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Research Article

Antiproliferative Activity of Xanthones Isolated from *Artocarpus obtusus*

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An investigation of the chemical constituents in *Artocarpus obtusus* species led to the isolation of three new xanthones, pyranocycloartobiloxanthone A (**1**), dihydroartoindonesianin C (**2**), and pyranocycloartobiloxanthone B (**3**). The compounds were subjected to antiproliferative assay against human promyelocytic leukemia (HL60), human chronic myeloid leukemia (K562), and human estrogen receptor (ER+) positive breast cancer (MCF7) cell lines. Pyranocycloartobiloxanthone A (**1**) consistently showed strong cytotoxic activity against the three cell lines compared to the other two with IC_{50} values of 0.5, 2.0 and 5.0 μg/mL, respectively. Compound (**1**) was also observed to exert antiproliferative activity and apoptotic promoter towards HL60 and MCF7 cell lines at respective IC_{50} values. The compound (**1**) was not toxic towards normal cell lines human nontumorigenic breast cell line (MCF10A) and human peripheral blood mononuclear cells (PBMCs) with IC_{50} values of more than 30 μg/mL.

1. Introduction

Tropical rain forests contain a lot of interesting pharmacologically active constituents and many more are still waiting to be discovered as they still offer undoubtedly valuable and amazing chemical entities. The World Health Organization (WHO) reported that 11% of the 252 drugs considered as basic and essential in this century were exclusively origin from flowering plants [1]. Many impressive modern drugs available in the market were derived from plants source and most of them were based on their ethnomedicinal uses. Hence, many works on tropical plants from Malaysia are being and have been extensively studied as well as their biological activity. One of them is *Artocarpus* species. The *Artocarpus* species is one of the genus in the Moraceae family [2] and is made of about 55 species. The genus is widely distributed throughout subtropical and tropical region of the Indian subcontinent south of the Himalayas, Sri Lanka, Burma, Thailand, Indo-China, Southern China, Taiwan, Hainan, Malesia, and Melanesia [2, 3]. Some parts of the plants are also used in traditional medicine preparations for the treatment of various diseases such as diarrhea, fever, liver cirrhosis, hypertension, diabetes, inflammation, malaria, ulcers, wound, and for tapeworm infection [4–8]. Many interesting biological activities and phytochemical work have been carried out on the plants with the identification of various classes of interesting phenolic compounds [9–18]. Our previous works on the stem bark of *A. altilis* and *A. obtusus* have led to the isolation of prenylated flavonoids and xanthones [19, 20].

In preliminary screening for cytotoxic, antioxidant and antimicrobial activity of thirteen Malaysian *Artocarpus* species has resulted in the potential of a few species for further examination in particular *A. obtusus* Jarret [13]. The plant is endemic to Sarawak, and, in continuation of our investigation on Malaysian plants, we now described the antiproliferative activity of three new xanthones isolated from the stem bark of *A. obtusus*. The cytotoxic activity

was tested using MTT assay against human promyelocytic leukemia (HL60), human chronic myeloid leukemia (K562), and human estrogen receptor (ER+) positive breast cancer (MCF7). The most active xanthone was further tested against mouse embryo fibroblast (3T3), human cervical cancer (HeLa), human estrogen receptor (ER−) negative (MDA-MB 231), human colon cancer (HT29) and human hepatocarcinoma (HepG2), human nontumorigenic breast cell line (MCF10A), and human peripheral blood mononuclear cells (PBMCs). The potential compound was also analyzed for its antiproliferative activity and effect on cell morphology of HL60 and MCF7 cell lines at selected concentrations using BrdU and acridine orange and propium iodide staining, respectively. Currently, there is no recognized ethnomedicinal data and reported biological activity for *A. obtusus*; thus, this is the first report on antiproliferative activity studies of the isolated xanthones from the plant as well as the isolation and structural elucidation of compound (**3**).

2. Materials and Methods

2.1. General Experimental Procedures. All melting points (m.p.) were determined using a hot stage melting point apparatus model Leica GALEN III equipped with microscope and are uncorrected. UV and IR spectra were measured with Shimadzu UV 2100 and Perkin Elmer FTIR (model 1725X) spectrophotometers, respectively. The ^{1}H-NMR and ^{13}C-NMR spectra were obtained with JEOL ECA-400 spectrometer operating at 400 and 100 MHz with tetramethylsilane (TMS) as an internal standard, respectively. The MS were obtained with Shimadzu GCMS-QP5050 spectrometer with Direct Induction Probe (DIP) using ionization induced by electron impact at 70 eV. Column chromatography was carried out using Merck Si gel 60 No. 1.07734, Darmstadt, Germany. Analytical thin layer chromatography (TLC) was performed on commercially available Merck TLC plastic sheet precoated Kieselgel 60 F_{254}, 0.2 mm thickness, and the chromatotron plates were coated with Kieselgel 60 F_{254} and scrapped to 1 mm thickness.

2.2. Plant Material. Sample of the air-dried stem bark of *Artocarpus obtusus* was collected from Sarawak in 2004, identified by Dr. Rusea Go, and a voucher specimen (S94402) has been deposited at the Herbarium, Department of Biology, Faculty of Science, Universiti Putra Malaysia.

