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Cytotoxicity of bark extracts from *Aglaia loheri* (Blanco) Merr. (Meliaceae) against human colorectal carcinoma cells (HCT116)

Norielyn N. Abalos[1, 2], Sonia D. Jacinto[1]

[1]*Institute of Biology, University of the Philippines Diliman, Quezon City, Philippines*

[2]*Department of Biology, University of San Carlos-Talamban Campus, Cebu City, Philippines*

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Abstract

Natural products, plant extracts or plant-derived chemicals have played a promising role in the treatment and prevention of cancer showing considerably less toxicity and lack the side effects of other chemotherapeutic agents. The cytotoxic activity of bark extracts from a Philippine native tree, *Aglaia loheri*, were tested against human colorectal cancer cell line HCT116 using 3-(4,5-dimethyl2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The methanolic crude extract was subjected to a bioassay guided solvent partitioning and fractionation by vacuum liquid chromatography (VLC) and gravity column chromatography (GCC). The apoptotic effect of the most active fraction was investigated using JC-1 assay to determine mitochondrial membrane alteration and TUNEL assay to detect DNA fragmentation. *A. loheri* crude extract demonstrated very high cytotoxicity against HCT116 with IC_{50} value of 0.49±0.07µg/mL. The hexane (ALBH) and ethyl acetate (ALBEA) partitions and most of the VLC and GCC fractions showed high to moderate cytotoxicity. The GCC fraction ALBEA9.2 demonstrated very low IC_{50} value of 0.04±0.01µg/mL and showed high selectivity index (SI) of 8.72. Morphological examination of HCT116 cells treated with ALBEA9.2 showed loss of membrane integrity and loss of contact with neighboring cells, condensed cytoplasm and detachment from the substratum suggestive of apoptosis. Reduced mitochondrial membrane potential and the presence of genome fragmentation in treated cells confirmed the apoptosis-inducing effect of ALBEA9.2. The study indicates that bark extracts of *A. loheri* shows cytotoxic activity that is highly selective against HCT116 cell line. The findings suggest the potential of ALBEA9.2 as an anticancer agent by inducing apoptosis.

* **Corresponding Author:** Norielyn N. Abalos ✉ norielynabalos@gmail.com

Introduction

Cancer is a non-communicable disease characterized by sustained proliferation and abnormal differentiation of cells. Colorectal cancer (CRC) is the fourth most commonly diagnosed cancer in the world with about 1.1 million new cases and the fifth most common cause of cancer mortality with 551, 269 deaths per year (Bray *et al*., 2018). The data suggest that CRC remains to be a major public health concern worldwide and high mortality estimate indicates limited efficiency of existing cancer therapies. Therefore, search for treatments that directly target cancer cells, but with little or no harmful side effects, is of major interest in health research as many of the available anticancer drugs have limited utility due to acquired resistance and negative side effects (Cragg and Newman, 2005; García-Varela *et al*., 2016). A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells (Sharma *et al*., 2009). Natural products, plant extracts or plant-derived chemicals have played a promising role in the treatment and prevention of various diseases throughout the world providing various health benefits, with considerably less toxicity and side effects which are the limitations of other chemotherapeutic agents (Yuet-Ping *et al*., 2012; Manson *et al*., 2005). Many commercially approved anticancer drugs and abundant compounds in various stages of clinical development are plant derived natural products which have been explored for centuries in treating diseases (Asare *et al*., 2012; Park, 2012).

Philippine biodiverse forests contain a plethora of plants with ethnomedicinal values and folkloric uses; potentially, they may contain anticancer compounds. *Aglaia* species (from family Meliaceae) demonstrated ethnomedicinal value, cytotoxic and insecticidal properties (Dreyer *et al*., 2001; Rivero-Cruz *et al*., 2004). *Aglaia* is the only source of a group of about 50 known representatives of compounds known as rocaglate or rocaglamide derivatives, or flavaglines that demonstrated potent insecticidal, antifungal, antiviral, antibacterial or anthelmintic properties along with distinct cytotoxic activity against a range of human cancer cell lines (Wang *et al*., 2001, Mohamad *et al*., 1999, Bohnenstengel *et al*., 1999, Pan *et al*., 2010). Rocaglamide was first isolated in 1982 from *Aglaia elliptifolia* showing antileukemic activity (Wu *et al*., 1997). The genus has attracted considerable attention toward discovery and isolation of compounds with interesting structures and biological activities.

