

# CYTOTOXIC CONSTITUENT IN THE FRUIT PEEL OF *Lansium* *domesticum*

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## CYTOTOXIC CONSTITUENT IN THE FRUIT PEEL OF *Lansium domesticum*

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### ABSTRACT

Phytochemical study of the hexane fruits peel extract of *Lansium domesticum* contributed to the discovery of onoceranoid-type triterpene, onoceradienedione **1**. The structure of the isolated compound was done by advance spectroscopic techniques, comprising IR, UV, 1D, 2D NMR and HR-ESI-MS spectra data. Moreover, hexane extract and isolated compound **1** were examined for their cytotoxic activity against HeLa, T47D, and A549 cells. The results revealed the significance cytotoxic activity in the hexane extract.

**Keywords:** Fruit Peel, Onoceranoid-type triterpene, Meliaceae, Cytotoxicity, Anticancer, Spectroscopic Analysis  
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### INTRODUCTION

Langsat fruit (*Lansium domesticum* Corr., family Meliaceae) is one of Southeast Asia's most common fruits, particularly in Indonesia. It is widely found in other Asian countries, including Malaysia, Vietnam, Cambodia, Myanmar, and the Philippines. Phytochemical analysis revealed that triterpenoids especially onoceranoids with rare and unique skeleton, onoceranoid glycosides, tetranortriterpenoid, and cycloartenoidtriterpenes are the major components in this plant. The extract and its major components from genus *Lansium* have shown significant biological activities such as antifeedant, anticancer, antimalarial, antidiabetic, antioxidant and antifungal.<sup>1-17</sup>

Our investigation on the hexane soluble portion of methanol fruits peel extract of *Lansium domesticum* Led to the isolation of an onoceradienedione, namely an *8,14-secogammacera-7,14(27)-dien-3,21-dione* (Fig.-1) Moreover, cytotoxic activity of hexane extract and the isolated compound was evaluated using MTT and XTT assay against cervical (HeLa), breast (T47D) and lung (A549) cancer cell lines *in vitro*.

### EXPERIMENTAL

#### Plant Materials

*Lansium domesticum* Corr. fruits have been brought from markets Nganjuk, East Java, Indonesia in December 2018. The sample was cleaned and dried. Fruit peels of *Lansium domesticum* were cut into small

parts and powder. The plant sample was identified by Herbarium Bogoriense, Biology Research Center, Cibinong, Indonesia.

### Extraction and Isolation

The powder of *Lansium domesticum* (4000 g) was extracted by methanol at room temperature for 3 days (3 × 2L) and filtered. Then, the combined filtrates were evaporated and partitioned between hexane and DCM (dichloromethane). The hexane soluble fractions (300 g) were then separated by VLC (vacuum liquid chromatography) using step gradient solvent mixtures of hexane-DCM to give five fractions (A-E). Fraction C (0.612 g) was then separated by using silica gel column chromatography and eluted with the solvent mixtures of hexane-EtOAc to produce six sub-fractions (L<sub>1</sub>-L<sub>6</sub>). Sub-fraction L<sub>3</sub> (174 mg) has been applied to the column chromatography using the hexane-EtOAc solvent mixtures to get compound **1** (5.1 mg).

### General Procedure

NMR spectra were measured on Bruker AVANCE III spectrometer using CDCl<sub>3</sub> as a solvent. HR-EI-MS was obtained on a JEOL JMS-700 spectrometer. IR spectrum was determined on a FTIR-8400S (Shimadzu) spectrophotometer, where UV spectrum was recorded in MeOH on UV-Vis Shimadzu spectrometer. The melting point was measured on a Fisher-Johns Melting Point Apparatus. Silica gel 60 (700-200 mesh ASTM) was used for column chromatography. Analytical TLC was conducted on a pre-coated silica gel 60 F<sub>254</sub> (Merck).

### Detection Method

#### Bioassay

#### Cell Culture

Three cancer celllines, Cervical (HeLa), breast (T47D) and lung (A459) obtained from American Type Culture Collection (ATCC). Cells were cultured at 37°C and 5% CO<sub>2</sub> for 24 hours and 100% humidified in medium supplemented with 10% FBS, 1%, L-glutamine and 1% penicillin/streptomycin.

#### MTT Assay

Cytotoxicity of test samples was used by the established method<sup>18</sup>. Briefly, cells were seeded in 96 well plates (10<sup>4</sup>-10<sup>5</sup> cells/well) and incubated in CO<sub>2</sub> incubator at 37°C for 24 hours. Following incubation, cells were served with specific assay concentrations (1.5625-100 µg/mL) at 37°C in the CO<sub>2</sub> incubator for 24 hours. Doxorubicin was used as a positive control. After incubation, a hundred µL of MTT reagent in phosphate-buffered saline (5 mg/mL in PBS) was transferred into every well, and the plates re-incubated for 3 hours at 37 °C. Then, the medium was removed, and the purple formazan was solvated in 0.1 N HCl. The absorbance was recorded on ELISA microplate reader at wavelength 560 nm. Each experiment was carried out in triplicates. The % of cell viability was calculated by the following equation :

$$\% \text{ Cell viability} = \frac{(a-c)}{(b-c)} \times 100\%$$

Where a = absorbance of the sample, b = absorbance of cells control and c = absorbance of media control.