2.3. Preparation of Plant Extracts and Isolation Procedure. The dried ground stem bark (640 g) of *Artocarpus obtusus* was sequentially extracted with *n*-hexane, chloroform and methanol at room temperature. The extracts were concentrated *in vacuo* to give 3 g (0.47%), 9 g (1.4%), and 6 g (0.94%) of dark viscous *n*-hexane, chloroform, and methanol extracts, respectively. The chromatographic isolation procedure to obtain pyranocycloartobiloxanthone A (**1**) and dihydroartoindonesianin C (**2**) from the chloroform extract has been described previously [20]. Similarly, the dark viscous methanol extract (6 g) was separated by gravity column chromatography and eluted with mixture of solvents of increasing polarity consisting of *n*-hexane, *n*-hexane/$CHCl_3$, $CHCl_3$, $CHCl_3$/MeOH, and MeOH to afford 45 fractions of 200 mL each. Another batch of compounds (**1**) and (**2**) were obtained from fractions 11–19 after a series of silica gel column chromatographic separation. Fractions 26–32 were combined and applied on column chromatography of Sephadex LH20 and eluted with methanol to give 45 fractions of 100 mL each. The combined fractions 18–28 were further chromatographed by a series of column chromatography using silica gel and eluted with solvent mixture of *n*-hexane, *n*-hexane/$CHCl_3$, $CHCl_3$, $CHCl_3$/MeOH, and MeOH to afford yellow solid (25.0 mg). The solid was recrystallized with chloroform as needle-shaped pyranocycloartobiloxanthone B (**3**) with R_f value of 0.5 (EtOAc : $CHCl_3$; 4 : 1) and m.p. 218–220°C.

2.4. Spectral Data. Pyranocycloartobiloxanthone A (**1**); $C_{25}H_{22}O_8$, yellow needle-shaped crystals, and m.p. 288–290°C. The spectral data of the compound are identical to literature values [20].

Dihydroartoindonesianin C (**2**); $C_{25}H_{24}O_6$, yellow needle-shaped crystals, and m.p. 210–212°C. The spectral data of the compound are identical to literature values [20].

Pyranocycloartobiloxanthone B (**3**), $C_{25}H_{22}O_8$, yellow needle-shaped crystal with m.p. 218–220°C; UV (Acetone) λ_{max} nm (log ε): 381 (0.57), 256 (4.00), and 229 (0.32); IR ν_{max} cm^{-1} (KBr): 3390 (OH), 1642 (C = O), 1474, 1270, 1160, 982; EIMS m/z (% intensity): 450 (26.50), 436 (28.00), 435 (100), 377 (60.00), 378 (18.00), 349 (6.00), 331 (4.50), 188 (14.00), and 166 (6.00); HREIMS m/z 450.1329 $[M]^+$ (calcd for $C_{25}H_{22}O_8$, 450.1314). ^{1}H-NMR (400 MHz, Acetone-d_6): 1.19 (3H, *d*, *J* = 6.4 Hz, H-15), 1.45 (6H, s, H-19, H-20), 1.91 (1H, *m*, H-11), 2.25 (1H, *dd*, *J* = 15.6, 7.7 Hz, H-13), 2.76 (1H, *m*, H-12), 3.32 (1H, *m*, H-11), 5.47 (1H, *s*, H-14), 5.65 (1H, *d*, *J* = 10.0 Hz, H-17), 6.12 (1H, *s*, H-6), 6.49 (1H, *s*, H-3′), 6.93 (1H, *d*, *J* = 10.0 Hz, H-16), 7.96 (1H, *brs*, OH-13), 8.44 (1H, *brs*, OH-2′), 8.84 (1H, *brs*, OH-4′), 13.37 (1H, *brs*, OH-5); ^{13}C-NMR (100 MHz Acetone-d_6): 16.5 (C-15), 24.2 (C-11), 29.8 (C-19), 30.0 (C-20), 33.9 (C-12), 38.8 (C-13), 80.2 (C-18), 96.5 (C-14), 101.3 (C-6), 103.6 (C-3), 105.5 (C-3′), 105.8 (C-10), 107.1 (C-8), 114.3 (C-1′), 117.6 (C-16), 127.3 (C-6′), 129.4 (C-17), 135.0 (C-5′), 153.2 (C-4′), 153.6 (C-2′), 153.8 (C-5), 161.0 (C-9), 163.1 (C-2), 164.1 (C-7), and 182.3 (C-4) (Figure 1).

2.5. In Vitro Assay for Antiproliferative Activity

2.5.1. Cell Lines. The cancerous and noncancerous cell lines (suspension and anchorage-dependent cells) were obtained from the American Type Culture Collection (ATCC) and the RIKEN Cell Bank (RCB). The cancerous cell lines used were HL-60 (human acute promyelocytic leukemia) (RCB), K562 (human chronic myeloid leukemia) (ATCC), MCF7 (human estrogen receptor (ER+) positive breast cancer) (ATCC), HeLa (human cervical cancer) (ATCC), HepG2 (human hepatocarcinoma) (ATCC), HT29 (human colon cancer) (RCI), and MDA-MB 231 (human estrogen receptor (ER−) negative) (ATCC). The noncancerous cell line and normal cells were 3T3 (Mouse embryo fibroblast) (ATCC),