Aglaia loheri locally known as balubar, is a shrub or tree of the family Meliaceae that grows in Indonesia, Papua New Guinea, Taiwan, China and the Philippines (Pannell, 1998). It is part of the diet of the Aetas, an indigenous tribe in the Philippines, that uses the plant for medicinal and nutritional purposes (Dapat *et al*, 2013). Earlier studies on crude extracts and isolated compounds from leaves of *A. loheri* reported high to moderate cytotoxicity against HCT116 and A549 cells as well as teratogenic activity against maternal mice (Ragasa *et al*., 2012; Canoy *et al*., 2011 and Herrera *et al*., 2011). Investigating *A. loheri* bark extract as a source of potential compounds to be utilized as medicine can make a significant contribution to the health care system and the society, especially in cancer drug research and development.

This study was designed to evaluate the cytotoxic activity of *A. loheri* bark extracts against HCT116 through a bioassay-guided fractionation. The apoptotic effect of the most active fraction was also investigated.

Materials and methods

Plant Collection and Crude Extraction

With the proper permit for collection, bark of *Aglaia loheri* was collected from Mt. Lamao, Bataan. Plant identification was verified at the Jose Vera Santos Herbarium of the Institute of Biology, University of the Philippines, Diliman, Quezon City, and voucher specimens were deposited in the same herbarium under accession number 21418. Bark samples were air dried then oven-dried at 40°C. Dried samples were cut to smaller pieces and pulverized using an electric blender. Pulverized bark was macerated in methanol (RCI Labscan Ltd., Thailand) for at least 48 hours. The extract was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator (Heidolph, Germany) at 40°C which yielded the methanolic crude extract.

Solvent Partitioning and Fractionation

The most cytotoxic methanolic crude extract from the bark of *A. loheri* was subjected to solvent-solvent partitioning between hexane (RCI Labscan Ltd., Thailand) and water yielding the hexane partition (ALBH) followed by ethyl acetate (RCI Labscan Ltd., Thailand) and water yielding the ethyl acetate partition (ALBEA). The solvent partitions were concentrated using rotary evaporator at 40°C. The remaining aqueous partition (ALBAq) was subjected to lyophilization (Alpha 1-2 LDplus Martin Christ Gefriertrocknungsanlagen GmbH, Germany). *A. loheri* ethyl acetate partition (ALBEA) which is the most active, was further separated into different fractions by vacuum liquid chromatography (VLC) using silica gel 60G (Merck, Germany) as stationary phase and gradient mixtures from 100% hexane to 100% ethyl acetate and ethanol as eluents to obtain 10 fractions (ALBEA06-ALBEA15). The first five eluents (ALBEA01-ALBEA05) had insignificant yield and were combined with ALBEA06. The most cytotoxic fractions, ALBEA09 and ALBEA13, were further purified by gravity column chromatography (GCC) using silica gel 60 0.063-0.200mm for column chromatography (Merck, Germany) and RP18 silica for liquid chromatography (Merck, Germany) as stationary phase for ALBEA9 and ALBEA13, respectively. ALBEA9 afforded three fractions (ALBEA9.1-ALBEA9.3) and another three fractions from ALBEA13 (ALBEA13.1-ALBEA13.3).

Cell Culture

Human colorectal carcinoma (HCT116) and non-cancer Chinese hamster ovarian cell line (AA8) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). HCT116 was grown in McCoy's 5a medium (Gibco, NY, USA) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco, NY, USA) and gentamicin (Gibco, NY, USA). AA8 was grown in Minimum Essential Eagle medium (Gibco, NY, USA) supplemented with 10% FBS and gentamicin. Cells were grown in T75 culture flasks maintained at 37°C, 5% CO_2 and 95% humidity.

Monitoring Cytotoxicity by MTT Assay

Cytotoxicity of bark crude extract and *A. loheri* partitions and fractions against HCT116 or AA8 was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay adapted from Mosmann (1983). MTT assay is a colorimetric test that measures the reduction of MTT by mitochondrial dehydrogenase to purple formazan crystals in live cells. HCT116 cells were seeded in 96-well plates at a density of 4 x 10^4cells/mL. Plates were then incubated for 24 hours at 37°C, 5% CO_2 and 95% humidity and were utilized in all MTT treatments.

