decreased the expression of Niemann-Pick C1-like 1 (NPC1L1) in human small intestine epithelial cells (FHs 74 Int), reducing intestinal cholesterol absorption at the cellular level[34]. It also promoted apoptosis mediated by the activation of ERK and the downregulation of Akt in murine fibrosarcoma cells (MCA-102)[35]. It was reported that **4** and stigmasterol exhibited anti-proliferative activities against human prostate cancer cells (DU-145) by increasing the expression of p53 protein and inhibiting carcinoma development by decreasing the expression of p21 and p27 proteins[36]. Thus, **4** was reported to exhibit cytotoxic properties against several cancer cell lines such as colon and breast cancer cell lines which corroborate our findings that **4** showed high cytotoxicity against colon cancer cells, HT-29, and breast cancer cells, MCF-7, with IC_{50} values of 1.41 and 2.277 µg/mL, respectively.

CONCLUSION

Squalene (**1**), polyprenols (**2**), triglycerides (**3**), and β-sitosterol (**4**), isolated from the dichloromethane extract of *Dysoxylum gaudichaudianum* leaves, exhibited varying cytotoxic activities against three human cancer cell lines, breast (MCF-7) and colon (HT-29 and HCT-116), and a normal cell line, human dermal fibroblast, neonatal (HDFn). The anti-proliferative activities of **1-4** were highest against HT-29, with IC_{50} values ranging from 0.84 to 2.738 µg/mL, followed by MCF-7, with IC_{50} values ranging from 2.277 to 16.12 µg/mL, and HCT-116, with IC_{50} values ranging from 7.859 to 13.05 µg/mL. All compounds (**1-4**) were moderately cytotoxic against HDFn with IC_{50} values >10 µg/mL. Overall, **1-4** were more cytotoxic to the breast and colon cancer cell lines than to the normal cell line.

ACKNOWLEDGEMENT

A research grant from the De La Salle University Science Foundation, through the University Research Coordination Office, is gratefully acknowledged.

REFERENCES

1. *Dysoxylum gaudichaudianum.* http://www.philippineplants.org/Families/Meliaceae.html. Downloaded on February 25, 2016.
2. *Dysoxylum gaudichaudianum.* http://keys.trin.org.au:8080/key-server/data/0e0f0504-0103-430d-8004-060d07080d04/media/Html/taxon/*Dysoxylum_gaudichaudianum*.htm. Downloaded on February 25, 2016.
3. Weiner MA. Secrets of Fijian Medicine. University of California, Berkeley, California 1984; 65-67.
4. Igiu-StuartXchange. http://www.stuartxchange.com/Igiu.html. Downloaded on February 25, 2016.
5. Bourdy G, Francois C, Andary C, Boucard M. Maternity and medicinal plants in Vanuatu II, Pharmacological screening of five selected species. J Ethnopharmacol 1996; 52(3):139-143.
6. Chen JL, Kernan MR, Jolad SD, Stoddart CA, Bogan M, Cooper R. Dysoxylins A-D tetranortriterpenoids with potent anti-RSV activity from *Dysoxylum gaudichaudianum*. J Nat Prod 2007; 70(2):312-315.
7. Odimegwu DC, Grunwald T, Esimone CO. Anti-respiratory syncytial virus agents from phytomedicine. 2011. In Resch, B. (Ed.), Human respiratory syncytial virus infection (179-196). Available from: http://www.intechopen.com/books/human-respiratory-syncytial-virus-infection/anti-respiratory-syncytial-virus-agents-from-phytomedicine.
8. Tukiran H, Hamdani B, Mahyudi R, Hidayati N. Several compounds isolated from stem bark of Kedoya (*Dysoxylum gaudichaudianum* (A. Juss.) Miq.) (Meliaceae). Jurnal Ilmu Dasa 2009; 10(2):236-244.
9. Nagakura Y, Yamanaka R, Hirasawa Y, Hosoya T, Rahman A, Kusumawati I, Zaini N, Morita H. Gaudichaudysolin A, a New Limonoid from the Bark of *Dysoxylum gaudichaudianum*. Heterocycles 2010; 80(2):1471-1477.
10. Ragasa CY, Torres OB, Bernardo LB, Mandia EH, Don M-J, Shen C-C. Glabretal-type triterpenoids from *Dysoxylum mollissimum*. Phytochem Lett 2013; 6(3):514-518.
11. Ragasa CY, Ng VAS, De Los Reyes MM, Mandia EH, Oyong GG, Shen C-C. Chemical constituents and cytotoxicity of the leaves of *Dysoxylum gaudichaudianum* (A. Juss.) Miq. Der Pharma Chemica 2014; 6(5):182-187.
12. Freshney RI, 2000. Culture of Animal Cells: A Manual of Basic Techniques. Wiley-Liss, Inc., New York, USA.
13. Jacinto SD, Chun EAC, Montuno AS, Shen C-C, Espineli DL, Ragasa CY. Cytotoxic cardenolide and sterols from *Calotropis gigantea*. Nat Prod Commun 2011; 6(6):803-806.
14. Geran RI, Greenberg NH, McDonald MM, Schumacher AM, Abbott BJ. Protocols for screening chemical agents and natural products against animal tumour and other biological systems. Cancer Chemother Rep 1972; 3:17-19.
15. Makizumi R, Yang W-L, Owen RP, Sharma RR, Ravikumar TS. Alteration of drug sensitivity in human colon cancer cells after exposure to heat: implications for liver metastasis therapy using RFA and chemotherapy. Int J Clin Exp Med 2008; 1:117-129.
16. Clarke PA, Hostein I, Banerji U, Di Stefano F, Maloney A, Walton M, Judson I, Workman P. Gene expression profiling of human colon cancer cells following inhibition of signal transduction by 17-allylamino-17-