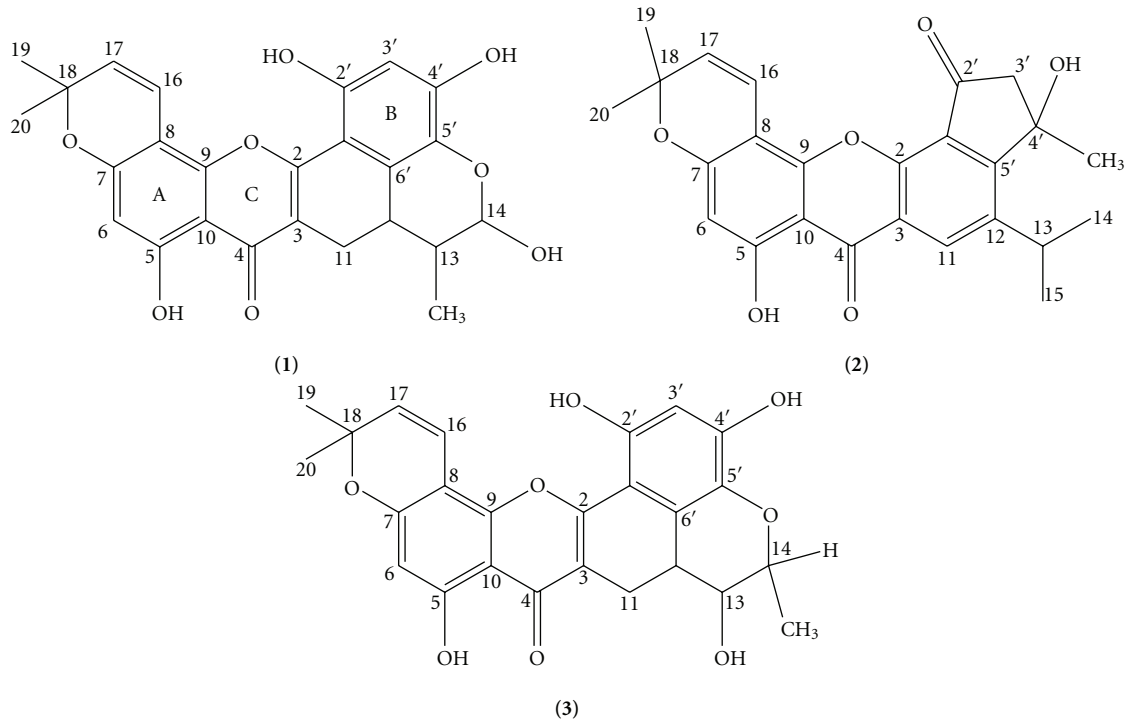


Figure 1: The chemical structure of the three isolated compounds tested for antiproliferative activity, pyranocycloartobiloxanthone A (**1**), dihydroartoindonesianin C (**2**), and pyranocycloartobiloxanthone B (**3**).

MCF10A (human nontumorigenic breast cell line) (ATCC) and PBMC (human peripheral blood mononuclear cells). PBMCs were obtained from one of the authors herself. Blood (10–12 mL) was taken from the donor by using the 25 mL syringe. The blood sample was diluted with same volume of PBS. The diluted blood sample was carefully layered on Ficoll-Paque Plus (Amersham Biosciences, USA) the ratio of 2 : 1. The mixture was centrifuged at 1500× g for 40 minutes at 18–20°C. The undisturbed lymphocyte layer was carefully transferred into centrifuge tube. The lymphocyte was washed and spun down three times by adding 10 mL sterile PBS and to get the pellet and resuspended in RPMI 1640 (Sigma, USA) with 100 IU/mL of penicillin, 100 μg/mL of streptomycin (Flowlab, Australia), and 10% v/v Fetal Bovine Serum (FBS) (PAA, Austria). Cell counting was performed to determine the PBMC number in equal volume of trypan blue.

2.5.2. Cytotoxic Analysis by the MTT Assay. The colorimetric assay was performed on the isolated compounds using a modified microculture tetrazolium salt (MTT) assay (Sigma, USA) [21]. Varying concentrations of 0.46, 0.93, 1.87, 3.75, 7.5, 15, and 30 μg/mL of pyranocycloartobiloxanthone A (**1**), dihydroartoindonesianin C (**2**), and pyranocycloartobiloxanthone B (**3**) were prepared from the substock solutions by serial dilution in RPMI 1640 to give a volume of 100 μL in each microtitre plate well (96-flat bottom microwell plates). Each well was then added with 100 μL of cell lines in complete growth media (RPMI 1640) 5×10^5 cells/mL. Untreated cells were used as control and included for each sample. The assay for each concentration of compounds was performed in triplicate. The culture plates were incubated for 72 hour at 37°C in a humidified (90%) incubator with 5% CO_2. After incubation, the fractions of surviving cells were determined relative to the untreated cell population using the colorimetric MTT assay. In this method, the viabilities of the cells were determined by measuring the amount of blue formazan crystals formed after 20 mL of freshly prepared MTT solution (5 mg in 1 mL PBS) was added to each well followed by four hours of incubation at 37°C. Then, 170 μL of the remaining supernatant was removed, 100 μL of DMSO was added to each well, and the mixture was stirred thoroughly to dissolve the blue crystal formazan. The plate was then incubated for 30 minutes to ensure that all the crystals were dissolved. Finally, the absorbance (OD) of each well at 570 nm of wavelength was read using the microplate reader (ELISA reader, EL340 Biokinetic Reader, Bio-Tek Instrumentation). Doxorubicin was used as a positive control. A graph was plotted for the percentage of cell viability against concentration of the extract, and the cytoxicity index used was IC_{50}, the concentration that yields 50% inhibition of the cell compared with untreated control. The percentage of cell viability was determined as follows:

$$\%\ \text{Viability} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100\%. \quad (1)$$

2.6. Cell Proliferation ELISA BrdU (5-bromo-2′-deoxyuridine) Assay (Colorimetric). This assay was conducted to determine the antiproliferative effects of the tested compound. In this assay, three different concentrations of

Table 1: ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectral data of pyranocycloartobiloxanthone B (3) and pyranocycloartobiloxanthone A (1).