Ten (10)mg/mL of crude extract, partitions or fractions were serially diluted two fold, in a Master Dilution Plate (MDP) and 10µL from the MDP was transferred to the plated cells to final concentrations of 100µg/mL, 50µg/mL, 25µg/mL, 12.5µg/mL, 6.25µg/mL, 3.125µg/mL, 1.5625µg/mL and 0.78125µg/mL. For highly cytotoxic extracts, three-fold or four-fold serial dilution was used in order to get a suitable concentration response curve and IC_{50} estimate. DMSO was used as negative control while the anticancer drug Doxorubicin served as positive control. The treated cells were then incubated for another 72 hours at 37°C, 5% CO_2 and 95% humidity.

After treatment, the media were withdrawn and 20µL MTT at 5mg/mL phosphate-buffered saline (PBS) was added to each well. Plates were incubated at same conditions for four hours and then 150µL DMSO was added per well to dissolve the formazan crystals. Absorbance of MTT reduced to formazan was detected at 570nm using LeDetect microplate reader (Labexim, EU). Three independent experiments were carried out with duplicate wells per concentration. The absorbance obtained at 570nm was used to calculate percentage of inhibition. Assuming 100% viability in control cells, percent inhibition was calculated using the formula:

% Inhibition = 100 [1- (Absorbance of extract treated cells/Absorbance of DMSO-treated cells)]

The concentration required to inhibit cell viability by 50% (IC_{50}) was determined using Graphpad Prism 6.0 software (San Diego, CA). All concentration response curves were constrained with 100% on top and 0% at the bottom.

Cell Selectivity of A. loheri

The selectivity index (SI) was calculated from the ratio of IC50 to AA8 versus IC50 to HCT116. SI value indicates selectivity of the sample for the cell line tested. SI value ≥ 2 indicates selectivity against cancer cell lines (de Oliveira *et al*., 2015).

Cellular Morphology after treatment with ALBEA 9.2

Activation of apoptosis alters cellular morphology. The typical morphological features of apoptotic cells after treatment with ALBEA 9.2 were observed by light microscopy. Cells were seeded in 96-well plates as described previously. The most cytotoxic, selective and high yielding GCC fraction, ALBEA9.2, was tested for its effect on cellular morphology of HCT116 cells and micrographs were captured after 8, 24, 48- and 72-hour incubation. DMSO was used as negative control while Doxorubicin served as positive control.

Analysis of Mitochondrial Membrane Depolarization by JC-1 Assay

The intrinsic apoptosis pathway is characterized by decreased mitochondrial membrane potential. This can be easily detected using fluorescent cationic dyes that accumulate in healthy mitochondria but diffuse to the cytoplasm in apoptotic cells. The effect of ALBEA9.2 on mitochondrial membrane potential was investigated using MitoProbe™ JC-1 Assay Kit (Invitrogen Corp, CA, USA). Cells were seeded in a 96 well plate and treated as described above. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) was used as positive control and DMSO as negative control. Cells were stained with JC-1 according to the manufacturer's instruction with some modifications. Briefly, after 24 hours exposure to ALBEA9.2 or controls, JC-1 solution was added to the wells to a final concentration of 2µM followed by 30 min incubation at 37°C, 5% CO_2 and 95% humidity. Cells were washed with PBS twice and fluorescence was measured using Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific). Fluorescence detection was set at Ex/Em 535/595nm for red and 485/535nm for green. Reduction in red to green fluorescence ratio is indicative of mitochondrial membrane depolarization. Three independent experiments were done with duplicates per experiment.