demethoxygeldanamycin, an inhibitor of the hsp90 molecular chaperone. Oncog 2000; 19:4125-4133.
17. Spanova M, Daum G. Review Article: Squalene - biochemistry, molecular biology, process biotechnology, and applications. Eur J Lipid Sci Technol 2011.
18. Rao CV, Mark HLN, Reddy RS. Chemopreventive effect of squalene on colon cancer. Carcinogenesis, 1998; 19:287–290.
19. Loganathan R, Radhakrisnan AK, Selvaduray KR, Nesaretnam K. Selective anti-cancer effects of palm phytonutrients on human breast cancer cells. Royal Soc Chem Adv 2015; 5:1745-1753.
20. Loganathan R, Selvaduray KR, Nesaretnam K, Radhakrisnan 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.
21. Desai KN, Wei H, Lamartiniere CA. The preventive and therapeutic potential of the squalene-containing compound. Cancer Lett 1996; 101:93–96.
22. 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.
23. Chudzik M, Korzonek-Szlacheta I, Król W. Triterpenes as potentially cytotoxic compounds. Molec 2015; 20:1610-1625.
24. Yang L, Wang C-Z, Ye J-Z, Li H-T. Hepatoprotective effects of polyprenols from Ginkgo biloba L. leaves on CCl4-induced hepatotoxicity in rats. Fitoter 2011; 82(6):834-40.
25. Wang C-Z, Shen Z-B, Gao L. The biological activeness of polyprenols from Ginkgo biloba L. on antitumor. Nat Prod Res Dev 2002; 14(5):18-20.
26. Kuznecovs S, Jegina K, Kuznecovs I. P9 Inhibition of P-glycoprotein by polyprenol in human breast cancer cells. The Breast 2007; 16(S1): S15.
27. Kuznecovs I, Kuznecovs S, Kuznecovs G. DPAGT1 regulation with polyprenol in MCF-7 breast cancer cells: possible therapeutic approach to E-cadherin loss prevention. Eur J Cancer 2012; 48(S1): S127.
28. Maeda Y, Sumiyoshi M, Kimura Y. Effects of tuna oil on tumor growth and metastasis to liver in intrasplenic Lewis lung carcinoma (ILL) implanted mice. J Trad Med 2004; 21(5):215-220.
29. Costantini S, Rusolo F, De Vito V, Moccia S, Picariello G, Capone F, Guerriero E, Castello G, Volpe MG. Potential anti-inflammatory effects of the hydrophilic fraction of pomegranate (*Punica granatum* L.) seed oil on breast cancer cell lines. Molecules 2014; 19:8644-8660.
30. Awad AB, Chinnman M, Fink CS, Bradford PG. β-Sitosterol activates Fas signaling in human breast cancer cells. Phytomed 2007; 14:747-754.
31. Jayaprakasha GK, Mandadi KK, Poulose SM, Jadegoud Y, Gowda GA, Patil BS. Inhibition of colon cancer growth and antioxidant activity of bioactive compounds from *Poncirus trifoliate* (L.) Raf. Bioorg Med Chem 2007; 15:4923-4932.
32. Choi YH, Kong KR, Kim YA, Jung KO, Kil JH, Rhee SH, Park KY. Induction of Bax and activation of caspases during beta-sitosterol-mediated apoptosis in human colon cancer cells. Int J Oncol 2003; 23(6):1657-1662.
33. 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.
34. 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):859–866
35. Moon DO, Kyeong JL, Yung HC, Gi-Young K. β-Sitosterol-induced apoptosis is mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells. Int Immunopharmacol 2007; 7:1044–1053.
36. Scholtysek C, Krukiewicz AA, Alonso J-L, Sharma KP, Sharma PC, Goldmann WH. Characterizing components of the Saw Palmetto Berry Extract (SPBE) on prostate cancer cell growth and traction. Biochem Biophys Res Commun 2009; 379:795–798.