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Full Length Research Article

PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT AND ANTIPROLIFERATIVE PROPERTIES OF *ARTOCARPUS ALTILIS* (SUKUN) FROM ENDAU ROMPIN, JOHOR, MALAYSIA

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ABSTRACT
This study aimed to investigate the phytochemical contents, antioxidant and antiproliferative activity of *Artocarpus altilis* collected from Endau Rompin, Malaysia. Phytochemical profiling was determined using GC-MS. The antioxidant properties were evaluated by using FRAP, ABTS and DPPH assays while the effects of *A. altilis* on the proliferation of MCF-7 and MDA-MB-231 breast cancer cells was evaluated by using MTT assay. Cell cycle arrest and apoptosis was evaluated using flow cytometry analysis. The results showed that there is significant antioxidant activity as compared to the standard L-ascorbic acid (methanol extract with 91.0±0.31, ethyl acetate extract 87.4±0.55 and hexane extract had 22±0.6mg ACEAC/g) in DPPH, in FRAP (methanol extract with 65.0±0.16, ethyl acetate extract 48.4±0.11 and hexane extract had 39.0±0.05mg ACEAC/g) and finally in ABTS (methanol extract with 31.3±1.84, ethyl acetate extract 20.2±0.69 and hexane extract had 9.6±0.71mg ACEAC/g). The GC-MS analysis of methanol extract revealed the presence of at least 39 compounds. The extract of *A. altilis* was found to induce cytotoxicity against both MCF-7 and MDA-MB-231 cell line with IC_{50} values of 18.00µg/ml, 4.80µg/mL, and 4.95µg/mL in MCF-7 and 17.16µg/mL, 14.88µg/mL and 4.93µg/mL, in MDA-MB-231 at 24, 48 and 72hr respectively. Cell cycle analysis showed that *A. altilis* induced apoptosis at various stages in both cell lines and significant reduction in number of viable cells was observed. Apoptosis occurred during the first 24 hours and significantly increased after 48 and 72 hours of treatment. These findings suggested that *A. altilis* extract has the potential as natural antioxidant and anticancer agents. Further study is needed to verify the efficacy of this extract in *in vivo* and isolation of active compounds.

Keywords: Apoptosis, Breast cancer, Cell cycle, Cytotoxicity, *Artocarpus altilis*

INTRODUCTION

Approximately 14.1 million new cancer cases and 8.2 million deaths occurred in 2012 globally and the number of deaths caused by cancer is likely to increase to 13.1 million by 2030 (Abu Bakar et al., 2010). Breast cancer is the second most common cancer in the world overall; among women, and the most common cause of cancer death. It accounts for more than 25% of all new diagnoses of cancer and 15% of all cancer deaths in women each year (DeSantis et al., 2016; Smith & DeSantis, 2018). Malaysia is a middle income country in the Asia Pacific region with a population of 28.1 million. In Malaysia, until the National Cancer Registry (NCR) was launched in June 2003, there was a lack of incidence data on cancers.

Breast cancer is the commonest cancer in Malaysia with the age standardised rate for females of 47.4 per 100,000 (Taib, Akmal, Mohamed, & Yip, 2011a, 2011b). The National Cancer Registry of Malaysia (NCR) records 21,773 Malaysians being diagnosed with cancer but estimates that almost 10,000 cases are unregistered every year. It is estimated that one in four Malaysians (1:4) will develop cancer by 75 years old. It is estimated that nearly 40% of all cancers are preventable, including colorectal, lung and cervical cancers, with smaller effects in breast and nasopharyngeal cancer that only improvement in early detection and proper treatment leads to better chance of survival rates for people with cancer (DeSantis, Ma, Goding Sauer, Newman, & Jemal, 2017). Therefore, discovering new drugs with minimal toxicity is a wide scientific challenge, and there is an urgent need to search for new agent to cartel the menace by the use of alternative evidence based herbal medicines.

The genus Artocarpus belongs to the family Moraceae which comprises of about 60 genera and over 1000 species. Many of these species are used as a source of food and in traditional medicinal practises (Hari, Revikumar, & Divya, 2014). Artocarpus species are known for its large edible fruit with high nutritive values. Various compounds isolated from *A. altilis* have high potential as chemotherapeutic agents. It has been reported that artocarpin, which was tested on other breast cancer cells, MCF-7 and MDA-MB-231, showed cytotoxicity effect with an IC_{50} of 3.3 and 3.8 µg/ml, respectively (Enos Tangke Arung, Wicaksono, et al., 2010). In another report, nine isoprenylated flavones isolated from *A. altilis* roots (cycloartocarpin, artocarpin, chaplashin, morusin, cudraavone B, cycloartobiloxanthone, artonin E, cudraavone C, and artobiloxanthone) displayed cytotoxicity against KB, breast cancer (BC), and Vero (African green monkey fibroblast) cell lines, with IC_{50} values in the range of 2.9-14.7 µg/mL (Shamaun et al., 2010). Similar report was conducted by (Etti, Abdullah, et al., 2017) in which Artonin E treatment caused significant (P,0.05) loss in MCF-7 cell viability.

So far, the chemical compounds and biological activities of *Artocarpus altilis* from this region have not been reported. Thus, this study was carried out to determine the phytochemical constituents and investigate the antioxidants and antiproliferative effects of a liverwort, *Artocarpus altilis* from Endau Rompin Johor Malaysia.

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Figure 1: Leaves, Stem and Fruits of *A. altilis*

Materials and Method

Sample collection for bioactive activities
Fresh leaves of the Artocarpus altilis was collected from Endau Rompin forest on 5th May 2017, (2◦25"12.94"N, 103◦15"40.9"E) Endau Rompin forest is one of the few remaining areas of virgin lowland rainforest in the southern part of Peninsular Malaysia.

Plant materials and sample preparation
Plant materials collected from Kampung Peta in Endau Rompin Johor Malaysia. Voucher specimen was identified and deposited in Herbarium Department Technology and Natural Resources Universiti Tun Hussein Onn Malaysia, plant sample was carefully clean and rinse by using distilled water in order to remove contaminant or soil debris. The sample was dried in the shade at room temperature and ground in a mortar or dried in the oven for two days at 40^{0c}. the dried sample was grinded to fine powder using electric blinder, the ground sample was kept in zip lock bag and stored in a freezer (-20^{0C}) for further analysis (Jaberian, Piri, & Nazari, 2013; Saad et al., 2006).

Preparation of plant
Preparation of extracts The aqueous extract of *A. altilis* was prepared according to traditional use by the Jakun population as an infusion. The yield of this extraction was 32.15%. Organic extraction with different solvents was also performed. The yields extraction with hexane, ethyl acetate, and methanol were 6.22 %, 8.63 %, and 20.6 % respectively.

Sample Extraction
Ground sample was extracted using the method from (Abu Bakar et al., 2010) with slight modification. The extraction of plant samples using organic solvents (methanol, ethyl acetate and hexane) was carried out by successive maceration extraction as previous described by Bhunu, Mautsa, and Mukanganyama (2017) with few modifications. The mixture was filtered through a filter paper. The extract was used for the determination of phytochemicals and antioxidant, antiproliferative analysis.

Gas Chromatography- Mass Spectrometry (GC- MS) analysis
Gas chromatography equipped with mass spectrometry (GC-MS-2010 Plus- Shimadzu) was used to analyze the selected crude in order to identify the secondary metabolites present. The column (30.0 m length, 0.25mm ID, 0.25µm thickness) temperature was set for 4 min at the temperature of 50◦C, which was then increased to 300◦C at the rate of 3◦C/min, and then sustained for 10 min. The temperature of the injector was set at 250◦C and the volume is 0.1 L. The flow rate of the helium carrier gas was set to 1 mL/min with a total run duration of 60 min. Mass spectra were attained from the range m/z 40 to 700 and the electron ionization at 70 eV. The identification of the compounds was done by matching their mass spectra with the available library data. The interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns.

Determination of antioxidant activities

DPPH radical scavenging assay
1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was purchased from Sigma–Aldrich (USA). Butylated hydroxytoluene (BHT) and α-tocopherol was purchased from Merck (India) and are of analytical standard, in order to determine the radical scavenging ability, the method reported by Mensor *et al.* as adopted by (A. Bakar et al., 2015; Ghasemzadeh, Jaafar, Rahmat, & Ashkani, 2015; Kinabalu, 2012) was used. Briefly, 0.3 mM alcohol solution of DPPH (1 mL) was added to samples (2.5 mL) containing different concentrations originating from different varieties' extracts. The samples were first kept in a dark place at room temperature and their absorbance was read at 518 nm after 30 min. The antiradical activity (AA) was determined using the following formula:
$AA\% = 100 - [(Abs_{sample} - Abs_{empty\ sample})/Abs_{control} \times 100$ where Abs is absorbance
Empty sample= 1 mL methanol + 2.5 mL extract Control sample= 1 mL 0.3 mM DPPH + 2.5 mL methanol. IC_{50}, the amount of sample extracted into 1 mL solution necessary to decrease by 50% the initial DPPH concentration, was derived from the % disappearance versus concentration plot (at this point concentration means mg of sample extracted into 1.0 mL solution).