H/C	(**3**) In acetone-d_6			(**1**) In DMSO [13]	
	δ_H (in ppm, J in Hz)	δ_C (in ppm)	HMBC	δ_H (in ppm)	δ_C (in ppm)
2	—	163.1	—	—	160.7
3	—	103.6	—	—	100.8
4	—	182.3	—	—	179.1
5	—	153.8	—	—	151.2
6	6.12 (1H, s)	101.3	C-5, 7, 8	6.17	98.7
7	—	164.1	—	—	160.7
8	—	107.1	—	—	104.3
9	—	161.0	—	—	157.9
10	—	105.8	—	—	103.6
11	1.91 (1H, *m*)	24.2	C-3, 6′	1.88	21.5
	3.32 (1H, *m*)		C-3, 4, C-12	3.32	
12	2.76 (1H, *m*)	33.9	C-13, 1′, 5′, 6′	2.55	31.2
13	2.25 (1H, *dd*, J = 15.6, 7.7)	38.8	C-12, 14, 6′	1.82	35.7
14	5.47 (1H, *s*)	96.5	C-13, 15	5.32	93.1
15	1.19 (3H, *d*, J = 6.4, CH_3)	16.5	C-12, 13, 14	1.08	14.6
16	6.93 (1H, *d*, J = 10.0)	117.6	C-7, 9, 18	6.86	114.8
17	5.65 (1H, *d*, J = 10.0)	129.4	C-16, 18, 19	5.74	127.3
18	—	80.2	—	—	78.0
19	1.45 (3H, *s*, CH_3)	29.8	C-17, 18	1.44	27.7
20	1.45 (3H, *s*, CH_3)	30.0	C-17, 18	1.42	27.9
1′	—	114.3	—	—	111.0
2′	—	153.6	—	—	151.2
3′	6.49 (1H, s)	105.5	C-2′, 4′, 5′	6.43	103.1
4′	—	153.2	—	—	151.1
5′	—	135.0	—	—	132.5
6′	—	127.3	—	—	124.6
OH-5	13.37 (1H, *brs*)	—	C-6, 7, 10	13.36	—
OH-13	7.96 (1H, *brs*)	—	—	—	—
OH-14	—	—	—	6.93	—
OH-2′	8.44 (1H, *brs*)	—	—	9.91	—
OH-4′	8.84 (1H, *brs*)	—	C-3′	9.81	—

compound or test sample were prepared together with control. The concentration chosen were 1 μg/mL, the IC_{50} concentration, and 20 μg/mL. Two types of cells were selected: HL-60 (suspension) and MCF7 (adherent) cells, since they gave the most significant results for the cytotoxic activity. The procedures are according to the instruction manual from Roche. Each sample was assayed in triplicate, and control samples include a blank (no cells) and background (without BrdU). The cells were treated by pyranocycloartobiloxanthone A (**1**) for 24, 48, and 72 hours. The cells were cultured together with various dilutions of test substance (tested compound) in 96-well MP flat bottom in a final volume of 100 μL/well, and 10 μL BrdU label (diluted by 1/2000 in media) was added to each well and incubated for 24 hour at 37°C. BrdU label was then replaced with 200 μL of fix/denaturing solution for 30 min at room temperature (25°C) and removed by inverting the plate. The anti-BrdU-POD working solution (100 μL/well) (diluted by 1/100 in dilution buffer) was added and incubated for one hour at room temperature. The cells were washed three times with wash buffer and once with dH_2O. Then, removed dH_2O and 100 μL of substrate solution was added to each well and incubated for 15 min at room temperature in the dark. The stop solution (100 μL/well) was added, and the absorbance was measured using ELISA reader at 450 nm, (reference wavelength 690). The activity was observed by the optical density (OD) of the absorbance at 450 nm, and graphs were plotted from the optical density obtained against time of exposure for each cell lines.

2.7. Acridine Orange (AO) and Propodium Iodide (PI) Staining. Cell suspensions were mixed with an equal volume of staining solution (1 : 1) containing 10 μg/mL acridine orange and 10 μg/mL propium iodide (dissolved in PBS) and observed under fluorescence microscope within 30 minutes. The viable (green intact cells), apoptotic (green

Table 2: The IC_{50} values of isolated compounds towards various cell lines.

Cell line	IC_{50} (μg/mL)			
	(**1**)	(**2**)	(**3**)	Doxorubicin
HL60	2 ± 0.7	26 ± 0.8	17 ± 0.8	0.2 ± 0.03
K562	0.5 ± 0.05	>30	>30	nt
MCF7	5 ± 1.2	27 ± 1.9	23 ± 1.3	0.2 ± 0.06
HeLa	8 ± 3.3	nt	nt	nt
MDA-MB 231	12 ± 1.1	nt	nt	nt
HT29	14 ± 1.5	nt	nt	nt
HepG2	20 ± 2.0	nt	nt	nt
3T3	7 ± 1.6	nt	nt	nt
MCF10	>30	nt	nt	nt
PBMC	>30	nt	nt	>30

Note: (nt)-not tested, (**1**)-pyranocycloartobiloxanthone A, (**2**)-dihydroartoindonesianin C, and (**3**)-pyranocycloartobiloxanthone B. Data represent mean ± SD of triplicate determinations from three independent experiments.

shrinking cells with condensed of fragmented nucleus), and necrotic (red cells) were the morphological changes that were examined under fluorescence microscope.

