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Foreword: On behalf of the organizing committee of the International Conference and Department of Chemistry, Universitas Sumatera Utara, we would like to express our warmest welcome for all our participants and speakers to the 1st International

Conference on Chemical Science and Technology Innovation (ICOCSTI) 2019. The conference itself was held in Medan, July 18-19th 2019, with the theme " A Chemical Breakthrough for Science Technology Innovation". With over 60 selected manuscripts presented in the conference from authors across the globe, this proceeding aimed to serve as a platform to exchange information of the research methods, results, and experiences from various field of chemical science and engineering all over the world, including organic chemistry, inorganic chemistry, natural product chemistry, material science, polymer chemistry, engineering chemistry, and environmental sciences. In addition to that, we hope that this proceeding could promote research opportunities for the **(More)**

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Cytotoxicity of Heterophyllene A, the Derivative of Arylbenzofuran from Stem Bark of *Artocarpus calophylla*

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Keywords: Artocarpus calophylla, Arylbenzofuran, Cytotoxicity

Abstract: Exploration of secondary metabolites was the focus of this research, especially of *Artocarpus calophylla* species to look for a potential cytotoxic agent. An arylbenzofuran derivative compound, namely heterophyllene A was isolated from the stem bark of *Artocarpus calophylla*. Structure determination of this compound has been elucidated using UV-Vis spectroscopy, 1D, and 2D NMR analysis. This compound has a lower IC₅₀ than ethyl acetate extract. The IC₅₀ of this compound (57,54 µg/mL) to HeLa and (25,80 µg/mL) to T47D cells, ethyl acetate extract (>100 µg/mL) to HeLa and (84,16 µg/mL) to T47D cells.

1 INTRODUCTION

Moraceae is a family of plants that is a source of a bioactive compound in large quantities. The main genus in Moraceae is *Artocarpus* which consists of more than 60 species. *Artocarpus* plants spread from Southeast Asia, South Asia, Northern Australia and Central America (Kochummen 1987; Verheij and Coronel, 1992). Some *Artocarpus* species commonly found in Indonesia include jackfruit (*A. heterophyllus* Lamk), cempedak (*A. champeden*), breadfruit (*A. altilis* [Park] Fosberg) (Ilyas, 2013) and others which are endemic in Myanmar such as *A. lakoocha* and *A. calophylla* KURZ (Takahashi et al., 2004).

There are some secondary metabolites which are proven capable to be produced by this genus, for instance, terpenoid, steroid, and phenolic compound (Barik et al., 1997; Wang et al., 2007; Chen et al., 2010; Nguyen et al., 2012). A number of pharmacologically active constituents have been isolated from *Artocarpus* species, with this having a variety of activities including antibacterial (Khan et al., 2003), antiplatelet (Weng et al., 2006), antifungal (Jayasinghe et al., 2004), antimalarial (Widyawruyanti et al., 2007; Boonlaksiri et al.,

2000) and cytotoxic (Ko et al., 2005; Hakim et al., 2002; Syah et al., 2006).

In this study, it was reported that heterophyllene A is an arylbenzofuran derivative compound isolated from ethyl acetate extract of the stem bark of *A. calophylla*. *A. calophylla* is one of the species in the genus *Artocarpus* that has not been widely studied both from the study of phytochemicals and its biological activity. The chemical structure of the compound was determined by UV, 1D, and 2D NMR. Cytotoxic activity of the compound and ethyl acetate extract to HeLa and T47D cells is also described.

2 EXPERIMENTALS

2.1 General

NMR spectra were recorded on JEOL 600 ECA spectrometer using CDCl₃ at 600 (¹H) and 125 (¹³C) MHz. The UV spectrum was recorded using UV-1800 Shimadzu spectrophotometer. Vacuum Liquid Chromatography (VLC) and Gravity Column Chromatography (GCC) were carried out using Si gel 60 GF254. Meanwhile, Si gel PF254 was used in

TLC analysis and pre-coated silica gel plates (Merck, Darmstadt, Germany, Kieselgel 60 GF254 0,25 mm thickness).

2.2 Plant Material

The sample collection of the stem bark of *A. Calophylla* was carried out in April 2018. The location where the samples were collected was in Myanmar particularly in Inaw Village, Myitkyina City. Afterward, before being dried up, the stem barks were cleaned out. The drying was done in the shade. After the cleaning and the drying process, it was then cut into small pieces before finally being ground into powder.

2.3 Extraction and Isolation

The stem barks of *A. calophylla* which were already in the form of powder were macerated with methanol for three whole days at indoor temperature. Afterward, the filtration step was done to obtain the necessary solvent. In order to acquire the methanol extract, the solvent was then evaporated using Rotary Vacuum Evaporator. The crude methanol extract was partitioned with n-hexane and ethyl acetate. A vacuum liquid chromatography was utilized to separate as much as 15g of Ethyl acetate extract. The separation process was done on a silica gel using a mixture of ethyl acetate and eluent n-hexane by intensifying the polarity of the gradient. The compound purification process was done using gravity column chromatography on silica gel by increasing the eluent polarity. The compound purity test was carried out using TLC analysis with at least three different system and anisaldehyde reagents.