Ferric Reducing Antioxidant Power (FRAP) assay:
FRAP of various extracts of *A. altilis* was perform based on the method of (Benzie & Devaki, 2017). The assay mixture contained 2.5mL of 300mM acetate buffer at pH 3.6, 0.25mL of 10mM TPTZ solution in 40mM HCl, 0.25mL of 20mM FeCl3 and test substances in 0.1mL water, ethanol or methanol. The absorbance was measured after 30min incubation at 593nm. Standard graphs were constructed using known concentrations of ferrous salt in water, ethanol or methanol to replace FeCl3. All tests were run in triplicate and mean values will be used to calculate EC1 values. EC1 is defined as concentration of an antioxidant having a ferric reducing ability equivalent to that of mM ferrous salt. The final result was expressed as the concentration of antioxidant having a ferric reducing ability.

ABTS- decolourization assay
2,2'-azinobis(3-ethylbenzthiazoline)-6-sulphonic acid or ABTS free radical decolourization assay was done according to (Re et al., 1999) with some modifications. The pre-formed radical monocation of ABTS was generated by reacting ABTS solution (7mM) with 2.45 mm potassium persulfate (K2 S2 O8). The mixture was allowed to stand for 15 hrs in the dark at room temperature. The solution was diluted with hexane, ethyl acatate or methanol to obtain the absorbance of 0.7 ± 0.2 units at 734 nm. The aliquot of 200µl of each sample was added to 2000µl of ABTS free radical cation solution. The absorbance was monitored

for 5 min and it was measured spectrophotometrically at 734 nm using a spectrophotometer. Appropriate solvent blanks were run in each assay. The percentage inhibition was calculated against a control and compared to a Trolox standard curve (10-100 mm). The radical-scavenging activity was expressed in IC_{50}, the amount of sample extracted into 1 mL solution necessary to decrease by 50% the initial ABTS concentration.

2.6 Anticancer activities

2.6.1 Cell line and culture conditions

Breast cancer MCF-7 and MDA-MB-231 cells (American Type Culture Collection) were grown in RPMI 1640 media with L-glutamine. Both cell lines were supplemented with 10% FBS, 1% penicillin-streptomycin and incubated in CO_2 incubator at 37°C.

Drug preparation

Plants extracts was initially solubilized in dimethyl sulphoxide (DMSO) at a concentration of 10 mM, stored at 4°C and protected from the light. In all experiments, DMSO concentration never exceeded 1%, which has no effect on the cells. Doxorubicin (DOX) (20 mg/10 ml) was diluted in complete culture medium freshly before use and added to the cells at different concentrations.

MTT Assay

A method described by (Bsharat, 2013; Ruiz-Montanez et al., 2015; Scherließ, 2011; Zhou et al., 2016), with some modifications 6.9 x 10^5 of MCF-7 and MDA-MB-231 were seeded in 200µl CGM in 96-well plate in each well and treated for 24hr, 48h and 72hr respectively, A serial dilution was added into each well of the plate starting from row A to H from a concentration of 100 µg/ml. The plate was incubated for another 72 h. After that, 20µl of MTT reagent was added into each well and incubated for four more hours. In order to dissolve and solubilize the coloured crystals, 100µl of solubilisation solution, dimethyl sulfoxide was added into each well. The absorbance was finally read at 570 nm using ELISA reader (AWARENESS-State Fax, USA) from which the cytotoxicity was determined by the following formula:

$$\text{Cytotoxicity \%} = \frac{\text{Optical density of sample}}{\text{Optical density of control}} \times 100$$

The inhibition concentration (IC_{50}), concentration of extract that able to inhibit cell proliferation by 50% was calculated graphically for each cell proliferation curve.

Cell Cycle Analysis by Propidium Iodide (PI) Staining.

The cell cycle analysis was done according to method described by (Queiroz et al., 2014). A total of 1 x 106 cells were incubated and treated with the sample extracts at IC_{50} value for 24, 48 and 72 h. All adhering and floating cells were harvested and transferred to a sterile centrifuge tube. The cells were centrifuged using centrifuge machine at 4°C with 1,200 rpm for 10 min. Cells were washed using cold PBS and resuspended in 0.5 ml cold PBS. Ice-cold 70% ethanol was added to the cell suspension and incubated in -20°C for 2 h. The sample was then centrifuged and the ethanol was removed. The cells were washed twice using cold PBS before they were stained with 500µl of 10 µg/ml propidium iodide in 100 µg/ml of RNase for 30 min at room temperature in the dark. Cell cycle distribution was detected with flow cytometry. The results were analysed using Summit 4.3 software

Annexin V/PI apoptosis assay

The procedure was carried out according to (Tor et al., 2014a). The apoptosis was determined using Annexin V-FITC Apoptosis Detection Kit (Catalog Number APOAF, Sigma). Cells at a concentration of 1 x 106 cells were incubated and treated with the sample extracts at IC50 value for 24, 48 and 72 h. All adhering and floating cells were harvested, washed twice using PBS and transferred to a sterile centrifuge tube. The cells were suspended in binding buffer (100 mM HEPES/NaOH, pH 7.5 containing 1.4 M NaCl and 25 mM CaCl2) at a concentration of 1 x 106 cells per ml. A total of 500 µl of the cells were transferred to a 5 ml culture tube. After that, 5 µl of Annexin V-FITC conjugate and 10µl of propidium iodide were added to the cell suspension. The cells were incubated for 10 min at room temperature in the dark. The fluorescence of the cells was determined by flow cytometer. The results were analysed using Summit 4.3 software.

RESULTS

Antioxidants activity of the plants extracts

Extracts from different solvents are able to scavenge the free radicals indicating their potential as radical scavengers. All extracts reduced radicals significantly as compared to the same concentration standard, ascorbic acid scavenged the DPPH, FRAP and ABST radical by 97.7%, 76% and 47% respectively. Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers therefore, the observed high free radical scavenging activity of the methanol extract may have accounted to its polarity in this study the radical scavenging potential of the plants extract was found to be 91%, 87% and 22% in methanol, ethyl acetate and hexane extracts in DPPH respectively. While in FRAP it was found to be 65%, 48% and 39% in methanol, ethyl acetate and hexane respectively, and lastly in ABTS it was found to be 31%, 20% and 9% in methanol, ethyl acetate and hexane respectively which is comparable to ascorbic acid used as the positive control. The radical scavenging activities may be due the presence of some flavonoids with free hydroxyl group that can donate hydrogen and electron, this is agrees well with the literature reported for the antioxidant activity of *A. altilis.*

Table 1: Radical scavenging activities of the extracts of the leaves of *A. altilis*

Sample Name	Absorbance at 570	% Inhibition (100µg/mL)		
		DPPH	FRAP	ABTS
Blank	0.58	-	-	-
Ascorbic acid	0.12	97.0±0.21	76.5 ± 0.5	47.1 ± 0.81
Methanol extract	0.30	91.0±0.31	65.0±0.16	31.3±1.84
Ethyl acetate extract	0.10	87.4±0.05	48.1±0.11	20.2±0.69
Hexane extract	0.35	22.6±0.12	39.0±0.05	9.6±0.71

Anticancer activities of *A. altilis*

MTT assay.

The pattern for the inhibition of cell proliferation was very similar for both cell lines, All the samples showed reduction in proliferation on the two cell lines with both dose and time dependent pattern which became more obvious especially at higher (100 and 50µg/mL) concentrations.

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The *in vitro* cytotoxic activity (IC_{50}) of extracts was evaluated against estrogen independent breast cancer cell line (MDA-MB-231) and estrogen dependent cell line (MCF-7) breast cancer cells lines using the MTT assay. The IC_{50} values of extracts on the viability of cancer cells after 24hr, 48hr and 72hr of incubation are; in MCF-7, DOX was found to be most cytotoxic against the tested cell line with IC_{50} value of 5.87µg/mL, 3.23µg/mL and 1.98µg/mL for 24, 48 and 72 hrs respectively. While methanol extract of *A. altilis* also exhibits both high and moderate IC_{50} value of 11.53µg/mL and 15.71µg/mL at 72hr and 48hr while at 24hr the IC_{50} value was 41.17µg/ml. In MDA-MB-231, DOX was found to be most cytotoxic against the tested cell line with IC_{50} value of 11.21µg/mL, 8.1µg/mL and 3.07µg/mL for 24, 48 and 72 hrs respectively. Also methanol extract of *A. altilis* exhibits strong anti-proliferation activity with 2.15µg/ml, 3.87µg/ml and 18.67µg/ml at 72hr, 48hr and 24hr respectively.

Cell cycle Arrest

Cell cycle distribution in MCF- 7 (Figure 2) cells were studied after exposure to AAM extract at IC_{50} concentration for 24, 48 and 72 hours as shown fig 3 above). At 24hr there was significant arrest at S and G_2/M phases while the number of cells at Sub G_1 and G_0/G_1 were reduced significantly ($p<0.05$). At 48hr (B) for AAM there was significant arrest at S and G_2/M phases while the number of cells at Sub G_1 and G_0/G_1 were reduced significantly ($p<0.05$), At 72hr (C) in AAM significant arrest was noticed in Sub G_1 and G_0/G_1 phases while in S and G_2/M phases significant reduction in number of cells was observed. While Cell cycle distribution in MDA-MB-232 cells (figure 3) were studied after exposure to AAM extract at IC_{50} concentration for 24, 48 and 72 hrs as shown fig 4). At 24hr there was significant arrest in Sub G_1 and G_2/M phases, while reduction in number of cells was also observed in S and G_0/G_1 phases. At 48hr there was significant arrest in Sub G_1, S and G_2/M phases while number of cells was reduced significantly in G_0/G_1. At 72hr AAM also shows a significant arrest in Sub G_1, S and G_2/M phases while number of cells was reduced significantly in G_0/G_1.