3. Results and Discussion

Based on the biological screening test results on 39 extracts from thirteen species of *Artocarpus* species, the three extracts of *Artocarpus obtusus* exhibited excellent cytotoxic activity against HL60 cells with IC_{50} values from 1.4 to 8.4 μg/mL [13]. However, only chloroform extract of *A. obtusus* exhibited cytotoxic activity towards MCF7 cells with IC_{50} values 22.60 μg/mL, while the other extracts were inactive towards the cell. These extracts were further phytochemically investigated with the isolation and identification of three new xanthones, pyranocycloartobiloxanthone A (**1**), dihydroartoindonesianin C (**2**), and pyranocycloartobiloxanthone B (**3**). The structural elucidation of two of xanthones, (**1**) and (**2**) isolated from the chloroform extract, has already been published recently [20], and the structure determination of the third xanthone (**3**) is included in this presentation. After chromatographic separation of the methanol extract, pyranocycloartobiloxanthone B (**3**) was obtained as yellow needle-shaped crystals with m.p. 218–220°C. The UV spectrum exhibited absorption bands at λ_{max} 381, 256, and 229 nm for a typical xanthone skeleton. Broad and strong absorptions were noted at 3390 and 1642 cm^{-1} in the IR spectrum due to the presence of hydroxyl and carbonyl functionalities. The EIMS gave molecular ion peak at m/z 450 which is consistent with molecular formula $C_{25}H_{22}O_8$. The high-resolution EIMS exhibited molecular ion peak at m/z 450.1329 (calculated $C_{25}H_{22}O_8$ for m/z 450.1314). The ^{1}H-NMR displayed characteristic signals for chelated phenolic hydroxyl group at δ 13.37 and a 2,2-dimethylchromene ring substituent with the observation of a pair of olefinic protons at δ 6.93 and 5.65 with identical coupling constant of 10.0 Hz and a sharp singlet of two overlapped methyl groups at δ 1.45. The aromatic region also revealed the occurrence of two

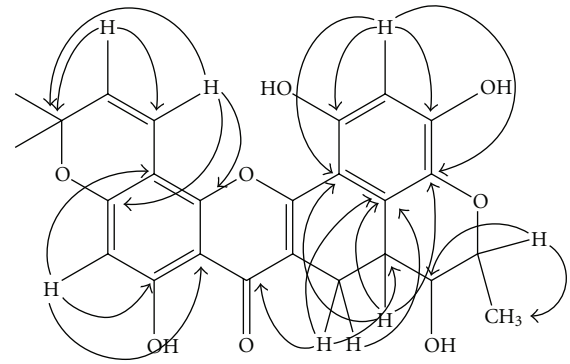


Figure 2: Selected HMBC of pyranocycloartobiloxanthone B (**3**).

singlets at δ 6.12 (H-6) and 6.49 (H-3′), and another high field resonance at δ 1.19 assigned to methyl group at C-15. The two broad chemical shifts noted at δ 8.44 and 8.84 were due to the hydroxyl groups attached to aromatic ring at C-2′ and C-4′.

The ^{13}C-NMR and DEPT spectra indicated the existence of twenty-five signals contributed by fourteen quaternary carbons including a conjugated carbonyl (δ 182.3), seven methine, one methylene (δ 24.2), and three methyl (δ 16.5, 29.8, and 30.0) carbon atoms. These ^{1}H-NMR and ^{13}C-NMR spectral data were quite similar to pyranocycloartobiloxanthone A (**1**) but with some obvious differences especially the location of one the hydroxyl groups at C-14 now shifted to C-13 which occurred at δ 38.8. The methyl group at C-13 shifted to C-14 and the chemical shift of this carbon now shifted upfield at δ 96.5 (Table 1). On the basis of HMQC and HMBC spectral analysis, the chemical shifts were fully assigned, and the positions of the substituents on the pyran and aromatic rings were determined. The ^2J and ^3J correlations of both protons at H-6 to -5, C-7, and C-8, H-3′ to C-2′, C-4′, and C-5′ confirmed the position of each respective aromatic protons. The two-bond and three-bond correlations were also used to locate the positions of both methyl and hydroxyl groups in the pyran ring (Figure 2). In (**1**), the methyl protons at C-15 exhibited correlations with C-12, -13, and -14 [20]. However, the methyl protons only showed correlations to C-13 and 14 in compound (**3**) which supported the attachment of this methyl group at C-14. Similarly, the methine proton at C-12 do not indicate any correlation to the methyl group. Based on these spectral data, the structure of this new xanthone was assigned and given trivial name pyranocycloartobiloxanthone B (**3**).