2.3.1 Heterophyllene A

Brown solids; UV (MeOH) λ_{\max} 219, 340, and 358 nm. $^1\text{H-NMR}$ (CDCl_3 , 600 MHz) δ_{H} 1.46 (3H (2x), s, Methyl-10,11), 5.64 (1H, d, $J=9.9$ Hz, H-6), 6.64 (1H, d, $J=9.9$ Hz, H-5), 6.77 (1H, dd, $J=8.3;2.0$, H-5'), 6.81 (1H, s, H-3), 6.85 (1H, s, H-3'), 6.89 (1H, s, H-9), 6.97 (1H, d, $J=2.0$, H-7'), 7.38 (1H, d, $J=8.3$ Hz, H-4'), 8.11 (1H, s, OH); $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz), δ_{C} 27.8 (CH_3 (2x), C-10;C-11), 76.3 (C, C-7), 98.1 (CH, C-7'), 101.5 (CH, C-3'), 103.9 (CH, C-3), 105.5 (CH, C-9), 109.6 (C, C-4a), 112.1 (CH, C-5'), 116.1 (CH, C-5), 117.6 (C, C-3a), 121.2 (CH, C-4'), 122.8 (C, C-3a'), 129.6 (CH, C-6), 151.2 (C, C-2), 151.4 (C, C-4), 153.6 (C (2x), C-2', C-7a'), 154.2 (C, C-8a), 154.7 (C, C-9a), 155.6 (C, C-6').

2.4 Cytotoxicity Bioassays

The Cytotoxic assay was carried out using MTT assay method in vitro against HeLa and T47D cells. Cytotoxic tests were carried out by planting cancer cell cultures that had been harvested into 96 well plates. Furthermore, the 96 well plates containing the cancer cells were treated with 100 μL of the isolated compound and were incubated for 24 hours. The test sample varied the concentration of the solutions by 7 variations starting from the concentration of 1,5625; 3,125; 6,25; 12,5; 25; 50 and 100 $\mu\text{g/mL}$ and repeated three times (triple). The positive control used was doxorubicin, a media control solution consisting of culture media, and cell control solution consisting of culture and cell media.

The next step was the administration of 100 μL of MTT reagents to each well after being incubated for 24 hours. Afterwards, it was incubated again for 3-4 hours in the CO_2 incubator (until formazan crystals were formed). When formazan crystals have been formed, the condition of the cell was observed with an inverted microscope, then as much as 10% of SDS stopper was added in 0,1 N HCl. Finally, the 96 well plates were wrapped with paper and were re-incubated overnight.

The next step was using ELISA reader to read the absorbance value. It was done to find out the IC_{50} value of each test sample. The reading process of each well's absorbance was done with a wavelength of 500-600 nm. By using absorbance data which were obtained from the measurements, then it was possible to determine the percentage of cells inhibited. The determination of IC_{50} values was carried out using linear regressions.

3 RESULTS AND DISCUSSIONS

Heterophyllene A (Figure 1) was obtained as brown solids (9 mg). The UV spectrum showed maximum absorbance at 219, 340, and 358 nm, which indicates the presence of an arylbenzofuran skeleton (Tan, et.al., 2012). The $^1\text{H-NMR}$ spectrum (Table 1) shows aromatic signals with the ABX system at δ_{H} 7.38 (d, $J = 8.3$ Hz, 1H), 6.97 (d, $J = 2.0$ Hz, 1H), 6.77 (dd, $J = 8.3$ Hz and $J = 2.0$ Hz, 1H). Two proton signals at δ_{H} 5.64 (d, $J = 9.9$ Hz, 1H) and 6.64 (d, $J = 9.9$ Hz) indicate the presence of dimethylchromene rings (Boonyaketgason, et.al., 2017). The spectrum shows three singlet aromatic protons at δ_{H} 6.85 (s, 1H), 6.89 (s, 1H) and 6.81 (s, 1H) and that there is a singlet signal at δ_{H} 8.11 (s, 1H) which is a hydroxy group. Spectrum $^{13}\text{C-NMR}$,

DEPT 90, and DEPT 135 show 21 carbon signals consisting of eleven quaternary carbon at δ_C 76.3; 109.6; 117.6; 122.8; 151.2; 151.4; 153.6 (2C); 154.2; 154.7; 155.6, two methyl at δ_C 27.8 (2C) and eight methine at δ_C 98.1; 101.5; 103.9; 105.6; 112.1; 116.1; 121.2; 129.6 (Table 1).