Detection of apoptosis by TUNEL Assay

One hallmark of apoptosis is the formation of deoxyribonucleic acid (DNA) fragments implying the activation of endonucleases. These DNA breaks can be easily detected by fluorescence microscopy. Apoptosis of cells treated with ALBEA9.2 was determined using terminal deoxynucleotidyl transferase (TdT) mediated-16-deoxyyuridine triphosphate (dUTP) Nick-End Labelling (TUNEL) to visualize nuclei with DNA fragmentation. DeadEnd™ fluorometric TUNEL system (Promega, WI, USA) was used according to the manufacturer's protocol with some modification. HCT116 cells were seeded in 96-well plate and treated with high concentration (5µg/mL) and IC_{50} (0.04µg/mL) of ALBEA9.2 for 72 hours. The cells were then fixed, permeabilized and labeled with TdT reaction mix for 60 minutes. Hoechst 33342 was used to counterstain the nuclei. Stained cells were viewed using fluorescence. Three independent experiments were done with duplicates per experiment. Five representative images per replicate were taken.

Statistical analysis

The results are presented as means ± standard deviation (SD) of three independent experiments in duplicates. The non-parametric Kruskal-Wallis test with a post-hoc Dunn's test was used on IC_{50} data. JC-1 assay data were log transformed and statistical differences were determined by one-way ANOVA with Tukey's test. Statistical analyses were performed using Graph Pad Prism 6.0. Differences were considered significant at $P \leq 0.05$.

Results

A. loheri Bark Extracts Exhibit Cytotoxicity against HCT116

The cytotoxic effect of methanolic crude extract and solvent partitions from the bark of *Aglaia loheri* was tested against HCT116 using MTT assay (Fig. 1A). According to the American National Cancer Institute plant screening program, a crude extract is considered to have *in vitro* cytotoxic activity if the IC_{50} is <30µg/mL (Suffness and Pezzuto, 1990). Bark crude extract of *A. loheri* showed notable cytotoxic activity against HCT116 with IC_{50} value of

0.49±0.07µg/mL comparable to that of doxorubicin with IC_{50} value of 0.18±0.1µg/mL. *A. loheri* crude extract was then subjected to bioassay-guided partitioning and fractionation.

The hexane and ethyl acetate fractions of *A. loheri* exhibited high cytotoxicity with IC_{50} of 0.97±0.65µg/mL and 0.20±0.05µg/mL, respectively (Fig. 1A). However, the aqueous fraction was considered nontoxic with IC_{50} greater than 100µg/mL. The ethyl acetate fraction (ALBEA) showed pronounced cytotoxic activity and promising selectivity (see Fig. 2) against HCT116 and was chosen for further purification using vacuum liquid chromatography (VLC).

Ten VLC fractions collected from ALBEA (ALBEA6-ALBEA15) exhibited good cytotoxic activity against HCT116 (Fig. 1B). Insignificant yield was collected from the first five fractions (ALBEA1-ALBEA5) hence were pooled with ALBEA6. ALBEA13 and ALBEA14 showed the lowest IC_{50} values against HCT116 at 0.06±0.02µg/mL and 0.08±0.01µg/mL, respectively. IC_{50} values of ALBEA15 (0.13±0.03µg/mL), ALBEA9 (0.22±0.04µg/mL) and ALBEA10 (0.1±0.02µg/mL) are also highly cytotoxic against HCT116. ALBEA13, ALBEA14, ALBEA10, ALBEA15 and ALBEA9 demonstrated high cytotoxicity against HCT116 comparable with the positive control, doxorubicin, with IC_{50} value of 0.14±0.14µg/mL.

The two most cytotoxic and high yielding VLC fractions, ALBEA9 and ALBEA13, were subjected to gravity column chromatography (GCC) for further fractionation. ALBEA9 afforded three fractions (ALBEA9.1 – ALBEA9.3). ALBEA13 was also separated into three fractions (ALBEA13.1 – ALBEA13.3). The cytotoxic activity of the GCC fractions are shown in Fig. 1C. The high yielding fractions, ALBEA9.2 and ALBEA13.1 demonstrated the lowest IC_{50} values against HCT116 at 0.04±0.01µg/mL and 0.08±0.04µg/mL, respectively. The remaining GCC fractions also exhibited promising cytotoxic activity but with much lower yield.