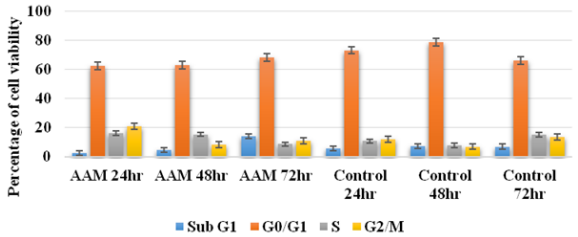

Figure 2: Cell cycle profile of MCF-7 cells treated with AAM extract

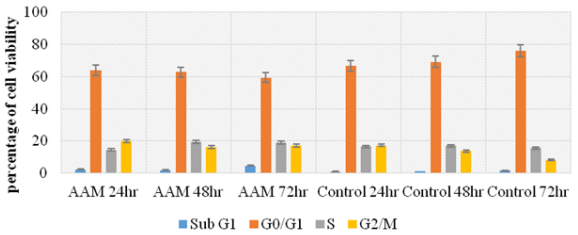

Figure 3: Cell cycle profile of MDA-MB-231 cells treated with AAM extract

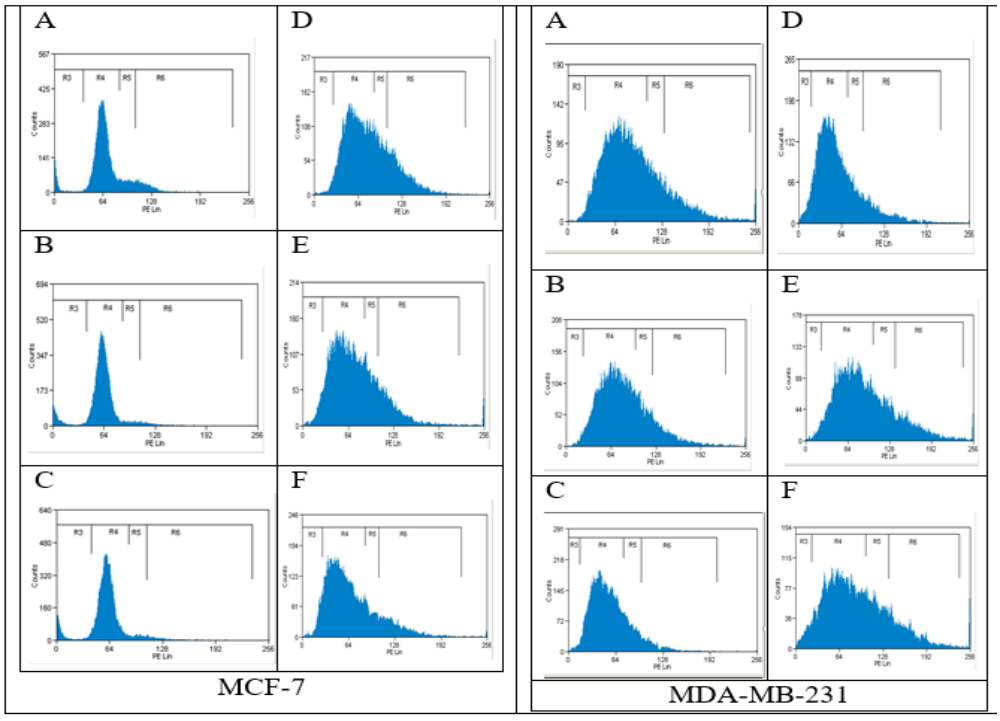

Figure 4: Flow cytometric scans of untreated (A-C) MCF-7 and MDA-MB-231 cancer cells those treated (D-F) with methanol crude extract of *A. altilis at* IC_{50} value for 24hr (A & D), 48hr (B & E) and 72hr (C & F). sectors P3-P6 represents the cells in sub G_1, G_0/G_1, S and G_2-M Phases respectively.

APOPTOSIS

Treatment with plants extracts causes apoptosis of both BCa. Cells were treated with IC_{50} value for 24, 48 and 72 hours. The cells were then stained with both FITC-conjugated Annexin V and PI. FACS was used to obtain the stained cell population. (Fig 5 and 6) Histograms from FACS analysis at each extract concentration. The FL1 channel was used to detect annexin-V-FITC staining and the FL2 channel was for PI staining. Graphic presentation (fig 5& 6) shows the percentage of early apoptotic cells stained with Annexin-V-FITC, apoptotic or necrotic and late apoptotic or late necrotic cells stained with both Annexin-V-FITC and PI at various durations of treatment. Plants extracts for the treatment was examined for 24, 48 and 72 hours in Bca.

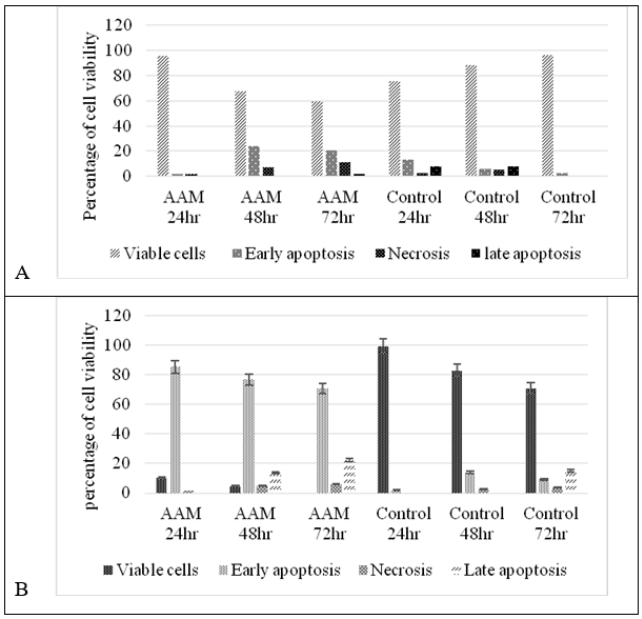

Figure 5: Apoptosis study of MCF-7 (A) and MDA- MB-231 (B) cells treated with methanol crude extract of *A. altilis* at IC50 value. Values are expressed as mean ± standard deviation (n=3). * showed a significant difference ($p < 0.05$) relative to their respective control. The distribution of cells undergoing early and late apoptosis together with those viable cells not in apoptosis and the total extent of apoptosis, was determined in MCF-7 cells treated with *A. altilis* extract for 24 h, 48 h and 72 h in comparison to their respective control, using Annexin-V FITC and propidium iodide flow cytometric analysis.

Figure 5 above shows the percentages of cell viability treated AAM extract in MCF-7 and MDA-MB-231 cells. In MCF-7 at 24hr 2.07% of the total cells were at early apoptotic stage, 1.64% at necrotic stage and 0.62% were in the late stage of apoptosis while 95.67% of cells were viable, and for the control culture 13.07% of the total cells were early apoptotic cells, 2.55% at apoptotic stage and 8.15% were in late stage of apoptosis while 75.43% of cells were viable. At 48hr AAM had 24.16% of the total cells were in early apoptosis, 7.37% at necrotic stage and 1.00% were in the late stage of apoptosis while 67.47% of cells were viable, while for the control culture 6.17% of the total cells were early apoptotic cells, 5.08% at apoptotic stage and 0.25% were in the late stage of apoptosis or necrosis while 88.49% of cells were viable. After 48hr of treatment the proportion of cells in the live cell group decreased significantly by 28.2%, while the proportion of cell at early apoptosis has increase with 22.09 %. So also the proportion of cells at necrotic stage has increased with 5.73%. Lastly, in late apoptotic stage the cells proportion also increases with 0.38%. But for the control culture the proportion of cell at early apoptosis has decrease with 6.9 %, late apoptotic stage also decreased with 7.9% while necrotic stage has increased with 2.53%, and number of viable cells also increased with 13.32%. At 72hr 20.57% of the total cells were early apoptotic cells, 11.50% at necrotic stage and 2.06% were in the late stage of apoptosis or necrosis while 59.69% of cells were viable. After 72hr of treatment the proportion of cells in the live cell group decreased significantly by 7.78%. While the proportion of cell at early apoptosis has increase with 3.99%. So also the proportion of cells at necrotic stage has increased with 4.13%. Lastly in late apoptotic stage the cells proportion also increases with 1.06%. But for the control culture at 72hr the proportion of cell at early apoptosis has decrease with 3.8 %, late apoptotic stage also decreased with 0.02% while apoptotic stage has increased with 4.08%, and number of viable cells also increased with 8.02**%.**

While in MDA-MB-231 at 24hr 4.07% of the total cells were early apoptotic cells, 85.47% at apoptotic stage and 0.04% were in the late stage of apoptosis or necrosis while 10.42% of cells were viable, for control culture 0.01% of the total cells were at early apoptotic cells, 0.01% at necrotic stage and 0.26% were in the late stage of apoptosis or necrosis while 99.72% of cells were viable. At 48hr 76.88% of the total cells were early apoptotic cells, 18.33% at apoptotic stage and 0.01% were in the late stage of apoptosis or necrosis while 4.78% of cells were viable, while for control culture 13.94% of the total cells were in early apoptotic phase, 2.48% at apoptotic stage and 0.62% were in the late stage of apoptosis or necrosis while 82.99% of cells were viable.

