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A Sesquiterpene Aldehyde Isolated From Ethyl Acetate Extract Of *Lansium Domesticum* Fruit Peel

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ABSTRACT

Lansium domesticum (fam. Meliaceae) contains various compounds with various biological activities. Based on the previous research, extracts from several parts of the plant have biological activity. This study aimed to isolate a compound from the fruit peel of *L. domesticum* and evaluate its cytotoxic activity against T47D, WiDr, and HepG2 cell lines. Powdered peels were macerated with ethyl acetate and the filtrate was evaporated to give EtOAc extract. The dried extract was triturated with n-hexane to give *n*-hexane soluble fraction (A) and an insoluble fraction (B). The fraction B was separated using vacuum liquid chromatography (VLC) with mobile phase *n*-hexane: ethyl acetate and given 5 fractions. Fractions B3-B5 were combined and separated using VLC with *n*-hexane and ethyl acetate as mobile phase. This VLC separation gave 18 subfractions, subfractions 6-9 with a similar TLC profile were combined. This subfraction was separated further using preparative thin layer chromatography to give compound 1. The Isolated compound (1) appeared as a liquid. The chemical structure of 1 was identified according to spectroscopic data and in comparison with literature. Cytotoxic bioassay was performed on T-47D, WiDr, and Hep G2 cell lines in a series of concentrations at 50, 40, 30, 20, 10, and 5µg/mL, with Doxorubicine used as a positive control. According to spectroscopic data, compound 1 was identified as 2-ethyl,3-(1'-hydroxy-2'-menthene) propenal, and demonstrated the strongest cytotoxicity against T-47D cell lines (IC_{50}=39.18±1.54µg/mL).

Keywords: *Lansium domesticum*, fruit peel, cytotoxic, cell lines

INTRODUCTION

Lansium domesticum is a plant from the Meliaceae family which contains various compounds having potential pharmacological values. Based on previous studies, extracts from several parts of the *L. domesticum* plant were shown to have biological activities. Chloroform extract of *L. domesticum* young fruit, for example, was known to have cytotoxic activity and induce apoptosis in KB and HT-29 cancer cells. The chloroform extract was also reported to be cytotoxic against B16F10 cells (IC_{50} 421.50±12.98µg/mL (Manosroi *et al.*, 2012). Another study reported that *L. domesticum* peel had antioxidant activity. Ethanol-ethylacetate fraction (50:50%, v/v) had anti-DNA damage activity in lymphoblast cells induced by H_2O_2 exposure (Klungsupya *et al.*, 2015). In addition, several compounds have been isolated from this plant. Dukunolide, tetranortriterpenoid compounds, and terpenoid glycosides lansioside A had been isolated, however, their biological activity had not been reported yet (Nishizawa *et al.*, 1982, 1985). Kokosanolide A-C compounds were isolated from seeds and had antifeedant activity against larvae of *Epilachna vigintioctopunctata* (Mayanti *et al.*, 2011). Langsatides A and B were also isolated from the seeds, but they had insignificant activity as antibacterial and cytotoxic (Rudiyansyah *et al.*, 2018)

In our previous study, a compound was isolated from n-hexane soluble fraction (fraction B) of L.domesticum fruit peel ethyl acetate extract using vacuum liquid chromatography. Based on ^{13}C-NMR and ^{1}H-NMR data, this compound was identified as Lamesticumin A and displayed

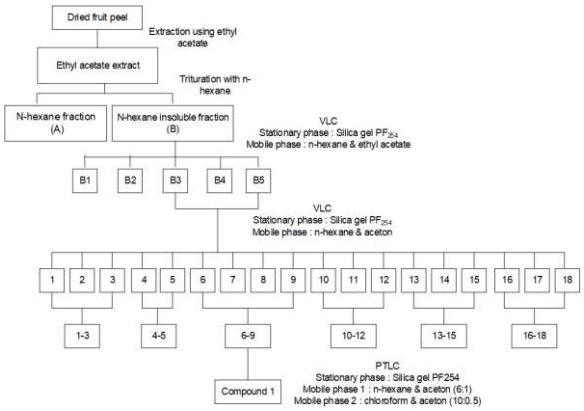
cytotoxic properties against T-47D cell line with IC_{50} 15.68±0.30µg/mL (Fadhilah *et al*., 2020). Lamesticumin A has been isolated from *L. domesticum* twigs and reported to have antibacterial activity (Dong *et al*., 2011). This study was a continuation of the compound isolation process from n-hexane soluble fractions of duku fruit peel extract and evaluation of the cytotoxic activity of the isolated compound against cancer cells.