Based on multiplicity and HSQC spectrum, three aromatic protons which resonate at δ_H 7.38, 6.77 and 6.97 are respectively assigned as H-4', H-5', H-7'. HMBC correlation shows that H-4' correlates with C-6' (Figure 2), H-5' correlates with C-3' and C-3a', while H-7' at δ_H (6.97) correlates with C-3a', C5' and C-6'. Singlet signals on aromatic protons at δ_H 6.85 correlates with quaternary carbon at C-3a', δ_H 6.89 correlates with methine at C-3 and quaternary carbon at C-9a, δ_H 6.81 correlates with C quaternary at C-2' and methine at C-9. This indicates that singlet aromatic protons are at position C-3', C-3 and C-9. The hydroxy position was confirmed to be C-4 and C-6' by HMBC. Two methyls (C-10 and C-11) were found by HSQC spectrum to be present in the compound. Afterward, the authors did additional evaluation to compare with the published data (Table 2) (Boonyaketguson et al., 2017), the structure of this compound was identified as Heterophyllene A.

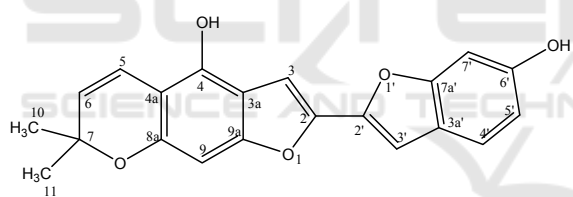


Figure 1: Structure of Heterophyllene A

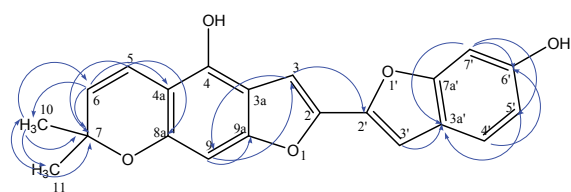


Figure 2: HMBC correlation for Heterophyllene A

Table 1: NMR spectroscopic data of Heterophyllene A in $CDCl_3$

No	1H (m, J in Hz)	^{13}C (type)	HMBC
1	-	-	-
2	-	151.2	-
3	6.81 (s)	103.9	2', 9
3a	-	117.6	-
4	-	151.4	-
4a	-	109.6	-
5	6.64 (d, 9.9)	116.1	7, 8a
6	5.64 (d, 9.9)	129.6	4a, 7, 10
7	-	76.3	-
8	-	-	-
8a	-	154.2	-
9	6.89 (s)	105.5	3, 9a
9a	-	154.7	-
10	1.46 (s)	27.8	6, 7, 11
11	1.46 (s)	27.8	6, 7, 10
1'	-	-	-
2'	-	153.6	-
3'	6.85 (s)	101.5	3a'
3a'	-	122.8	-
4'	7.38 (d, 8.3)	121.2	6'
5'	6.77 (dd, 8.3; 2.0)	112.1	3', 3a'
6'	-	155.6	-
7'	6.97 (d, 2.0)	98.1	3a', 5', 6'
7a'	-	153.6	-

Table 2: The data of the chemical shift which compares Heterophyllene A from *A. heterophyllus* (right) and Heterophyllene A from *A. calophylla* (left)

No	1H (m)	^{13}C	No	1H (m)	^{13}C
1	-	-	1	-	-
2	-	151.2	2	-	154.2
3	6.81 (s)	103.9	3	6.87 (s)	105.6
3a	-	117.6	3a	-	113.8
4	-	151.4	4	-	151.5
4a	-	109.6	4a	-	101.2
5	6.64 (d)	116.1	5	6.64 (d)	116.2
6	5.64 (d)	129.6	6	5.64 (d)	129.6
7	-	76.3	7	-	76.3
8	-	-	8	-	-
8a	-	154.2	8a	-	154.0
9	6.89 (s)	105.5	9	6.85 (s)	101.6
9a	-	154.7	9a	-	154.7
10	1.46 (s)	27.8	10	1.40 (s)	27.8
11	1.46 (s)	27.8	11	1.40 (s)	27.8
1'	-	-	1'	-	-
2'	-	153.6	2'	-	153.6
3'	6.85 (s)	101.5	3'	6.97 (s)	98.2
3a'	-	122.8	3a'	-	122.8
4'	7.38 (d)	121.2	4'	7.38 (d)	121.1
5'	6.77 (dd)	112.1	5'	6.76 (dd)	112.1
6'	-	155.6	6'	-	155.6
7'	6.97 (d)	98.1	7'	6.81	103.9
7a'	-	153.6	7a'	-	153.6

Cytotoxic activity on HeLa and T47D cells showed that Heterophyllene A compounds were more toxic than ethyl acetate extracts. The IC₅₀ of Heterophyllene A compound was 57.54 µg/mL to HeLa and was 25.80 µg/mL to T47D cells. The IC₅₀ of ethyl acetate extract was >100 µg/mL to HeLa and was 84.16 µg/mL to T47D cells. The IC₅₀ of doxorubicin was 2.72 µg/mL to HeLa and was 0.01 µg/mL to T47D cells.

4 CONCLUSIONS

In conclusions, Heterophyllene A, the derivative of arylbenzofuran compound was isolated from the stem bark of *A. calophylla*. The biological activity of this compound was investigated using cytotoxicity test to HeLa and T47D cells. Although it did not have strong activity, this compound had better activity than ethyl acetate extract.

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