After 48hr of treatment the proportion of cells in the live cell group decreased significantly by 5.66%. While the proportion of cell at early apoptosis has increase with 12.81%, so also the proportion of cells at necrotic stage has increased with 6.14%. Lastly in late apoptotic stage the cells proportion also increases with 0.03%. But for the control culture the proportion of cell at early apoptosis has decrease with 12.93%, late apoptotic stage also decreased with 0.36% while apoptotic stage has increased with 2.47%, and number of viable cells also decreased with 16.73%. At 72hr, 70.79% of the total cells were early apoptotic cells, 5.84% at necrotic stage and 22.15% were in the late stage of apoptosis or necrosis while 1.22% of cells were viable, for control culture 9.80% of the total cells were early apoptotic cells, 3.56% at apoptotic stage and 15.24% were in the late stage of apoptosis or necrosis while 71.07% of cells were viable. After 72hr of treatment the proportion of cells in the live cell group decreased significantly by 33.0%. While the proportion of cell at early apoptosis has increase with 56.89%. So also the proportion of cells at necrotic stage has increased with 10.46%. Lastly in late apoptotic stage the cells proportion also increases with 11.01%. But for the control culture at 72hr the proportion of cell at early apoptosis has decrease with 4.14%, late apoptotic stage also decreased with 14.62% while apoptotic stage has increased with 1.08%, and number of viable cells also decreased with 11.92%.

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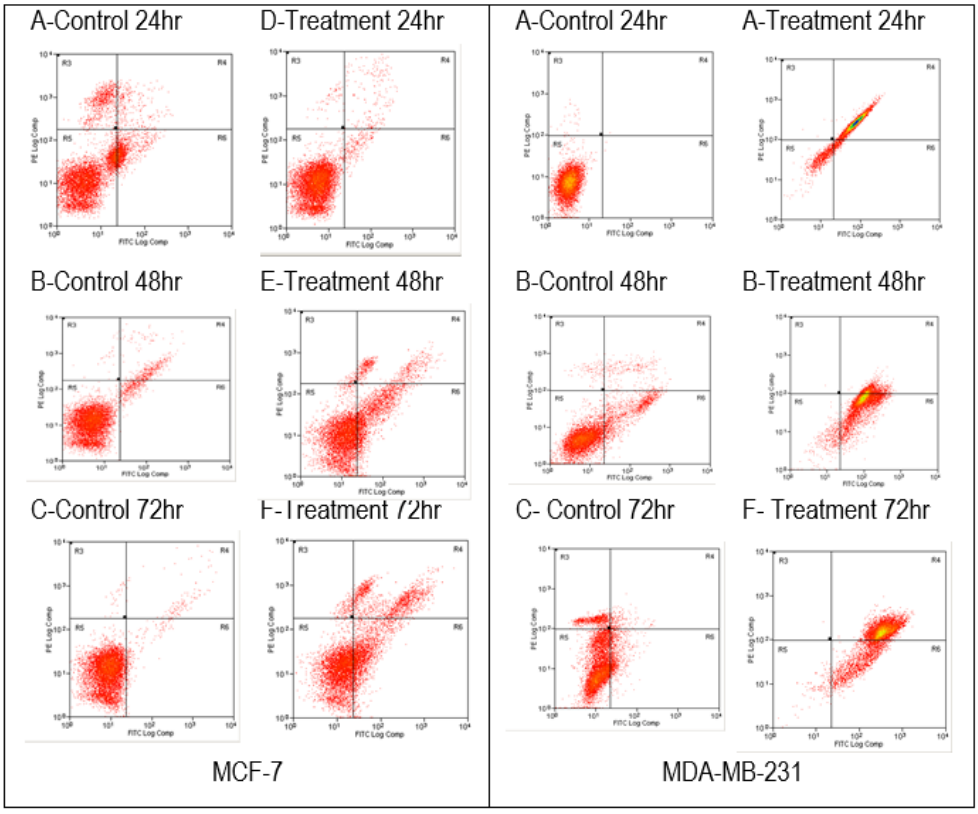

Figure 6: Annexin V FTIC of MCF-7 and MDA-MB-231 treated with AAM extract

Gas chromatography-mass spectroscopy (GC-MS) Analysis

The gas chromatography-mass spectroscopy (GC-MS) analysis was conducted on *Artocarpus altilis* methanol extract. The peaks (Figure 4.9) in the chromatogram were integrated and compared with the database of spectra of known compounds stored in the GC-MS libraries of National Institute Standard and Technology (NIST), WILEY229.LIB, Pfleger-Maurer-Weber-Drugs-and-. Pesticides Library for toxicology (PMW_tox2) and Flavour, Fragrance, Natural and Synthetic Compounds (FFNSC1.3.lib).

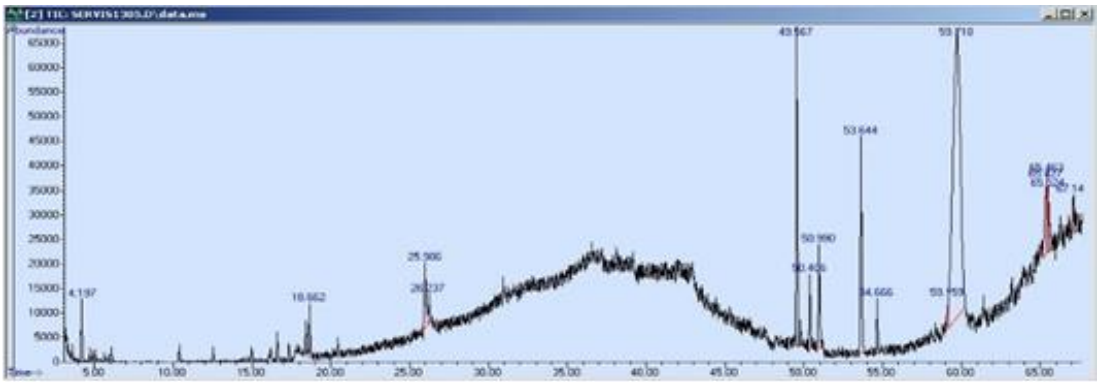

Figure 7: GC chromatograms of *A.altilis* methanol crude extract

The result of AAM revealed 50 peaks, with 45 compounds identified, representing 98.49% of the entire extract. The major among them were Acetic acid (34.68%), n-Hexadecanoic acid (14.89%), 1,2,3-Propanetriol, 1-acetate (Acetin) (7.28%), Hexadecanoate <methyl-> (4.01%), 7-Tetradecenal, (Z)- (2.92%) Glycerol. alpha. -monoacetate (2.80%), Phytol (2.46%), Octadecanoic acid (2.26%), Cholesterol (2.10%), Palmitic acid (1.35%), Linolenate <methyl-> (1.32%), Megastigmatrienone and 8-Oxabicyclo-oct-5-en-2-ol, 1,4,4-trimethyl (1.28%) each, 1,2,3-Propanetriol (1.23%) and 4,4,5,8-Tetramethylchroman-2-ol (1.22%).

DISCUSSION

Many studies have shown that many polyphenols contribute significantly to the antioxidant activity of *A. altilis* (Enos Tangke Arung, Wicaksono, et al., 2010) and act as highly effective free radical scavengers which is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Jalal et al., 2015).

The present *in vitro* study quantified in terms of percentage inhibition of a pre-formed free radical by antioxidants in each sample. Antioxidant was conducted to evaluate the antioxidant activities of the methanol, ethyl acetate and hexane extracts of *A. altilis* leaves All the extract demonstrated scavenging of stable DPPH, FRAP, and ABTS. The antioxidant potential of this species might be due to the phytochemical contents by acting as reducing agents or free radical scavengers and/or the synergistic effects by phenolics and flavonoids (Adaramola & Onigbinde, 2017; Ruiz-Montanez et al., 2015; Wan-Ibrahim, Sidik, & Kuppusamy, 2010; Zhang et al., 2017).

The GC-MS analysis of the *A. altilis* extract showed that Acetic acid has the highest concentration among detected secondary metabolites, in comparism with other previous studies, it showed that the major compound of *A. altilis* possess was Artocarpin, Artonin E and Phytol, and these are known to poses vast bioactivity such as antibacterial, anti-inflammatory, anti-fungal (Aparna *et al.,* 2012; Hameed *et al.,* 2015), antioxidant (Jaradat, Hussen, & Al Ali, 2015) pesticide, antioxidant, hypocholesterolemic nematicide, and 5-Alpha reductase inhibitor (Hamid *et al.,* 2017; Kumar, Kumaravel, & Lalitha, 2010).

Some previous studies proved that phytol exhibited cancer preventive and antioxidant (Martínez et al., 2012; Song & Cho, 2015; Thakor *et al.,* 2017), and breast cancer specifically (Balachandran *et al.,* 2016; Enriquez-Navas, Wojtkowiak, & Gatenby, 2015; Sheeja *et al.,* 2016). Thus, the effective anti-proliferative activities demonstrated by the selected crude extracts in this study might be contributed by the synergistic effect of this compound with the other compounds identified.