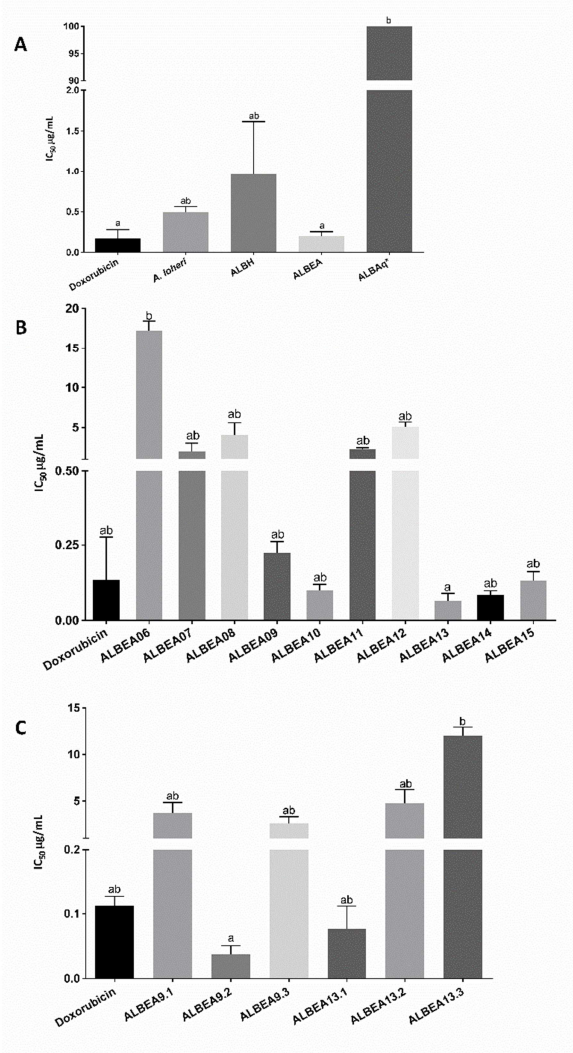

Fig. 1. Cytotoxic effects of *A. loheri* bark extracts against human colorectal carcinoma cell line, HCT116. Cytotoxic activity of *A. loheri* (A) crude extract and solvent partitions, (B) VLC fractions and (C) GCC fractions of ALBEA9 and ALBEA13 after 72 hours of treatment. Results are means of IC_{50} ± standard deviation, n=3. Means with the same letter are not significantly different ($P \leq 0.05$), n=3. Values >30µg/mL are considered inactive. Doxorubicin, a standard anticancer drug, was used as positive control. *IC_{50} value is greater than 100µg/mL.

Selective cytotoxicity of A. loheri extracts against HCT116

The use of high-dose chemotherapeutic drugs has been limited due to their systemic toxicity, affecting both cancer and non-cancer cells. The specificity of the active *A. loheri* extract and fractions was tested

against non-cancer Chinese hamster ovarian cell line (AA8). Selectivity index was computed as IC_{50} ratio on AA8 versus IC_{50} on HCT116. SI value greater than or equal to 2 is considered promising for exhibiting twice more cytotoxicity to cancer cells than that of the non-cancer cells (de Oliveira *et al.*, 2015).

Fig. 2 shows that the most active extracts and fractions of *A. loheri* display selectivity against HCT116. Bark crude extract of *A. loheri* is twice more cytotoxic against HCT116 (SI=2.08). The ethyl acetate solvent partition (ALBEA) showed higher selective cytotoxicity (SI=5.48) while the hexane partition was non-selective. Fractionation of the extract lead to an increase in selectivity. Computed selectivity index of ALBEA fractions showed considerably higher selectivity than doxorubicin. Selectivity of the ALBEA9 and ALBEA9.2 was enhanced from 5.96 to 8.72 against HCT116 indicating better selectivity. ALBEA13 and ALBEA13.1 however are less selective at 3.84 and 2.45, respectively.

