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Cytotoxic activities of chemical constituents from *Mesua daphnifolia*

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Abstract. Detail chemical investigations on the stem bark of *Mesua daphnifolia* gave three triterpenoids and four xanthones. They are friedelin (**1**), friedelan-1,3-dione (**2**), lup-20(29)-en-3β-ol (**3**), cudraxanthone G (**4**), ananixanthone (**5**), 1,3,5-trihydroxy-4-methoxyxanthone (**6**) and euxanthone (**7**). These chemical constituents were tested *in vitro* for their cytotoxic activities against four cell lines, MDA-MB-231 (human estrogen receptor negative breast cancer), HeLa (cervical carcinoma), CEM-SS (T-lymphoblastic leukemia) and CaOV3 (human ovarian cancer). Compound **4** showed a broad spectrum of activity against the MDA-MB-231, HeLa and CEM-SS cell lines with IC_{50} values of 1.3, 4.0 and 6.7 µg/ml respectively. Meanwhile, the other compounds **1**, **2**, **3**, **5**, **6** and **7** gave only selective activities against the cell lines.

INTRODUCTION

Mesua is a rather large genus consisting of about 48 species of stove evergreen shrubs or trees that are widely distributed in many tropical countries e.g. India, Burma, Thailand, Indochina and New Guinea (Dassanayake, 1980). So far, not many studies have been carried out on this genus but there are some reports on *Mesua ferrea*. Previous phytochemical studies have revealed plants from this genus to be rich in many classes of secondary metabolites including phenylcoumarins, xanthones and triterpenoids (Govindachari *et al.*, 1967; Chow & Quon, 1968; Bala & Seshadri, 1971; Bandaranayake *et al.*, 1975; Raju *et al.*, 1976). Some of the phenylcoumarins isolated were reported to show cytotoxic and antibacterial activities (Morel *et al.*, 1999, Verotta *et al.*, 2004). *Mesua daphnifolia* is a new species to the phytochemical and pharmacological studies. Our recent study on the stem bark extracts of the plant has led to the isolation of three triterpenoids (**1**, **2** and **3**) and four xanthones (**4, 5, 6** and **7**). These compounds were screened for their biological activities towards MDA-MB-231, HeLa, CEM-SS and CaOV3 cell lines. This paper reports the isolation and biological activities of these compounds. These activities have not been reported before.

MATERIALS AND METHODS

General. Column chromatography (CC) was performed on Merck Kieselgel (40-63 µm) or *Sephadex LH-20*. EIMS were recorded on a Shimadzu GCMS-QP5050A spectrometer. NMR spectra were obtained using a Unity INOVA 500MHz NMR/ JEOL 400MHz FT NMR spectrometer using tetramethylsilane (TMS) as internal standard.

Plant Material. The stem bark of *M. daphnifolia* was collected from Fraser's Hill, Pahang, Malaysia. The plant materials were identified by Mr. Shamsul from the

Institute of Bioscience, Universiti Putra Malaysia where a voucher specimen was deposited (specimen no. SK96/02).

Extraction and Isolation. Dried and powdered stem bark of plant material (2.0 kg) was extracted twice with n-hexane for more than forty-eight hours at room temperature. Both the n-hexane extracts were combined and concentrated under reduced pressure to yield a residue. Extractions were continued using chloroform ($CHCl_3$) and finally acetone (Me_2CO). This resulted in three different crude extracts. The hexane extract was chromatographed on a silica gel column using a stepwise gradient system (hexane/ $CHCl_3$ and $CHCl_3/Me_2CO$) to give 50 fractions (*Fr.*). *Fr. 20* and *Fr. 27* afforded **1** (65 mg) and **2** (33 mg), respectively. *Fr. 23-26* were combined and purified by CC (SiO_2; hexane/$CHCl_3$ gradient) to furnish 20 subfractions. Subfraction 8 yielded **3** (23 mg) while subfractions 9-10 were combined and further purified by column chromatography (CC) (*Sephadex LH-20*; MeOH) to afford **4** (10 mg). *Fr. 31-32* were combined and subjected to CC (*Sephadex LH-20*; MeOH) to yield **5** (12 mg). Meanwhile, fractionation of the acetone extract over a silica gel column (hexane/ $CHCl_3$, $CHCl_3$/EtOAc and EtOAc/MeOH gradient) provided 40 fractions. *Fr. 8-10* were combined and further purified by silica gel column chromatography and eluting with the same solvent system as above to give **7** (8 mg). *Fr. 14-15* were combined and separated in a silica gel column (hexane/$CHCl_3$ and $CHCl_3/Me_2CO$ gradient) to give **6** (5 mg). The structures of these compounds were derived based on spectroscopic evidence, mainly 1D and 2D NMR spectroscopy and mass spectrometry.