With respect to cytotoxicity and antiproliferative activity of the plants crude extracts, the results of cytotoxicity activities from this study is comparable to other similar data obtained by previous research (Foo *et al.,* 2015; Nur & Nugroho, 2018; Ruiz-Montanez et al., 2015). It is very crucial to know that according to the American National Cancer Institute (NCI), the criteria of cytotoxic activity for the crude extracts is an IC_{50} < 30 µg/ml (Talib & Mahasneh, 2010) while for pure compound or drug, IC_{50} value less than 4 µg/mL is considered potent (Nordin *et al.,* 2018). The less the IC_{50} value, the higher the potential of the tested extract to inhibit cell proliferation (Artika *et al.,*2017). The methanol extract of the plants extracts exhibited high antiproliferative potential against the tested cell lines with some significant differences in selectivity ($p < 0.05$).

It was reported in the study by (Wang et al., 2004) that five geranyl dihydrochalcones from the ethylacetate extract of A. altilis leaves had cytotoxic effects on some human cancer cell lines, such as human lung adenocarcinoma (SPC-A-1 cells), human colon carcinoma (SW-480 cells), and human hepatocellular carcinoma (SMMC-7721 cells). In a similar study by Arung et al. (2009) A. altilis extract shown cytotoxic effect on breast cancer cells (T47D) in a concentration dependent manner, with an extract IC50 6.19 µg/ml. A similar finding that artocarpin, which was tested on other breast cancer cells, MCF-7 and MDA-MB-231, showed cytotoxicity effect with an IC50 of 3.3 and 3.8 µg/ml, respectively (Enos *et al.,* 2018).

In this study, *A. altilis* methanol extract on MCF-7 has shown a significant arrest at S and G_2/M phases at 24 hr, while the number of cells at Sub G_1 and G_0/G_1 were reduced significantly ($p<0.05$). Similarly, at 48hr there was significant arrest at S and G_2/M phases while the number of cells at Sub G1 and G_0/G_1 were reduced significantly ($p<0.05$), and at 72hr significant arrest was noticed in Sub G1 and G_0/G_1 phases while in S and G2/M phases significant reduction in number of cells was observed. So also on MDA-MB-231 *A. altilis* methanol extract has shown a significant arrest at various phases on time dependent manner, at 24hr there was significant arrest in Sub G1 and G_2/M phases, while reduction in number of cells was also observed in S and G_0/G_1 phases. Similarly, at 48hr there was significant arrest in Sub G1, S and G_2/M phases while number of cells was reduced significantly in G_0/G_1. And also at 72hr there was significant arrest in Sub G1, S and G_2/M phases while number of cells was reduced significantly in G_0/G_1. Basically for the duration of treatment (72hr) in both the tested cells there was significant arrest at all the phases, this is clear indication that *A. altilis* effectively induced cell cycle arrest in a time-dependent manner.

This study is in line with previous studies on anti-proliferation activities of A. altilis, research by (Enos *et al.,* 2018) confirmed that A. altilis (Sukun wood extract) induced apoptosis in T47D cells in a concentration-dependent fashion at sub-G_1 phase. In another study by (Pardo-de-Santayana & Macía, 2015) revealed that artelastin (isolated from Artocarpus elasticus) showed accumulation of treated cells in S phase rather than a mitotic arrest in synchronized MCF-7 cells, and demonstrated that artelastin impairs the progression of G_0/G_1 and /S cells through S-phase, which is in accordance with the accumulation in S-phase detected by flow cytometry. Furthermore, the study shows that artelastin interferes with DNA replication hindering the progression of cells to complete duplication. This important effect may be another mechanism by which artelastin exerts its antiproliferative activity in MCF-7 cells. Similar study was carried out in Artonin E also isolated from *A. elasticus* by (Etti *et al.,* 2017) which shows that there was transiently arrested in treated MCF-7 at G_0/G_1 phase of the cell cycle upon treatment with 3 and 10 µM Artonin E. So also dose-dependent increase was observed in population of cells in the sub- G_0/G_1 phase when the exposure time was increased to 48 hours. This G_0/G_1 arrest was attributed to the upregulation of p21 that led to the downregulation of cyclin D as evident by the gene expression studies. Likewise, it was reported that, incubation of MDA-MB-231 with artonin E after 12 hours the percentage of cells in the G_2/M. phase increased marginally from compared to untreated control. There was also a significant ($p<0.05$) accumulation of cells in the sub G_0/G_1 phase, indicating the population of cell death. Upon increasing the time of exposure to 24 hours, the cells in the sub G_0/G_1 population increased significantly with a transient accumulation at the G_2/M phase (Etti *et al.,* 2017) Similar study was also conducted by (Rahman *et al.,* 2016) showed that Artonin E isolated from the

stem bark of *A. elasticus* induced a depletion of SKOV-3 cells in the S phase in a time-dependent manner. The accumulation of cells arrested at the S phase was significantly ($p<0.05$) increased at 72 h after treatment with a concurrent decrease in the proportion of cells in the G_0/G_1 phase. Results also indicate that the proportion of cells in the apoptosis stage increased significantly ($p<0.05$) in a time-dependent manner. It was reported that artocarpin isolated from A. heterophyllus induced a significant G_1 phase cell cycle arrest on colorectal cancer (CCD-18Co) which was followed by apoptosis, evidence by the sub-G_1 phase. Most likely, the persistent G_1 phase cell arrest mediated antiproliferative effect of artocarpin, and the following apoptotic and autophagic events should be secondary events of such mitotic block (Sun *et al.,* 2017; Sun & Tanumihardjo, 2007).

Comparable studies were carried out using different plants extract and on breast cancer cell line or different cancer cells. (Hu *et al.,*2010) reported that stained DNA of MCF-7 cells after treatment with 20-IM salidroside demonstrated that 67% of the cells were in the G1/G0 phase, 21% in the S phase, and 12% in the G_2/M phase. Stained DNA of MDA-MB-231 cells following treatment with 10-IM salidroside demonstrated that 48% of the cells were in the G_0/G_1 phase, 18% in the S phase, and 34% in the G_2/M phase. These suggested that 20-IM salidroside caused the G_0/G_1 arrest of MCF-7 cells and that 10-IM salidroside induced the G_2/M arrest of MDA-MB-231 cells. It's also reported that MCF-7 treated with *L. borneensis* extract altered the activity of protein and caused cell cycle arrest in G_0/G_1 phase and a parallel reduction in G_2 phase, an increase in number of cells in sub-G_1 indicated apoptosis event (Amaral *et al.,* 2018). According to (Yun *et al.,* 2005) tumor cell growth inhibitory effect of panduratin A in androgen-independent human prostate cancer cells PC-3 and DU145 has shown a remarkable cytotoxicity. It mediated apoptosis via both mitochondrial and death receptor pathways. Treatment by panduratin A led to G_2/M phase arrest in a dose-dependent manner by modulating expression of G_2/M regulatory molecules, including induction of p21/WAF1 and p27/Kip1 and downregulation of CDKs 2, 4, and 6 as well as decrease in cyclins D1 and E (Musthapa *et al.,* 2009). Similarly, isoliquiritigenin exhibited significant inhibitory effects against prostate cancer cell lines DU145 and LNCaP and caused S and G_2/M phase arrest due to the expression enhancement of GADD153 mRNA and protein associated with cell cycle arrest. This compound also stimulated transcriptional activity of GADD153 promoter dose dependently, Isoliquiritigenin also demonstrated the anti-proliferation of U87 glioma cells in vitro, and it caused cell cycle arrest at S and G_2/M phases and induced caspase mediated apoptosis pathway. Similar activity of isoliquiritigenin was also found in human lung cancer cells A549, in which cell cycle arrest at G_2/M phase was observed. Another study by Kang et al. (2005) indicates that adiponectin has an activity to arrest MDA-MB-231 cell growth at G_0/G_1.

A. altilis in both MCF-7 and MDA-MB-231 Most cells were found in G_1 this may be due the fact that G_1 phase is much longer than G_2 phase. Arrest in G_1 is thought to prevent aberrant replication of damaged DNA and arrest in G2 allows cells to avoid segregation of defective chromosomes (Agami & Bernards, 2000) Exposure of cells to DNA-damaging agents can result in perturbations of cell cycle progression and in cell death. The molecular mechanisms controlling these endpoints has important implications for both cancer causation and tumor responses to cytotoxic therapies (Xu, Kim, & Kastan, 2001) And the remaining arrest were found in S phase and G_2/M this is clear that S-phase arrest by potent inhibitors of microtubule assembly have been attributed to their interaction with microtubule proteins and/or spindle assembly checkpoints in different cells (Pedro *et al.,* 2005).