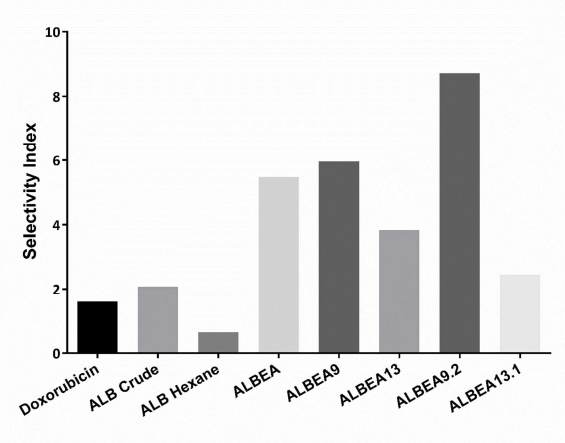

Fig. 2. Selectivity of *A. loheri* crude extracts, partitions and most active ethyl acetate fractions. SI value calculated from the ratio of mean IC_{50} value to AA8 versus mean IC_{50} value to HCT116 (n=3). SI value ≥ 2 indicates high selectivity against HCT116. Doxorubicin, a standard anticancer drug, was used as positive control.

Morphological changes in HCT116 cells treated with ALBEA9.2

ALBEA9.2 showed high cytotoxic activity and selectivity against HCT116 compared with ALBEA13.1 and was used in the succeeding assays. The morphological changes of HCT116 cells treated with ALBEA9.2 were observed under the microscope after 8, 24, 48, and 72hours treatment and were compared with the cells treated with DMSO and Doxorubicin. Representative micrographs are shown in Fig. 3. The cells treated with ALBEA9.2 appeared less uniform compared with DMSO. Significant change in the morphology of treated cells is evident after 72-hour exposure with the extracts at high concentration. Loss of membrane integrity and loss of contact with neighboring cells is apparent. Apoptotic like features such as condensed nuclei, rounding of cells and detachment from the plate were observed. However, cells treated at lower concentrations and the computed IC_{50} showed less remarkable changes.

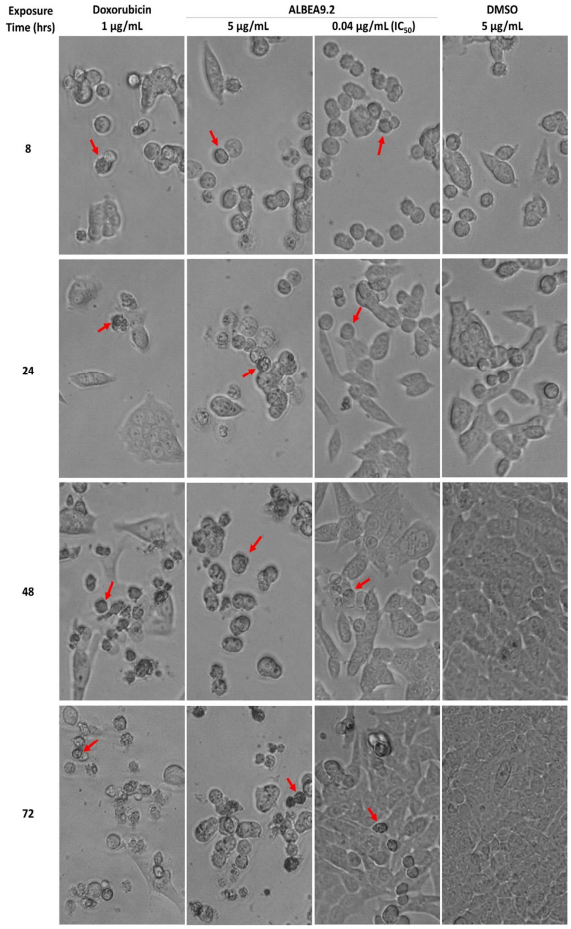

Fig. 3. Morphological features of HCT116 after 8, 24, 48, and 72 hours treatment with indicated concentrations of ALBEA9.2, Doxorubicin and DMSO. Rounding cells with condensed nuclei are pointed by red arrows. Images are representative of three trials and were captured under brightfield microscope (100x).

A. loheri extract induces apoptosis in HCT116 cells

To determine the apoptotic effect of ALBEA9.2 on HCT116 cells, two apoptosis assays were carried out. JC-1 assay determines early apoptosis event characterized by mitochondrial membrane destabilization and TUNEL assay that distinguishes late apoptosis marked by DNA fragmentation.

The effect of ALBEA9.2 on mitochondrial membrane potential is summarized in Fig. 4. HCT116 cells treated with 5µg/mL and 1µg/mL ALBEA9.2 displayed disrupted mitochondrial membrane potential indicated by lower red to green fluorescence ratio of 10.65 and 16.19, respectively. The negative control, DMSO, showed significantly higher red to green fluorescence ratio of 20.34 compared with cells treated with 5µg/mL ALBEA9.2.