By using three tumor cell lines (HL60, K562, and MCF7), a comparison was made between the three new xanthones to establish which has greater cytotoxicity. The cytotoxic activity was assayed at various concentrations, expressed in IC_{50} values (μg/mL) under continuous exposure for 72 hours, and is summarized in Table 2. The estimated IC_{50} values were obtained from the plotted graphs of percentage cell viability (%) against various concentrations of the compounds (μg/mL) (Figures 3 and 4). The study showed pyranocycloartobiloxanthone A (**1**) exhibited potent cytotoxic activity against the three cell lines with IC_{50} ranged from

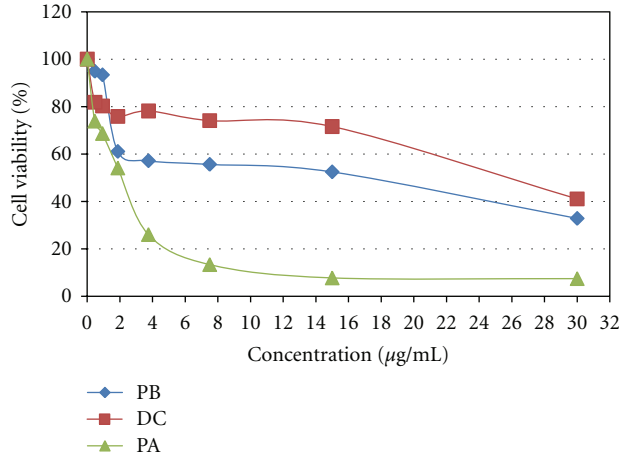


Figure 3: Percentage viability of HL60 cells treated with different concentration of pyranocycloartobiloxanthone A (**1**) (PA), dihydroartoindonesianin C (**2**) (DC), and pyranocycloartobiloxanthone B (**3**) (PB) measured after 72 hours using MTT assay.

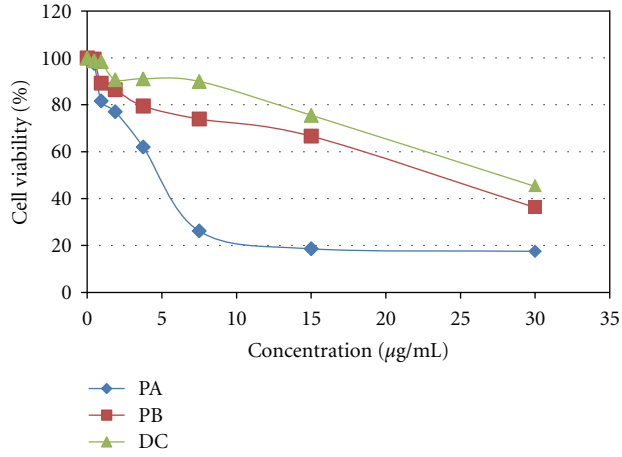


Figure 4: Percentage viability of MCF7 cells treated with different concentration of pyranocycloartobiloxanthone A (**1**) (PA), dihydroartoindonesianin C (**2**) (DC), and pyranocycloartobiloxanthone B (**3**) (PB) measured after 72 hours using MTT assay.

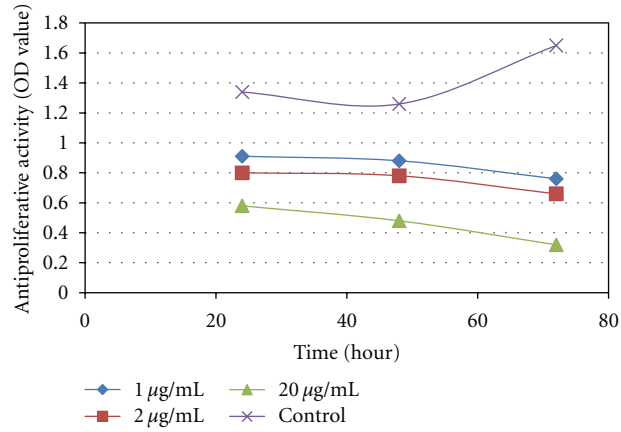


Figure 5: Effect of pyranocycloartobiloxanthone A (**1**) on the proliferation of HL60 cells *in vitro*. After treatment with 1 μg/mL, 2 μg/mL (IC_{50} based on MTT results) and 20 μg/mL for 24, 48, and 72 hours cellular proliferation of HL60 cells was assayed using BrdU incorporation ELISA.

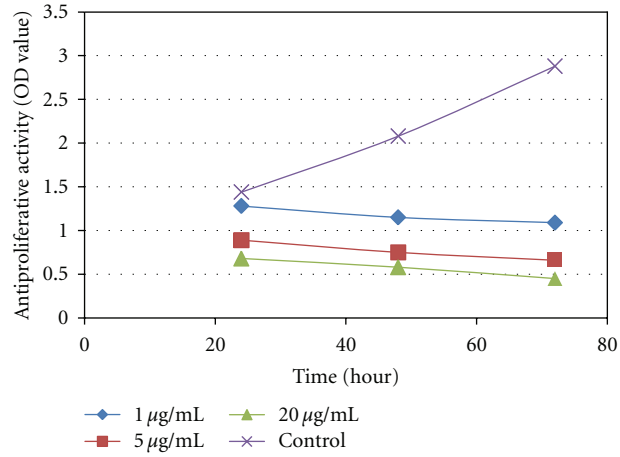
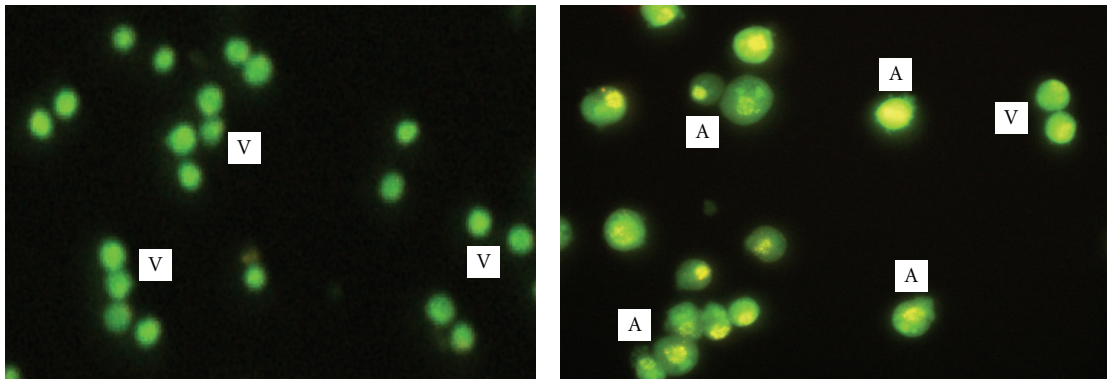