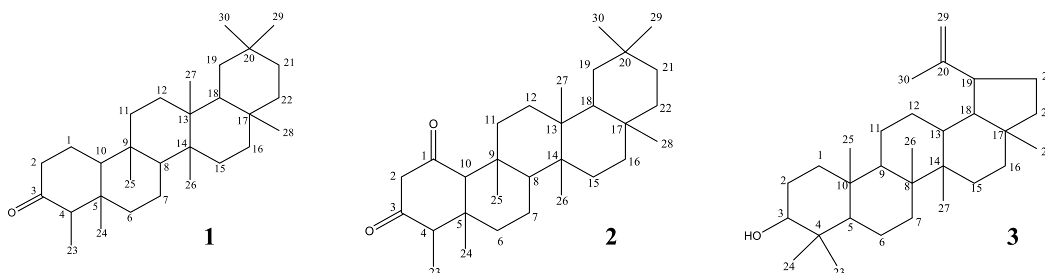
Cytotoxicity Assay. The stock solution was prepared at a concentration of 1 mg/ ml by dissolving 1 mg of sample (compound) in 1 ml of dimethylsulfoxide (DMSO). Serial dilution of the stock solution in the growth medium provided seven sample solutions at concentrations of 2.5, 5.0, 7.5, 10.0, 20.0, 30.0 and 40.0 µg/ml. Cells were grown in a 96 well microliter plate by filling each well with 100 µl of stock culture (1 x 10^5 cells/ml) and incubated at 37ºC for 24 hours. Growth medium was removed from the wells and each well was then treated with 100 µl of varying concentrations of sample solution. Controls were made containing only untreated cell population in 100 µl of growth medium. The assay for each concentration of sample was performed in triplicate and the culture plate was incubated for 3 days at 37ºC, 5% CO_2 and 90% humidity. After 3 days, 10 µl of the MTT labeling reagent (0.5 mg/ml) (Roche Diagnostics, USA) was added to each well. The plate was then incubated for a further 4 hours at 37ºC with 5% CO_2. After that, 100 µl of the solubilization solution was added to each well and the plate was allowed to stand overnight in the incubator at 37ºC with 5% CO_2. Cell viability was measured using ELISA spectrophotometer (EL_x 800) at a wavelength of 550 nm. The inhibitory concentration that killed cells by 50% (IC_{50}) was determined from absorbance (OD) versus concentration curve (Rahmat *et al.*, 2002).

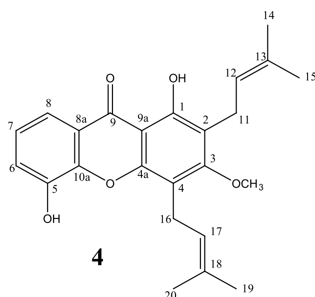
RESULTS AND DISCUSSION

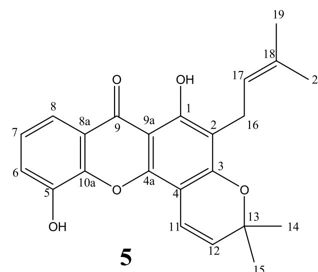
All the compounds **1**-**7** were tested for their biological activities towards MDA-MB-231, HeLa, CEM-SS and CaOV3 cell lines and the results are shown in Table 1. The MDA-MB 231 cell line was found to be very susceptible towards compound **4** and **5** with IC_{50} values of 1.3 and 4.6 µg/ml, respectively. Meanwhile, strong inhibitory activities were also observed for compounds **2** and **4** against the HeLa cell line with IC_{50} values of 4.6 and 4.0 µg/ml, respectively and medium activity was observed for compound **3** with an IC_{50} value of 8.6 µg/ml. The CEM-SS cell line was found to be moderately susceptible towards compounds **3** and **4** with IC_{50} values of 13.8 and 6.7 µg/ml respectively while other compounds gave only weak activities (IC_{50} > 25.0 µg/ml) against the cell line. Besides that, it was also found that most of the compounds tested were

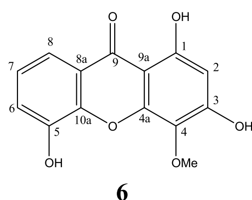
Table 1. Cytotoxic activities of compounds **1-7** from *Mesua daphnifolia*.

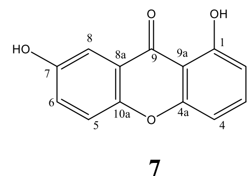
Compound	Cell line IC_{50} (µg/ml)			
	MDA-MB-231	HeLa	CEM-SS	CaOV3
1	39.6	35.0	> 40.0	> 40.0
2	> 40.0	4.6	> 40.0	> 40.0
3	28.5	8.6	13.8	28.2
4	1.3	4.0	6.7	28.7
5	4.6	> 40.0	> 40.0	29.7
6	35.6	> 40.0	> 40.0	> 40.0
7	31.9	34.5	27.0	9.0











weakly cytotoxic towards the CaOV3 cell line except for compound **7** which gave a moderate activity (IC_{50} = 9.0 µg/ml) in the assay. Compound **1** and **6** were found to be weakly active against all the cell lines tested with IC_{50} values of more than 34.0 µg/ml.

In the assays, all compounds tested indicated selective activity towards the cancer cell lines except for compound **4** which was found to have a broad spectrum of activity. This compound showed good inhibitory activities against MDA-MB-231, HeLa and CEM-SS cell lines with IC_{50}

values of 1.3, 4.0 and 6.7 µg/ml, respectively. A simple structure-activity relationship study was done on compounds **1** and **2** which have a friedelane skeleton. Structurally, it was observed that compound **2** is different from **1** by only an additional keto group at carbon C-1. However it was found that these two compounds showed a large difference in their cytotoxic activity towards the HeLa cell line with IC_{50} values of 35.0 and 4.6 µg/ml for compounds **1** and **2**, respectively. This means that the keto group at carbon C-1 in compound **2** might be the functional group which is responsible for a strong inhibitory activity against the cell line. Meanwhile for the xanthones (**4**, **5**, **6** and **7**), it was found that compound **4** which carries 2,4-diprenyl substituents in the xanthone ring A gave a strong inhibitory activity (1.3 µg/ml) towards the MDA-MB-231 cell line. Cyclization of one of the prenyl substituents at carbon C-4 shown in compound **5** led to a decrease in activity (4.6 µg/ml) and the absence of prenyl substituents in compounds **6** and **7** led to an almost complete loss of inhibitory activity (IC_{50} = 35.6 µg/ml and IC_{50} = 31.9 µg/ml, respectively) towards the cell line. Therefore, it was concluded that xanthones with 2,4-diprenylated skeleton are essential for the outstanding activity towards MDA-MB-231 cell line.

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