The cytotoxic effect of *A. altilis* may be due to the presence of compounds that possess cytotoxic effect like artocarpin, which was tested on breast cancer cells, MCF-7 and MDA-MB-231 and showed cytotoxicity effect (Wang *et al.*, 2004), and from other members of the same genus which includes; afforded friedelinol, squalene, bsitosterol, stigmasterol, phytol, polyprenol, cycloartenol, cycloartenol acetate and β-Sitosterol isolated form *A. camansi* has been shown to induce apoptosis in human tumors for colon and breast cancers (Tsai *et al.,* 2013) artelastin, artelastocarpin and carpilastofuran (from *A. elasticus*) (Cidade et al., 2001; Pedro et al., 2005). Norartacarpetin and Cudflavone B (from *A. communi)* (Hsu *et al.,* 2011; Ko et al., 2013), artoheterophyllin E-J (from *A. heterophyllus*) (Zheng *et al.,* 2013). On present study the morphological analysis has revealed that plants extracts treated MCF-7 and MDA-MB- 231 cells experienced cellular shrinkage, suggesting induction of apoptosis in the cells. Inhibition of the cell growth at IC_{50} concentration between 24 to 72 hours was due to cell cycle arrest at S and G_2/M, Sub G1 and, sub G1 and S phases in MCF-7 at 24and 48hr, at 72hr arrest at Sub G1 and G_0/G_1 in AAM as being confirmed by the flow cytometry analysis. On the other hand, MDA-MB- 231 cells also experienced cellular shrinkage, suggesting induction of apoptosis in the cells at arrest was made at Sub and G_2/M phases at 24hr. while at 48hr there was arrest at Sub G1, S and G_2/M also in 72 hr. However, crude extracts doses at 24-72hr arrest is a suggestion that the time of treatment may influence the effects on cell cycle.

The induction of apoptosis by plants crude in both MCF-7 cells and MDA-MB-231 was ascertained by the Annexin-V-FITC/PI-flow cytometry analysis (Figure 6). Apoptosis is characterized by distinct biochemical features in elimination of damaged cells or tumour cells without causing inflammation. The activation of enzymatic and catabolic processes in apoptosis thereby facilitate cell morphological changes such as externalization of plasma membrane phos- phatidylserine (PS), cellular shrinkage, membrane blebbing, chromatin condensation, nuclear fragmentation and formation of apoptotic bodies (Baunthiyal, Singh, & Dwivedi, 2017; Doonan & Cotter, 2008; Foo *et al.,* 2014). PS is a phospholipid component which has a strong binding affinity towards Annexin-V (Foo et al., 2014). In a normal cell, PS is positioned on the inner surface of the cell membrane and therefore inaccessible to Annexin-V. At an early stage of apoptosis, PS is translocated to the outside of the cell membrane and bind with Annexin-V. PS translocation is an irreversible event. The apoptotic bodies formed are eventually engulfed by phagocytes such as neutrophils and macrophages in in vivo. Since the in vitro system is lack of phagocytes, the membrane of the apoptotic bodies will rupture (also known as secondary necrosis) and accessible to the PI dye. Therefore, secondary necrotic cells are stained by both Annexin-V and PI (Tor *et al.,* 2014b). Even though plants crude extracts were confirmed to induce apoptosis in MCF-7 BCa cells, some other distinct morphological features of apoptosis such as membrane blebbing,

chromatin condensation, nuclear fragmentation and formation of apoptotic bodies were not observed. Subsequently here was no induction of caspase 3 observed when cells were exposed to extract. There might be no involvement of caspase 8 or caspase 9 which will lead to the activation of caspase 3. This is in line with previous finding by (Bakar *et al.,* 2015; Cao & Tait, 2018; Jaeschke, Duan, Akakpo, Farhood, & Ramachandran, 2018; Kutscher & Shaham, 2017; Mondal & Bennett, 2016; Sharif-Askari et al., 2001; Wei, Xu, Zhang, & Luo, 2018). On a similar study conducted by Arung *et al.,* (2010) stated that characteristics of cells undergoing apoptosis include the formation of sharply delineated, uniformly fine granular masses adjacent to the nuclear envelope and cytoplasmic condensation; breaking up of the nucleus into discrete fragments surrounded by a double layered envelope; and cell budding to produce membrane-bounded apoptotic bodies. In line with morphological analysis, a sub-G_1 apoptosis assay also confirmed that *A. altilis,* extracts induced apoptosis in MCF-7 and MDA-MB-231 cells in a time-dependent manner.

Similar cytotoxic effects were found on other Artocarpus species like *A. elasticus* (Musthapa et al., 2009), *A. obtusus* (Hashim et al., 2012), *A. heterophyllus* (Patel & Patel, 2011; Ruiz-Montanez et al., 2015), *A. odoratissimus* (Bakar & Bakar, 2018), *A. camansi* (Tantengco & Jacinto, 2015), *A. integrifolia* (Ahmed, Mohammed, & Veerappan, 2015), *A. chama* (Wang et al., 2004), *A. rotunda* (Suhartati et al., 2001) *A. lanceifolius* (Hakim *et al.,* 2002) and *A. fretessi* (Soekamto *et al.,*2003). This may be due the presence of identified compounds that was reported to be highly cytotoxic against some human cancer cells, and possess some biological activities such as inhibition of 5a-reductase, antioxidant, antiplatelet, and antibacterial activities, antiherpetic activity, melanin inhibition , inhibition of lipopolysaccharide-induced nitric oxide production, and skin whitening activity (Enos *et al.*, 2009).

Conclusion

In conclusion, the work presented herein has demonstrated that the methanol extract of A. altilis had strong anti-proliferative activities when compared with standard drug. the antioxidant activities displayed by the methanol, ethylacetate and hexane were significant compared with ascorbic acid indicating the potential of the leaves of this species as natural antioxidants. Therefore, biological activities displayed by the leaves extracts corroborate the traditional uses of this plant against various ailments including braest cancer.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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REFERENCES

Abu Bakar et al. (2010). Cytotoxicity, cell cycle arrest, and apoptosis in breast cancer cell lines exposed to an extract of the seed kernel of Mangifera pajang (bambangan). *Food and chemical toxicology, 48*(6), 1688-1697.

Adaramola, B., & Onigbinde, A. (2017). Influence of extraction technique on the mineral content and antioxidant capacity of edible oil extracted from ginger rhizome. *Chem Int, 3*(1), 1-7.

Agami, R., & Bernards, R. (2000). Distinct initiation and maintenance mechanisms cooperate to induce G1 cell cycle arrest in response to DNA damage. *Cell, 102*(1), 55-66.

Ahmed, K. B. A., Mohammed, A. S., & Veerappan, A. (2015). Interaction of sugar stabilized silver nanoparticles with the T-antigen specific lectin, jacalin from Artocarpus integrifolia. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 145*, 110-116.

Amaral, I., Silva, C., Correia-Branco, A., & Martel, F. (2018). Effect of metformin on estrogen and progesterone receptor-positive (MCF-7) and triple-negative (MDA-MB-231) breast cancer cells. *Biomedicine & Pharmacotherapy, 102*, 94-101.

Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., & Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical biology & drug design, 80*(3), 434-439.

Artika, I. M., Julistiono, H., Bermawie, N., Riyanti, E. I., & Hasan, A. E. Z. (2017). Anticancer activity test of ethyl acetate extract of endophytic fungi isolated from soursop leaf (Annona muricata L.). *Asian Pacific journal of tropical medicine, 10*(6), 566-571.

Arung, E. T., Muladi, S., Sukaton, E., Shimizu, K., & Kondo, R. (2018). Artocarpin, a promising compound as whitening agent and anti-skin cancer. *Jurnal Ilmu dan Teknologi Kayu Tropis, 6*(1), 33-36.

Arung, E. T., Wicaksono, B. D., Handoko, Y. A., Kusuma, I. W., Shimizu, K., Yulia, D., & Sandra, F. (2010). Cytotoxic effect of artocarpin on T47D cells. *Journal of natural medicines, 64*(4), 423-429.

Arung, E. T., Wicaksono, B. D., Handoko, Y. A., Kusuma, I. W., Yulia, D., & Sandra, F. (2009). Anti-cancer properties of diethylether extract of wood from sukun (Artocarpus altilis) in human breast cancer (T47D) cells. *Tropical Journal of Pharmaceutical Research, 8*(4).

Arung, E. T., Yoshikawa, K., Shimizu, K., & Kondo, R. (2010). Isoprenoid-substituted flavonoids from wood of Artocarpus heterophyllus on B16 melanoma cells: cytotoxicity and structural criteria. *Fitoterapia, 81*(2), 120-123.

Bakar, A., Fadzelly, M., Abdul Karim, F., Suleiman, M., Isha, A., & Rahmat, A. (2015). Phytochemical constituents, antioxidant and antiproliferative properties of a liverwort, Lepidozia borneensis Stephani from Mount Kinabalu, Sabah, Malaysia. *Evidence-based Complementary and Alternative Medicine, 2015*.

Bakar, F. I. A., & Bakar, M. F. A. (2018). Tarap—Artocarpus odoratissimus *Exotic Fruits* (pp. 413-418): Elsevier.

Balachandran, P., V Ajay Kumar, T., & Parthasarathy, V. (2016). Screening of potential anticancer compounds from Sargassum wightii to target breast Cancer specific HER2 receptor using in-silico analysis. *The natural products journal, 6*(2), 108-115.

Baunthiyal, M., Singh, V., & Dwivedi, S. (2017). Insights of

Antioxidants as Molecules for Drug Discovery. *International Journal of Pharmacology, 13*(7), 874-889.

Benzie, I. F., & Devaki, M. (2017). The 5 ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications. *Measurement of Antioxidant Activity and Capacity: Recent Trends and Applications*, 77.