#### XTT Assay

XTT assay of extract and isolated compound were done according to the established protocol.<sup>18</sup>

## RESULTS AND DISCUSSION

Compound **1** was isolated as white needle-like crystals, and the molecular formula C<sub>30</sub>H<sub>46</sub>O<sub>2</sub> was determined by HREI-MS (*m/z* 438.3498, [M+H]<sup>+</sup>) (Calcd. 438.6960), <sup>1</sup>H NMR, and <sup>13</sup>C NMR spectra data (Table-1). The UV spectrum of compound **1** in MeOH displayed absorption maxima at 214.5 nm. The presence of carbonyl and olefinic groups were suggested by IR spectra data. The proton NMR (600 MHz, CDCl<sub>3</sub>) displayed seven methyl singlet signals at δ<sub>H</sub> 0.84 (H<sub>3</sub>-28), 0.94 (H<sub>3</sub>-25), 1.01 (H<sub>3</sub>-30), 1.04 (H<sub>3</sub>-23), 1.08 (H<sub>3</sub>-24), 1.10 (H<sub>3</sub>-29), and 1.72 (H<sub>3</sub>-26); eight multiplet methylene signals at δ<sub>H</sub> 1.42, 2.06 (H<sub>2</sub>-1), 2.24, 2.70 (H<sub>2</sub>-2), 1.92, 2.40 (H<sub>2</sub>-11), 1.92, 2.40 (H<sub>2</sub>-12), 2.42 (H<sub>2</sub>-15), 1.67, 1.49 (H<sub>2</sub>-16), 1.54, 2.00 (H<sub>2</sub>-19),

and 2.21, 2.61 (H<sub>2</sub>-20); one doublet of doublet methylene signals at  $\delta_H$  1.35, 1.26 (H<sub>2</sub>-6); an olefinic broad singlet signal at  $\delta_H$  5.42 (H-7); three multiplet methine signals at  $\delta_H$  1.58 (H-17), 1.60 (H-13), and 1.65 (H-9); and the remaining one set of olefinic singlet methylene signals 4.92, 4.61 (H<sub>2</sub>-27). The <sup>13</sup>C NMR (150 MHz) revealed the presence of seven methyl carbon signals at  $\delta_C$  13.5 (C-25), 14.2 (C-28), 21.6 (C-30), 22.0 (C-24), 22.2 (C-26), 25.0 (C-23), and 26.1 (C-29); ten methylene carbon signals (9 sp<sup>3</sup> and 1 sp<sup>2</sup>) at  $\delta_C$  24.0 (C-11), 24.1 (C-12), 25.2 (C-16), 25.3 (C-6), 34.6 (C-2), 34.7 (C-20), 37.8 (C-19), 37.9 (C-15), 38.1 (C-1), and 107.7 (C-27); five methine carbon signals (including 4 sp<sup>3</sup> and 1 sp<sup>2</sup>) at  $\delta_C$  51.6 (C-5), 54.3 (C-13), 55.2 (C-17), 56.0 (C-9), and 121.8 (C-7); eight quaternary carbon signals (including 2 carbonyl, 2 sp<sup>2</sup>, 4 sp<sup>3</sup>) at  $\delta_C$  14.3 (C-14), 39.2 (C-18), 36.5 (C-10), 47.4 (C-4 and C-22), 135.4 (C-8), 216.6 (C-21), and 216.8 (C-3). According to the above spectra data, compound **1** has an onoceranooid-type triterpene. The structure of compound **1** was further confirmed by COSY and HMBC correlations (Fig.-2). Compound **1** was then established by comparison with the reporter data as an onoceradienedione (Fig.-1).<sup>19</sup> Moreover, cytotoxicity of hexane extracts and compound **1** were evaluated against HeLa, T47D and A549 cell lines. The results are shown in Table-2. According to the Cancer Institute standards, crude extracts with IC<sub>50</sub> values of < 20  $\mu$ g/mL, and a natural compound that has IC<sub>50</sub> values of < 4 or 10  $\mu$ g/mL are considered active<sup>20</sup>. Therefore, hexane extract displayed potential cytotoxic activity against T47D (IC<sub>50</sub> 0.10  $\mu$ g/mL) and A549 (IC<sub>50</sub> 18.83  $\mu$ g/mL) cell lines, but compound **1** demonstrated weak activity against only A549 (IC<sub>50</sub> 13.71  $\mu$ g/mL) cell lines.

