

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

RASĀYAN *J. Chem.*, Vol. 14, No.2, 2021

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.¹⁻¹⁷

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 (L₁-L₆). Sub-fraction L₃ (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 CDCl₃ 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 F₂₅₄ (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% CO₂ 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 method¹⁸. Briefly, cells were seeded in 96 well plates (10⁴-10⁵ cells/well) and incubated in CO₂ 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 CO₂ 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.¹⁸

RESULTS AND DISCUSSION

Compound **1** was isolated as white needle-like crystals, and the molecular formula C₃₀H₄₆O₂ was determined by HREI-MS (*m/z* 438.3498, [M+H]⁺) (Calcd. 438.6960), ¹H NMR, and ¹³C 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, CDCl₃) displayed seven methyl singlet signals at δ_H 0.84 (H₃-28), 0.94 (H₃-25), 1.01 (H₃-30), 1.04 (H₃-23), 1.08 (H₃-24), 1.10 (H₃-29), and 1.72 (H₃-26); eight multiplet methylene signals at δ_H 1.42, 2.06 (H₂-1), 2.24, 2.70 (H₂-2), 1.92, 2.40 (H₂-11), 1.92, 2.40 (H₂-12), 2.42 (H₂-15), 1.67, 1.49 (H₂-16), 1.54, 2.00 (H₂-19),

and 2.21, 2.61 (H₂-20); one doublet of doublet methylene signals at δ_H 1.35, 1.26 (H₂-6); an olefinic broad singlet signal at δ_H 5.42 (H-7); three multiplet methine signals at δ_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 (H₂-27). The ¹³C NMR (150 MHz) revealed the presence of seven methyl carbons signals at δ_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 sp³ and 1 sp²) at δ_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 sp³ and 1 sp²) at δ_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 sp², 4 sp³) at δ_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).¹⁹ 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 IC₅₀ values of < 20 $\mu\text{g/mL}$, and a natural compound that has IC₅₀ values of < 4 or 10 $\mu\text{g/mL}$ are considered active²⁰. Therefore, hexane extract displayed potential cytotoxic activity against T47D (IC₅₀ 0.10 $\mu\text{g/mL}$) and A549 (IC₅₀ 18.83 $\mu\text{g/mL}$) cell lines, but compound **1** demonstrated weak activity against only A549 (IC₅₀ 13.71 $\mu\text{g/mL}$) cell lines.

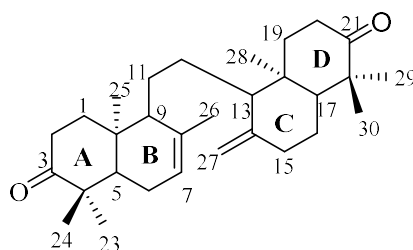


Fig.-1: Chemical Structure of Compound 1 from the Fruit Peel of *Lansium domesticum*

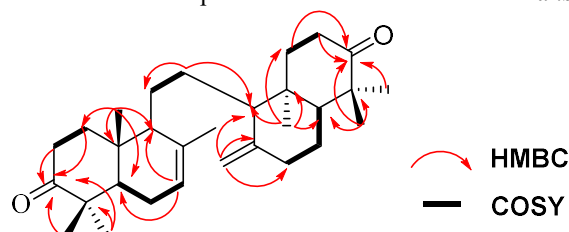


Fig.-2: HMBC and COSY Experiments of Compound 1

Table-1: NMR Spectral Data of Compound 1

Position	δ_H (ppm) (mult, J in Hz)	δ_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> , $J = 7.0, 5.0$ Hz)	51.6	C-9, C-10, C-24
6	1.35 (1H, <i>dd</i> , $J = 7.0, 10.0$ Hz)	25.3	C-7, C-10
	1.26 (1H, <i>dd</i> , $J = 5.0, 10.0$ 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, <i>m</i>)		
12	1.92 (1H, <i>m</i>)	24.1	C-13
	2.40 (1H, <i>m</i>)		
13	1.60 (1H, <i>m</i>)	54.4	C-28
14	-	147.3	-
15	2.42 (2H, <i>m</i>)	37.9	C-14, C-16, C-27
16	1.67 (1H, <i>m</i>)	25.2	C-17
	1.49 (1H, <i>m</i>)		
17	1.58 (1H, <i>m</i>)	55.2	C-13, C-18; C-29
18	-	39.2	-
19	1.54 (1H, <i>m</i>)	37.8	C-18, C-20, C-21
	2.00 (1H, <i>m</i>)		
20	2.21 (1H, <i>m</i>)	34.7	C-18, C-19, C-21
	2.61 (1H, <i>m</i>)		
21	-	216.6	-
22	-	47.7	-
23	1.04 (3H, <i>s</i>)	25.0	C-3, C-4, C-5
24	1.08 (3H, <i>s</i>)	22.0	C-3, C-4, C-5
25	0.94 (3H, <i>s</i>)	13.5	C-1, C-5, C-9, C-10
26	1.72 (3H, <i>s</i>)	22.2	C-7, C-8, C-9
27	4.92 (1H, <i>s</i>)	107.7	C-13, C-14, C-15
	4.61 (1H, <i>s</i>)		
28	0.84 (3H, <i>s</i>)	14.2	C-13, C-17, C-18, C-19
29	1.10 (3H, <i>s</i>)	26.1	C-17, C-21, C-22
30	1.01 (3H, <i>s</i>)	21.6	C-17, C-21, C-22