Bhunu, B., Mautsa, R., & Mukanganyama, S. (2017). Inhibition of biofilm formation in Mycobacterium smegmatis by Parinari curatellifolia leaf extracts. *BMC complementary and alternative medicine, 17*(1), 285.

Bsharat, M. M. M. (2013). *In vitro cytotoxic and cytostatic activities of plants used in traditional Arabic herbal medicine to treat cancer in Palestine.* Faculty of Graduate Studies In vitro cytotoxic and cytostatic activities of plants used in traditional Arabic herbal medicine to treat cancer in Palestine By Myasar Mohammed Mahmoud Bsharat Supervisors Prof. Mohammed S. Ali-Shtayeh Co-Supervisor Prof. Bashar Saad This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master in Life Sciences (Biology), Faculty of Graduate studies, An-Najah National University.

Cao, K., & Tait, S. W. (2018). Apoptosis and Cancer: Force Awakens, Phantom Menace, or Both? *International review of cell and molecular biology* (Vol. 337, pp. 135-152): Elsevier.

Cidade, H. M., Nacimento, M. S. J., Pinto, M. M., Kijjoa, A., Silva, A. M., & Herz, W. (2001). Artelastocarpin and carpelastofuran, two new flavones, and cytotoxicities of prenyl flavonoids from Artocarpus elasticus against three cancer cell lines. *Planta medica, 67*(09), 867-870.

DeSantis, C. E., Fedewa, S. A., Goding Sauer, A., Kramer, J. L., Smith, R. A., & Jemal, A. (2016). Breast cancer statistics, 2015: Convergence of incidence rates between black and white women. *CA: a cancer journal for clinicians, 66*(1), 31-42.

DeSantis, C. E., Ma, J., Goding Sauer, A., Newman, L. A., & Jemal, A. (2017). Breast cancer statistics, 2017, racial disparity in mortality by state. *CA: a cancer journal for clinicians, 67*(6), 439-448.

Doonan, F., & Cotter, T. G. (2008). Morphological assessment of apoptosis. *Methods, 44*(3), 200-204.

Enriquez-Navas, P. M., Wojtkowiak, J. W., & Gatenby, R. A. (2015). Application of evolutionary principles to cancer therapy. *Cancer research*.

Etti, I. C., Abdullah, R., Kadir, A., Hashim, N. M., Yeap, S. K., Imam, M. U., . . . Etti, U. (2017). The molecular mechanism of the anticancer effect of Artonin E in MDA-MB 231 triple negative breast cancer cells. *PloS one, 12*(8), e0182357.

Etti, I. C., Rasedee, A., Hashim, N. M., Abdul, A. B., Kadir, A., Yeap, S. K., . . . Etti, C. J. (2017). Artonin E induces p53-independent G1 cell cycle arrest and apoptosis through ROS-mediated mitochondrial pathway and livin suppression in MCF-7 cells. *Drug design, development and therapy, 11*, 865.

Foo, J. B., Yazan, L. S., Tor, Y. S., Armania, N., Ismail, N., Imam, M. U., . . . Ismail, M. (2014). Induction of cell cycle arrest and apoptosis in caspase-3 deficient MCF-7 cells by Dillenia suffruticosa root extract via multiple signalling pathways. *BMC complementary and alternative medicine, 14*(1), 197.

Foo, J. B., Yazan, L. S., Tor, Y. S., Wibowo, A., Ismail, N., How, C. W., . . . Cheah, Y. K. (2015). Induction of cell cycle arrest and apoptosis by betulinic acid-rich fraction from Dillenia suffruticosa root in MCF-7 cells involved p53/p21 and mitochondrial signalling pathway. *Journal of ethnopharmacology, 166*, 270-278.

Ghasemzadeh, A., Jaafar, H. Z., Rahmat, A., & Ashkani, S. (2015). Secondary metabolites constituents and antioxidant, anticancer and antibacterial activities of Etlingera elatior (Jack) RM Sm grown in different locations of Malaysia. *BMC complementary and alternative medicine, 15*(1), 1.

Hameed, I. H., Hussein, H. J., Kareem, M. A., & Hamad, N. S. (2015). Identification of five newly described bioactive chemical compounds in methanolic extract of Mentha viridis by using gas chromatography-mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy, 7*(7), 107-125.

Hamid, A., Kaushal, T., Ashraf, R., Singh, A., Gupta, A. C., Prakash, O., . . . Khan, F. (2017). (22β, 25R)-3β-Hydroxy-spirost-5-en-7-iminoxy-heptanoic acid exhibits anti-prostate cancer activity through caspase pathway. *Steroids, 119*, 43-52.

Hari, A., Revikumar, K., & Divya, D. (2014). Artocarpus: A review of its phytochemistry and pharmacology. *Journal of Pharma Search, 9*(1), 7.

Hashim, N. M., Rahmani, M., Ee, G. C. L., Sukari, M. A., Yahayu, M., Amin, M. A. M., . . . Go, R. (2012). Antioxidant, antimicrobial and tyrosinase inhibitory activities of xanthones isolated from Artocarpus obtusus FM Jarrett. *Molecules, 17*(5), 6071-6082.

Hsu, C.-L., Shyu, M.-H., Lin, J.-A., Yen, G.-C., & Fang, S.-C. (2011). Cytotoxic effects of geranyl flavonoid derivatives from the fruit of Artocarpus communis in SK-Hep-1 human hepatocellular carcinoma cells. *Food Chemistry, 127*(1), 127-134.

Hu, X., Zhang, X., Qiu, S., Yu, D., & Lin, S. (2010). Salidroside induces cell-cycle arrest and apoptosis in human breast cancer cells. *Biochemical and biophysical research communications, 398*(1), 62-67.

Jaberian, H., Piri, K., & Nazari, J. (2013). Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. *Food Chemistry, 136*(1), 237-244.

Jaeschke, H., Duan, L., Akakpo, J. Y., Farhood, A., & Ramachandran, A. (2018). The role of apoptosis in acetaminophen hepatotoxicity. *Food and chemical toxicology, 118*, 709-718.

Jalal, T. K., Ahmed, I. A., Mikail, M., Momand, L., Draman, S., Isa, M. L. M., . . . Wahab, R. A. (2015). Evaluation of antioxidant, total phenol and flavonoid content and antimicrobial activities of Artocarpus altilis (breadfruit) of underutilized tropical fruit extracts. *Applied biochemistry and biotechnology, 175*(7), 3231-3243.

Jaradat, N., Hussen, F., & Al Ali, A. (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of Ephedra alata Decne. *J Mater Environ Sci, 6*(6), 1771-1778.

Kang, J. H., Lee, Y. Y., Yu, B. Y., Yang, B.-S., Cho, K.-H., Yoon, D. K., & Roh, Y. K. (2005). Adiponectin induces growth arrest and apoptosis of MDA-MB-231 breast cancer cell. *Archives of pharmacal research, 28*(11), 1263-1269.

Kinabalu, K. (2012). Phytochemicals and antioxidant properties of

different parts of Camellia sinensis leaves from Sabah Tea Plantation in Sabah, Malaysia.

Ko, H.-H., Tsai, Y.-T., Yen, M.-H., Lin, C.-C., Liang, C.-J., Yang, T.-H., . . . Yen, F.-L. (2013). Norartocarpetin from a folk medicine Artocarpus communis plays a melanogenesis inhibitor without cytotoxicity in B16F10 cell and skin irritation in mice. *BMC complementary and alternative medicine, 13*(1), 348.

Kumar, P. P., Kumaravel, S., & Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. *African Journal of Biochemistry Research, 4*(7), 191-195.

Kutscher, L. M., & Shaham, S. (2017). Non-apoptotic cell death in animal development. *Cell death and differentiation, 24*(8), 1326.

Martínez, R., Torres, P., Meneses, M., Figueroa, J., Pérez-Álvarez, J., & Viuda-Martos, M. (2012). Chemical, technological and in vitro antioxidant properties of cocoa (Theobroma cacao L.) co-products. *Food Research International, 49*(1), 39-45.

Mondal, A., & Bennett, L. L. (2016). Resveratrol enhances the efficacy of sorafenib mediated apoptosis in human breast cancer MCF7 cells through ROS, cell cycle inhibition, caspase 3 and PARP cleavage. *Biomedicine & Pharmacotherapy, 84*, 1906-1914.

Musthapa, I., Juliawaty, L. D., Syah, Y. M., Hakim, E. H., Latip, J., & Ghisalberti, E. L. (2009). An oxepinoflavone from Artocarpus elasticus with cytotoxic activity against P-388 cells. *Archives of pharmacal research, 32*(2), 191.

Nordin, M. L., Kadir, A. A., Zakaria, Z. A., Abdullah, R., & Abdullah, M. N. H. (2018). In vitro investigation of cytotoxic and antioxidative activities of Ardisia crispa against breast cancer cell lines, MCF-7 and MDA-MB-231. *BMC complementary and alternative medicine, 18*(1), 87.

Nur, R. M., & Nugroho, L. H. (2018). Cytotoxic Activities of Fractions from Dioscorea bulbifera L. Chloroform and Methanol Extracts on T47D Breast Cancer Cells. *Pharmacognosy Journal, 10*(1).

Pardo-de-Santayana, M., & Macía, M. J. (2015). Biodiversity: the benefits of traditional knowledge. *Nature, 518*(7540), 487.