Figure 6: Effect of pyranocycloartobiloxanthone A (**1**) on the proliferation of MCF7 cells *in vitro*. After treatment with 1 μg/mL, 5 μg/mL (IC_{50} based on MTT results) and 20 μg/mL for 24, 48, and 72 hours cellular proliferation of MCF7 cells was assayed using BrdU incorporation ELISA.

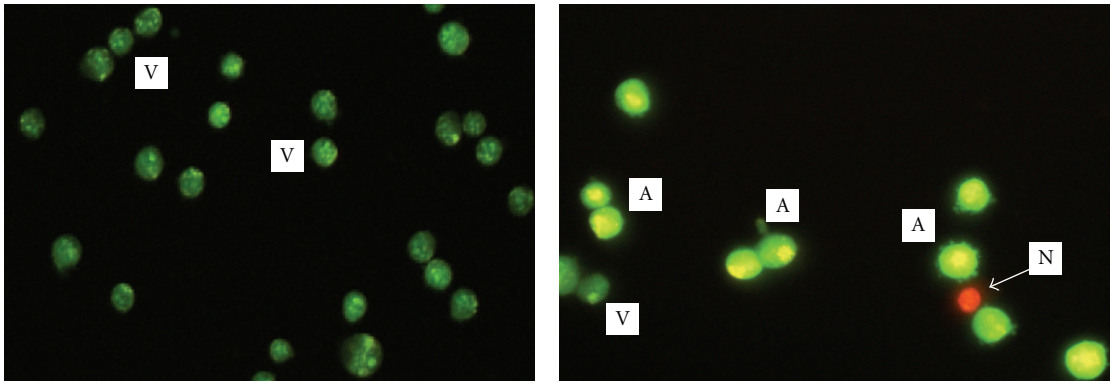
0.5–5.0 μg/mL in comparison with the other two compounds which only displayed moderate cytotoxic activities towards HL60 and MCF7 cell lines. Both compounds also failed to inhibit the growth of K562 cell line. In order to establish the potential of pyranocycloartobiloxanthone A (**1**) as a good cytotoxic agent, the compound was further tested against seven other cell lines (HeLa, MDA-MB 231, HT29, HepG2, 3T3, MCF10A, and PBMC). Due to insufficient amount of samples, compounds (**2**) and (**3**) were not further tested against these cell lines. The IC_{50} values on the sensitivity of MDA-MB 231, HT29, and HepG2 cell lines towards pyranocycloartobiloxanthone A (**1**) were obtained at 12.0, 14.0, and 20.0 μg/mL, respectively. However, the compound was not toxic towards normal cell lines, MCF10A and PBMC except for 3T3. The toxic effect of the compound displayed against 3T3 may be due to genetic changes occurred in the cell, or the compound was toxic to mouse cells. However further tests are needed to be carried out to underline the effect. The sensitivity of the compound towards the cell lines were comparable to doxorubicin used in the test.

The investigation on the potential cell proliferation inhibition activity of pyranocycloartobiloxanthone A (**1**) on HL60 and MCF7 cells was conducted due to their significant sensitivity (based from MTT assay) towards the compound. The results showed that at high and IC_{50} concentrations of pyranocycloartobiloxanthone A (**1**) on treated HL60 and MCF7 populations, the degree of cell killing or antiproliferative activity was significant, denoted by slight declined of OD value of absorbance over prolonged exposures in comparison to the untreated control population (Figures 5 and 6). The OD values for the IC_{50-} treated HL60 populations were 0.8, 0.78, and 0.66 for 24, 48, and 72 hours, respectively. On the other hand, OD values of the untreated



(a) (b)

Figure 7: Morphology features of HL60 cells incubated with pyranocycloartobiloxanthone A (**1**) after being stained with acridine orange and propidium iodide were observed under fluorescence microscope (magnification 200x) (a) untreated HL60 cells (b) treated with pyranocycloartobiloxanthone A (**1**) at 2 μg/mL (IC_{50} value), after 72 hours. Note: V: viable, A: apoptosis, and N: necrosis.



(a) (b)

Figure 8: Morphology features of MCF7 cells incubated with pyranocycloartobiloxanthone A (**1**) were observed under fluorescence microscope (magnification 200x) (a) untreated MCF7cells (b) treated with pyranocycloartobiloxanthone A (**1**) at 5 μg/mL (IC_{50} value), after 72 hours. Note: V: viable, A: apoptosis, and N: necrosis.