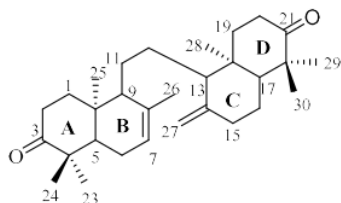


Fig.-1: Chemical Structure of Compound **1** from the Fruit Peel of *Lansium domesticum*

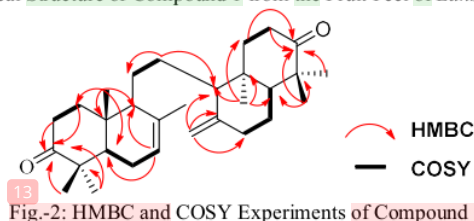


Fig.-2: HMBC and COSY Experiments of Compound **1**

Table-1: NMR Spectral Data of Compound **1**

Position	$\delta_H$ (ppm) (mult, <i>J</i> in Hz)	$\delta_C$ (ppm)	HMBC
1	1.42 (1H, <i>m</i> )	38.1	C-2, C-3, C-10
	2.06 (1H, <i>m</i> )		
2	2.24 (1H, <i>m</i> )	34.6	C-1, C-3, C-10
	2.70 (1H, <i>m</i> )		
3	-	216.8	-
4	-	47.4	-
5	1.59 (1H, <i>dd</i> , <i>J</i> = 7.0, 5.0 Hz)	51.6	C-9, C-10, C-24
	1.35 (1H, <i>dd</i> , <i>J</i> = 7.0, 10.0 Hz)		
6	1.26 (1H, <i>dd</i> , <i>J</i> = 5.0, 10.0 Hz)	25.3	C-7, C-10
	1.35 (1H, <i>dd</i> , <i>J</i> = 7.0, 10.0 Hz)		
7	5.42 (1H, <i>s br</i> )	121.8	C-5, C-9, C-26
8	-	135.4	-
9	1.65 (1H, <i>m</i> )	56.0	C-26
10	-	36.5	-
11	1.92 (1H, <i>m</i> )	24.0	C-9

	2.40 (1H, m)		
12	1.92 (1H, m)	24.1	C-13
	2.40 (1H, m)		
13	1.60 (1H, m)	54.4	C-28
14	-	147.3	-
15	2.42 (2H, m)	37.9	C-14, C-16, C-27
16	1.67 (1H, m)	25.2	C-17
	1.49 (1H, m)		
17	1.58 (1H, m)	55.2	C-13, C-18; C-29
18	-	39.2	-
19	1.54 (1H, m)	37.8	C-18, C-20, C-21
	2.00 (1H, m)		
20	2.21 (1H, m)	34.7	C-18, C-19, C-21
	2.61 (1H, m)		
21	-	216.6	-
22	-	47.7	-
23	1.04 (3H, s)	25.0	C-3, C-4, C-5
24	1.08 (3H, s)	22.0	C-3, C-4, C-5
25	0.94 (3H, s)	13.5	C-1, C-5, C-9, C-10
26	1.72 (3H, s)	22.2	C-7, C-8, C-9
27	4.92 (1H, s)	107.7	C-13, C-14, C-15
	4.61 (1H, s)		
28	0.84 (3H, s)	14.2	C-13, C-17, C-18, C-19
29	1.10 (3H, s)	26.1	C-17, C-21, C-22
30	1.01 (3H, s)	21.6	C-17, C-21, C-22

\* Recorded at 600 MHz for  $^1\text{H}$  and 150 MHz for  $^{13}\text{C}$ -NMR.

Table-2: The  $\text{IC}_{50} \pm \text{S.D.}$  ( $\mu\text{g/ml}$ ) of the Compound and Crude Extract from the Peel of *Lansium domesticum* and Doxorubicin

No.	Compound	$\text{IC}_{50} \pm \text{S.D.}$ ( $\mu\text{g/ml}$ )		
		HeLa	T47D	A549
1	8,14-Secogammacer-7,14(27)-dien-3,21-dione (onoceradienedione)	32.39 $\pm$ 1.17	30.69 $\pm$ 1.87	13.71 $\pm$ 0.42
2	n-Hexane extract	59.55 $\pm$ 4.29	0.10 $\pm$ 5.88	18.83 $\pm$ 2.58
3	Doxorubicin	2.83 $\pm$ 0.18	0.04 $\pm$ 0.03	-

\* All data represent the mean of  $\text{IC}_{50} \pm \text{S.D.}$  of triplicates.

### CONCLUSION

In conclusion, onoceradienedione **1** was produced from the hexane soluble portion of methanol fruits peel extract of *Lansium domesticum*. The structure of the isolated compound was done by advance spectroscopic techniques, including IR, UV, NMR, and HR-ESI-MS spectra data. Moreover, cytotoxicity of hexane extract and the isolated compound was evaluated using MTT and XTT assay against HeLa, T47D, and A459 cell lines. The results showed that hexane extract displayed significant cytotoxic activity against T47D and A459 cell lines, but the isolated compound has weak activity against A459 cell lines.

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