* Recorded at 600 MHz for ^1H and 150 MHz for ^{13}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	<i>8,14-Secogammacera-7,14(27)-dien-3,21-dione</i> (onoceradienedione)	32.39 ± 1.17	30.69 ± 1.87	13.71 ± 0.42
2	<i>n</i> -Hexane extract	59.55 ± 4.29	0.10 ± 5.88	18.83 ± 2.58
3	Doxorubicin	2.83 ± 0.18	0.04 ± 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.

ACKNOWLEDGEMENT

The author expresses high appreciation to: (1) Institute for Research and Community Services, Universitas Airlangga, Surabaya, Indonesia for "HIBAH RISET MANDAT KOLABORASI MITRA LUAR NEGERI" funding support. (2) Faculty of Pharmacy, Meijo University, Nagoya, Japan for NMR data. (3) Cancer Research Lab, Institute of Biosciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia for the cytotoxic analysis.

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

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Rasayan J. Chem.
ISSN: 0974-1496(Print)
ISSN: 0976-0083(Online)
CODEN: RJCABP
Frequency: March, June, September & December



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Publisher and Managing Editor
RASĀYAN Journal of Chemistry
23 'Anukampa', Janakpuri, O
Stn., Ajmer Road, Jaipur-302022 (India)

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Research Interest: Water treatment



Pankaj KUMAR

Professor and Head, Department of Chemistry, University of Energy and Petroleum studies, Dehradun, India

Contact: +917351958165

Email: pkumar@ddn.upes.ac.in

Research Interest: Biofuels and Bioenergy, Chemical sensors, Nanomaterials, Minimization of industrial wastes



Ramesh. L. GARDAS

Department of Chemistry Indian Institute of Technology Madras Chennai-600 036, India

Contact: +91 9884996125

Email: _gardas@iitm.ac.in

Research Interest: Physical Chemistry, Chemical Thermodynamics, Alternative Solvents



Susheel MITTAL

Senior Professor, School of Chemistry & Biochemistry, Thapar Institute of Engineering & Technology (Deemed to be University), Bhadson Road, Patiala-147004, India

Contact: +91-9815653261

Email: smittal2001@yahoo.com

Research Interest: Voltammetric Sensors, Potentiometric Sensors, Biosensors, Ambient Air Quality and Human Health



Willian Aperador CHAPARRO

School of Engineering, Universidad Militar Nueva Granada, Bogotá-111121, Colombia

Contact: + 57 3142220552

Email: william.aperador@unimilitar.edu.co

Research Interest: Materials, batteries, corrosion, coatings, tribology

Professor, Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Contact: +2348038198753

Email: nabukeddy@yahoo.com

Research Interest: Physical Chemistry, Computational Chemistry, Nanochemistry, Industrial Chemistry, Environmental Chemistry



R.V. SINGH

Ex Professor, Department of Chemistry, Rajasthan, Jaipur, India

Contact: +91 941406975

Email: rvsivr@hotmail.com

Research Interest: Inorganic Chemistry



Soro YAYA

Laboratoire des Procédés Industriels de Synthèse, de l'Environnement et des Energies Nouvelles (LAPISEN), Institut National Polytechnique (INP-HB), Yamoussoukro, BP 991 Yamoussoukro (Côte d'Ivoire)

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Research Interest: Organic synthesis, Natural Products, waste management



V.K. GARG

Professor and Dean Centre for Environmental Science and Technology School of Environment and Earth Sciences Central University of Punjab, Bathinda- 151001, India

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Email: vinodkgarg@yahoo.com

Research Interest: Pollution Monitoring and abatement, Solid Waste Management, Radioecology