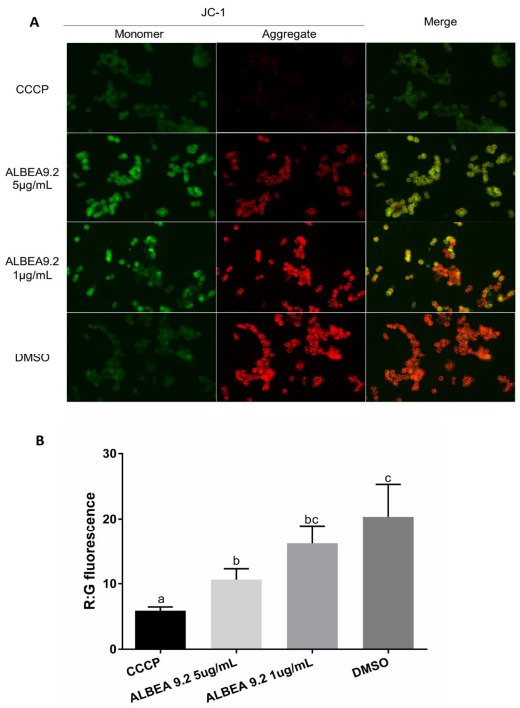

Fig. 4. Effect of ALBEA9.2 on mitochondrial membrane potential (ΔΨm) of HCT116 cells. Cells were seeded in 96-well plate followed by JC-1 staining 24 hours after treatment with carbonyl cyanide 3-chlorophenylhydrazone (CCCP), ALBEA9.2 (5µg/mL and 1µg/mL) or DMSO. (A) Cells were viewed using fluorescence microscope (200X) and representative images from three independent experiments are shown. (B) Ratio of red to green fluorescence was computed and values shown are means ± SD. Means with the same letter are not significantly different ($P \leq 0.05$), n=3.

Late apoptosis is characterized by DNA fragmentation detected by TUNEL assay as shown in Fig. 5. Nick ends of DNA fragments are marked with green fluorescence and Hoechst 33342 was used to counter stain the nuclei. Intense green fluorescence indicative of late apoptosis is clearly detected in the nuclei of cells treated with ALBEA9.2.

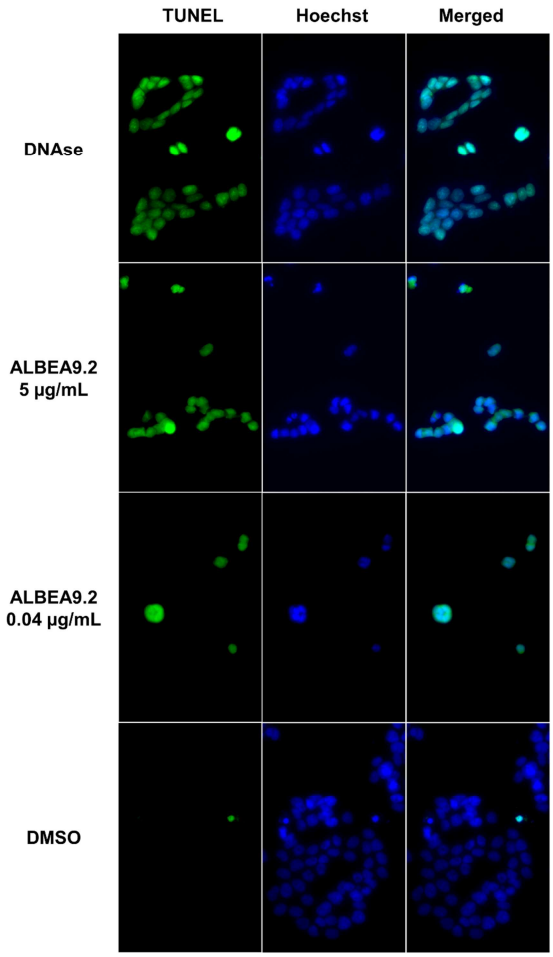

Fig. 5. Apoptotic effect characterized by DNA fragmentation in HCT116 treated with ALBEA9.2. Cells were seeded in 96-well plates and treated with selected concentrations of ALBEA9.2, DMSO (negative control), or DNAse (positive control) for 72 hours. Cells were then subjected to TUNEL assay and nuclei were counter stained with Hoechst 33342. Cells were viewed using fluorescence microscope and representative images (400X) from three independent experiments are shown.