HL60 populations were 1.34, 1.26, and 1.65 for 24, 48, and 72 hours, respectively. Therefore, the exposure of HL60 cells to pyranocycloartobiloxanthone A (**1**) at IC_{50} concentration for 24, 48, and 72 hours resulted in a reduction from 60 to 40% of BrdU incorporation in comparison with the untreated population. A slight decline of OD values for IC_{50}–treated MCF7 populations was observed from 0.89, 0.75, and 0.66 at 24, 48, and 72 hours, respectively. In the untreated populations, the OD values were 1.44, 2.08, and 2.88 for 24, 48, and 72 hours, respectively. The exposure has resulted in reduction from 61.8 to 23% of BrdU incorporation in comparison with untreated population. As indicated above, the data exhibited the exposure time remarkably influenced on the growth of HL60 and MCF7 cells in the ELISA BrdU assay. Both cells experienced a noteworthy decline in cell proliferations compared to the control populations at any concentration as the time of exposure increased. The time course studies indicated that pyranocycloartobiloxanthone A (**1**) was sensitive to HL60 and MCF7 cells by inhibiting the cell proliferation. Hence, the inhibition of cellular proliferation for both cells treated with pyranocycloartobiloxanthone A (**1**) was consistent with the decline in cell viability from the MTT assay evaluation. Hence, the reduction of OD or viability which is observed in MTT assay may reflect cell death and/or reduced rate of proliferation.

In contrast, by utilizing AO/PI staining, we were able to determine the percentage and mode of cell death by uptake of different fluorescence dyes and morphological changes of individual cell in the treatment group. In the morphological study, pyranocycloartobiloxanthone A (**1**) was chosen for further study as it has shown significant activity against selected cancer cell lines compared to other isolated compounds. The cells, HL60 and MCF7, were treated with respective IC_{50} values, 2 μg/mL, and 5 μg/mL of pyranocycloartobiloxanthone A (**1**) after 72 hours of incubation. The cells were observed under fluorescence microscope prior AOPI staining. Both apoptotic and necrotic features were observed in HL60 and MCF7 cells population after treatment with pyranocycloartobiloxanthone A (**1**) at their respective IC_{50} dose (Figures 7 and 8). However, the compound seemed to kill both cells in apoptotic manner at IC_{50} dose, whereby upon swelling of the cells, little buds

started to appear around the cells. The buds disintegrate from the mother cell to form apoptotic bodies while the mother cell will shrunk. Cell rupture was observed after the prolonged exposure (72 hours) and resulted in the formation of cellular debris. There are several genes involved with apoptosis in mammalian cells, and the apoptotic response is determined by a balance between antiapoptotic genes (Bcl-2 and p53) and proapoptotic genes (C-myc and Bax proteins) [22]. In cancer, there is a lack of equilibrium between the rate of cell division and cell death (apoptosis), therefore, any compounds or agents that are able to promote or suppress apoptosis are potential to be developed as anticancer drugs.

The current study showed that the compounds were sensitive or toxic towards the selected cell lines, and the response of each cell lines towards the compounds was different. Differential antiproliferative activity of the compounds indicated that the cells may have different particular molecular site or receptor for selected compounds to interact, which may influence the response of the activity [22]. Pyranocycloartobiloxanthone A (**1**) exhibited poor toxicity with high IC_{50} values (>30 μg/mL) against PBMC and MCF10A cells which is a good indication that normal human cell lines may be resistant to the compound. The suggested effective doses for a 50% inhibition in cell viability for plant extracts and pure compound to be considered active according to National Cancer Institute (NCI) guidelines should be less than 20 and 4 μg/mL, respectively [23]. It is interesting to note that, pyranocycloartobiloxanthone A (**1**), at low concentration, exhibited its antiproliferative effect against most of the selected cancer cell lines. On the other hand, the other two compounds, dihydroartoindonesianin C (**2**) and pyranocycloartobiloxanthone B (**3**) require high concentrations to exert their antiproliferative effect towards HL60, K562, and MCF7 cells. This finding is possibly due to the presence of active sites in the structure of the compounds, whereby the presence of the resorcinol moiety in ring B and isoprenyl substituent at C-3 of the flavones skeleton may contribute to the interesting activity [9, 24]. Therefore, by having the above two features together in the chemical structure of pyranocycloartobiloxanthone A (**1**), they have substantiated the remarkable antiproliferative activity displayed. Surprisingly, in spite of having both active features together and also an isomer to compound (**1**), pyranocycloartobiloxanthone B (**3**) displayed lower antiproliferative activity towards the target cell lines. This may be due to the migration of the hydroxyl group from C-14 to C-13 and methyl group in the opposite direction in compound (**3**). As for dihydroartoindonesianin C (**2**), position C-3 is also substituted with isoprenyl group but without the resorcinol skeleton in the chemical structure, and this may be caused much lower antiproliferative effect than compound (**1**). These findings indicated that both resorcinol moiety in ring B and isoprenyl substituent at C-3 position must be the most important features for revealing the potent activity. Thus, the continuing phytochemical investigation of Malaysian *Artocarpus* species cannot only identify new lead compounds as anticancer agents but also provide a pool of chemicals for future biological target studies.

4. Conclusion

The extracts *of A. obtusus* were found to exhibit good cytotoxic and potential antiproliferative activity against some cell lines and further isolation work on the extracts resulted in the isolate ion of three new xanthones. The structures of the compounds were established by spectroscopic method and comparison with literature values. One of the xanthones, pyranocycloartobiloxanthone A (**1**), showed potent antiproliferative activity towards various cell lines and inactive when tested on normal cell lines. The compound (**1**) was able to induce apoptosis against HL60 and MCF7 cell lines at its respective IC_{50} values.

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