Patel, R. M., & Patel, S. K. (2011). Cytotoxic activity of methanolic extract of Artocarpus heterophyllus against A549, Hela and MCF-7 cell lines. *Journal of Applied Pharmaceutical Science, 1*(7), 167-171.

Pedro, M., Ferreira, M. M., Cidade, H., Kijjoa, A., Bronze-da-Rocha, E., & Nascimento, M. S. J. (2005). Artelastin is a cytotoxic prenylated flavone that disturbs microtubules and interferes with DNA replication in MCF-7 human breast cancer cells. *Life sciences, 77*(3), 293-311.

Queiroz, E. A., Puukila, S., Eichler, R., Sampaio, S. C., Forsyth, H. L., Lees, S. J., . . . Khaper, N. (2014). Metformin induces apoptosis and cell cycle arrest mediated by oxidative stress, AMPK and FOXO3a in MCF-7 breast cancer cells. *PloS one, 9*(5), e98207.

Rahman, M. A., Ramli, F., Karimian, H., Dehghan, F., Nordin, N., Ali, H. M., . . . Hashim, N. M. (2016). Artonin E induces apoptosis via mitochondrial dysregulation in SKOV-3 ovarian cancer cells. *PloS one, 11*(3), e0151466.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine, 26*(9-10), 1231-1237.

Ruiz-Montanez, G., Burgos-Hernandez, A., Calderon-Santoyo, M., Lopez-Saiz, C., Velazquez-Contreras, C., Navarro-Ocana, A., & Ragazzo-Sánchez, J. (2015). Screening antimutagenic and antiproliferative properties of extracts isolated from Jackfruit pulp (Artocarpus heterophyllus Lam). *Food Chemistry, 175*, 409-416.

Saad, B., Dakwar, S., Said, O., Abu-Hijleh, G., Battah, F. A., Kmeel, A., & Aziazeh, H. (2006). Evaluation of medicinal plant hepatotoxicity in co-cultures of hepatocytes and monocytes. *Evidence-Based Complementary and Alternative Medicine, 3*(1), 93-98.

Scherließ, R. (2011). The MTT assay as tool to evaluate and compare excipient toxicity in vitro on respiratory epithelial cells. *International journal of pharmaceutics, 411*(1-2), 98-105.

Shamaun, S. S., Rahmani, M., Hashim, N. M., Ismail, H. B. M., Sukari, M. A., Lian, G. E. C., & Go, R. (2010). Prenylated flavones from Artocarpus altilis. *Journal of natural medicines, 64*(4), 478-481.

Sharif-Askari, E., Alam, A., Rhéaume, E., Beresford, P. J., Scotto, C., Sharma, K., . . . Lieberman, J. (2001). Direct cleavage of the human DNA fragmentation factor-45 by granzyme B induces caspase-activated DNase release and DNA fragmentation. *The EMBO Journal, 20*(12), 3101-3113.

Sheeja, L., Lakshmi, D., Bharadwaj, S., & Parveen, K. S. (2016). Anticancer activity of phytol purified from Gracilaria edulis against human breast cancer cell line (MCF-7). *Int J Curr Sci, 19*(4), 36-46.

Smith, R. A., & DeSantis, C. E. (2018). Breast cancer epidemiology. *Breast Imaging*.

Soekamto, N. H., Achmad, S. A., Ghisalberti, E. L., Hakim, E. H., & Syah, Y. M. (2003). Artoindonesianins X and Y, two isoprenylated 2-arylbenzofurans, from Artocarpus fretessi (Moraceae). *Phytochemistry, 64*(4), 831-834.

Song, Y., & Cho, S. K. (2015). Phytol induces apoptosis and ROS-mediated protective autophagy in human gastric adenocarcinoma AGS cells. *Biochemistry and Analytical Biochemistry, 4*(4), 1.

Suhartati, T., Achmad, S. A., Aimi, N., Hakim, E. H., Kitajima, M., Takayama, H., & Takeya, K. (2001). Artoindonesianin L, a new prenylated flavone with cytotoxic activity from Artocarpus rotunda. *Fitoterapia, 72*(8), 912-918.

Sun, G., Zheng, Z., Lee, M.-H., Xu, Y., Kang, S., Dong, Z., . . . Chen, W. (2017). Chemoprevention of colorectal cancer by artocarpin, a dietary phytochemical from Artocarpus heterophyllus. *Journal of agricultural and food chemistry, 65*(17), 3474-3480.

Sun, T., & Tanumihardjo, S. (2007). An integrated approach to evaluate food antioxidant capacity. *Journal of Food Science, 72*(9), R159-R165.

Taib, N. A., Akmal, M., Mohamed, I., & Yip, C.-H. (2011a). Improvement in Survival of Breast Cancer Patients–Trends in Survival over Two Time Periods in a Single Institution in an Asia Pacific Country, Malaysia. *Asian Pacific J Cancer Prev, 12*, 345-349.

Taib, N. A., Akmal, M., Mohamed, I., & Yip, C.-H. (2011b). Improvement in survival of breast cancer patients—trends in survival over two time periods in a single institution in an Asia Pacific Country, Malaysia. *Asian Pacific J Cancer Prev, 12*, 345-349.

Talib, W. H., & Mahasneh, A. M. (2010). Antiproliferative activity of plant extracts used against cancer in traditional medicine. *Scientia pharmaceutica, 78*(1), 33.

Tantengco, O. A. G., & Jacinto, S. D. (2015). Cytotoxic activity of crude extracts and fractions from Premna odorata (Blanco), Artocarpus camansi (Blanco) and Gliricidia sepium (Jacq.) against selected human cancer cell lines. *Asian Pacific Journal of Tropical Biomedicine, 5*(12), 1037-1041.

Thakor, P., Subramanian, R. B., Thakkar, S. S., Ray, A., & Thakkar, V. R. (2017). Phytol induces ROS mediated apoptosis by induction of caspase 9 and 3 through activation of TRAIL, FAS and TNF receptors and inhibits tumor progression factor Glucose 6 phosphate dehydrogenase in lung carcinoma cell line (A549). *Biomedicine & Pharmacotherapy, 92*, 491-500.

Tor, Y. S., Yazan, L. S., Foo, J. B., Armania, N., Cheah, Y. K., Abdullah, R., . . . Ismail, M. (2014a). Induction of apoptosis through oxidative stress-related pathways in MCF-7, human breast cancer cells, by ethyl acetate extract of Dillenia suffruticosa. *BMC complementary and alternative medicine, 14*(1), 55.

Tor, Y. S., Yazan, L. S., Foo, J. B., Armania, N., Cheah, Y. K., Abdullah, R., . . . Ismail, M. (2014b). Induction of apoptosis through oxidative stress-related pathways in MCF-7, human breast cancer cells, by ethyl acetate extract of Dillenia suffruticosa. *BMC complementary and alternative medicine, 14*(1), 1.

Tsai, P.-W., De Castro-Cruz, K. A., Shen, C.-C., Chiou, C.-T., & Ragasa, C. Y. (2013). Chemical constituents of Artocarpus camansi. *Pharmacognosy Journal, 5*(2), 80-82.

Wan-Ibrahim, W., Sidik, K., & Kuppusamy, U. (2010). A high antioxidant level in edible plants is associated with genotoxic properties. *Food Chemistry, 122*(4), 1139-1144.

Wang, Y.-H., Hou, A.-J., Chen, L., Chen, D.-F., Sun, H.-D., Zhao, Q.-S., . . . Lee, K.-H. (2004). New Isoprenylated Flavones, Artochamins A− E, and Cytotoxic Principles from Artocarpus c hama. *Journal of natural products, 67*(5), 757-761.

Wei, R., Xu, Y., Zhang, J., & Luo, B. (2018). Programmed Cell Death in CIRI *Cerebral Ischemic Reperfusion Injuries (CIRI)* (pp. 57-82): Springer.

Xu, B., Kim, S.-t., & Kastan, M. B. (2001). Involvement of Brca1 in S-phase and G2-phase checkpoints after ionizing irradiation. *Molecular and cellular biology, 21*(10), 3445-3450.

Yun, J.-M., Kwon, H., Mukhtar, H., & Hwang, J.-K. (2005). Induction of apoptosis by panduratin A isolated from Kaempferia pandurata in human colon cancer HT-29 cells. *Planta medica, 71*(06), 501-507.

Zhang, Z., Lin, D., Li, W., Gao, H., Peng, Y., & Zheng, J. (2017). Sensitive bromine-based screening of potential toxic furanoids in Dioscorea bulbifera L. *Journal of Chromatography B, 1057*, 1-14.

Zheng, X.-l., Wei, J.-h., Sun, W., Li, R.-t., Liu, S.-b., & Dai, H.-f. (2013). Ethnobotanical study on medicinal plants around Limu Mountains of Hainan Island, China. *Journal of ethnopharmacology, 148*(3), 964-974.

Zhou, J.-B., Peng, G., Li, J., Jia, Y.-C., Wang, J., Nie, S., & Zhang, Q.-Y. (2016). Anticancer Activity of Tetrahydrocorysamine against Pancreatic Adenocarcinoma Cell Line PANC-1 In vitro and In vivo. *Tropical Journal of Pharmaceutical Research, 15*(1), 141-148