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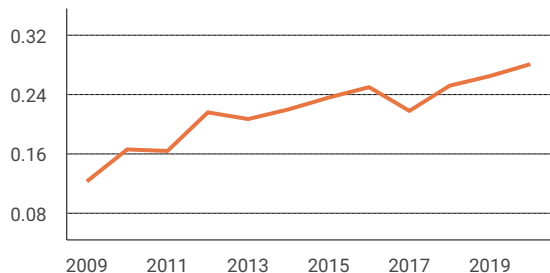
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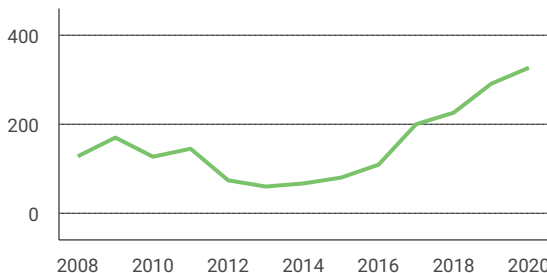
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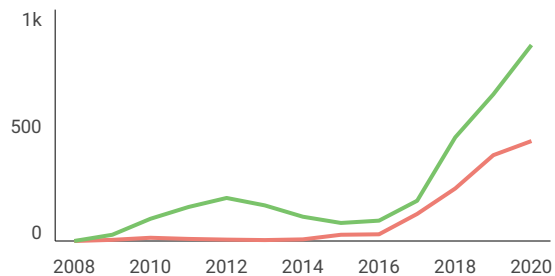
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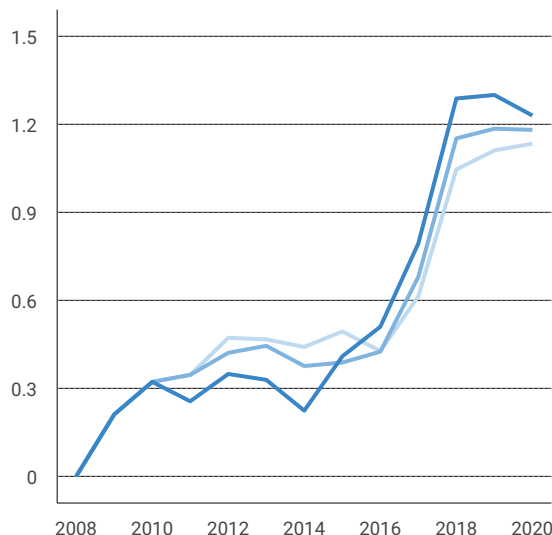
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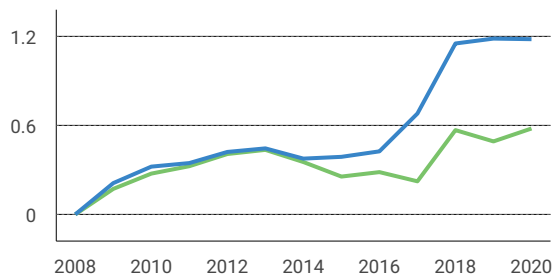
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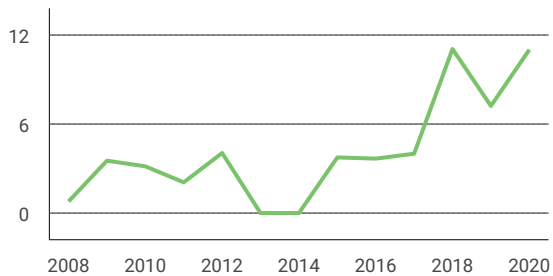


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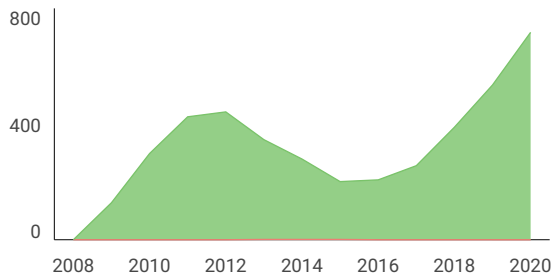
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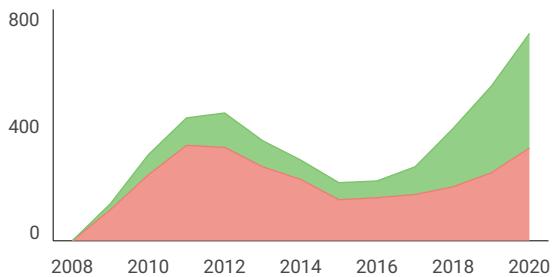
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Assoc. Professor on Natural Product Chemistry
Dept. of Chemistry
Fac. of Science and Technology
Universitas Airlangga

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Q. Labibah, K. N. W. Tun, N. S. Aminah*, A. N. Kristanti, R. Ramadhan, Y.
Takaya, C.A.C. Abdullah, M.J. Masarudin

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Name: NANIK SITI AMINAH

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