Discussion

Sustained cell proliferation along with evasion of apoptosis are important traits that promote cancer

development (Hanahan and Weinberg, 2011). Hence, compounds that inhibit proliferation and induce apoptosis have become the target of many researches in pursuit of potential anticancer agents (Feitelson *et al*, 2015). The present study revealed that bark extracts from *A. loheri* exhibit selective antiproliferative activity against HCT116 cell line. Interestingly, ALBEA9.2 is more than eight times more selective against HCT116 than on non-cancer cell line, AA8. Bezivin *et al.* (2003) and Prayong *et al.* (2008) consider that SI values greater than three indicate high selectivity against cancer cell lines. Investigation on the properties of the semi-purified fraction showed that ALBEA9.2 effectively demonstrated induction of apoptosis confirmed by disruption of mitochondrial membrane potential and DNA fragmentation.

The cytotoxic activity of the bark extracts is comparable to the previously reported cytotoxicity (IC_{50}<20µg/mL) of ethanolic crude extract from *A. loheri* leaves collected from Morong Bataan (Ragasa *et al.*, 2012) against HCT116 cells. In addition, the ethyl acetate extract of the leaves of *A. loheri* in the previous study, (Dapat *et al.*, 2013) exhibited an IC_{50} concentration of 5.47µg/mL which is remarkably less cytotoxic than the activity of the ethyl acetate extract recorded in this study. Their result on fractionation of the ethyl acetate leaf extract afforded subfractions that are also highly cytotoxic against HCT116. Compared to the results of this study, VLC fractions of the bark yielded five fractions with IC_{50} less than 1µg/mL. This variation in cytotoxic activity among plant organs and plants from different locations can be attributed to chemical polymorphism as protection against herbivore grazing was previously seen in *Aglaia leptantha* (Greger *et al.*, 2000). In addition, plants harvested from different seasons and environmental conditions show varying levels of metabolites (Paravini *et al.*, 2012).

The cytotoxic activity of the extracts and isolates from *A. loheri* bark could possibly be related to bisamides and flavaglines. Aglafolin, a flavagline isolated from *Aglaia elliptifolia* stems previously showed high cytotoxicity against human colon carcinoma (HCT8) with IC_{50} value of 0.05µg/mL and against A549 with IC_{50} of <0.001µg/mL (Wu *et al.*, 1997). Silvestrol, another derivative of flavagline isolated from stem bark of *Aglaia foveolata*, exhibited very high cytotoxicity against human colon cancer cell line (HT-29) (Pan *et al.*, 2010).

Morphological examination of the treated cells showed obvious apoptotic features such as detachment from the culture plate, condensation of the cytoplasm, cell shrinkage and loss of contact with neighboring cells (Delie and Rubas, 1997). Apoptosis, a highly controlled type of natural cell death, has been considered as the most effective non-surgical treatment for cancer (Pfeffer and Singh, 2015). This leads to the development of anticancer therapies that induce activation of the apoptosis pathway (Pfeffer and Singh, 2015). Induction of the intrinsic apoptosis pathway causes mitochondrial membrane permeabilization that leads to the release of cytochrome c and eventually, to cell death (Pfeffer and Singh, 2015; Lopez and Tait, 2015). The present study reveals clear apoptotic property of ALBEA9.2 that is initiated by mitochondrial membrane permeabilization which eventually ends in DNA fragmentation.

Conclusion

Bark crude extract and fractions from *A. loheri* exhibit notable cytotoxic activity with high selectivity against HCT116 cell line. The findings suggest the potential therapeutic activity of *A. loheri* extract and its isolated fraction ALBEA9.2 in inhibiting the growth of HCT116 cell line through promoting cell death by apoptosis. Isolation and identification of the active compounds as well as identification of their mode of action merit further investigation.

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Declaration of interest

The authors declare no conflict of interest related to this study.

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