MATERIALS AND METHODS

Solvents used were p.a. grade from E Merck. Silica gel F_{254} was used for thin-layer chromatography dan silica gel PF_{254} (E Merck) was used for preparative thin layer chromatography, cerium(IV)sulfate tetrahydrate (E Merck), RPMI 1640, Fetal Bovine Serum, Penicillin-Streptomycin, Fungizon, Sodium bicarbonate (Gibco), HEPES (Invitrogen), Phosphate Buffered Saline, MTT, Doxorubicin (Sigma Aldrich). Infrared (KBr) spectrum was obtained using IR spectrophotometer (Shimadzu), ultraviolet spectrum ($CHCl_3$) was obtained from UV spectrophotometer (Hitachi UH 5300), mass spectra were obtained from GC-MS (Shimadzu), ^{1}H- and ^{13}C- NMR spectra were obtained from JEOL JNM-ECZ 500R/S1, 500MHz.

Separation method

Lansium domesticum rind was obtained from Purbalingga, Central Java. The fruit peels were separated from the fruit and dried in an oven at 50ºC. The dried peel fruit was macerated with ethyl acetate (EtOAc) at room temperature for 24 h. The macerate was evaporated with a rotary evaporator at a temperature of 50°C to obtain a thick EtOAc extract. This study was a continuation study of the previous one (Fadhilah *et al*., 2020). The EtOAc extract obtained was triturated with n-hexane to give n-hexane (A) and insoluble fractions (B). Fraction B was further fractionated by vacuum liquid chromatography to give fractions B1-B5. Fractions B3-B5, showed similar TLC profiles (having similar major compounds) were combined and separated using VLC (*n*-hexane: acetone, increasing polarity) to give 18 fractions (Fr1–Fr18). Fractions 6-9 which had the same chromatographic profile were combined and the combined fractions were separated by preparative thin-layer chromatography (PTLC), developed twice [silica gel PF_{254}, 1st mobile phase (n-hexane: acetone 6:1) and 2nd mobile phase (chloroform: acetone 10: 0.5)], compound 1 was obtained (Figure 1).



Cytotoxic activity

The cytotoxic activity test was carried out by the previous method with modifications (Bahuguna *et al*., 2017). The cancer cells (T-47D, WiDr, Hep G2) used were a collection from Parasitology Laboratory, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. A hundred µl of cells in RPMI media were placed into 96 well microplates to obtain 10^4 cells / well and incubated for 24h (temperature 37°C and 5% CO_2). Cells were treated with the test sample and incubated for 24h. After the treatment process, the culture media was discarded, and the cells were given 100µL/well MTT reagent (5mg/ml) and incubated for 4 h. After incubation, 100µl of stopper solution (SDS 10%) was added and let stand at room temperature overnight. Absorbance measurement was conducted with a microplate reader (BioRad) at 595nm. The test sample (in DMSO) was made in a series of concentrations at 50, 40, 30, 20, 10, 5µg/mL and doxorubicin as a positive control with concentrations of 1, 0.5, 0.25, 0.125, 0.0625, 0.0312µg/mL. The data generated were used to plot a dose-response curve and IC_{50} of compound 1 was determined. The IC_{50} values were analyzed by one-way ANOVA with statistical significance $P<0.05$ using IBM SPSS ver.23.

RESULTS AND DISCUSSION

Compound 1 appeared as a colorless oil, displayed maximum absorption (UV λ_{max}) at 260nm indicating the presence of a chromophoric group. The IR spectrum showed absorption bands at 3426, 3000, 1679, 1464cm^{-1} indicating the presence of –OH, -C-H, α, β-unsaturated C=O respectively. The mass spectrum showed a molecular ion at m/z 236.

The ^{1}H- and ^{13}C- NMR ($CDCl_3$) spectra were obtained from JEOL JNM-ECZ 500R/S1, 500 MHz (Figure 2, Table I). The ^{13}C-NMR ($CDCl_3$) (Figure 2a) spectrum of compound 1 showed the presence of

15 carbon atoms, the spectrum displayed carbon signals attribute to four methyl groups (δ_c15.4 (C-5), 21.5 (C-10'), 21.5(C-9'), 26.4 (C-7'), three methylene groups (δ_c 22.4 (C-5'), 22.2 (C-4), 42 (C-6'), four alkene carbons, also the presence of α,β-unsaturated carbonyl (194.8 ppm) two methines (δ_c 45.7 (C-8'), 49.8 (C-4') and one tertiary carbon attached to an oxygen atom (δ_c 72.36 (C-1')

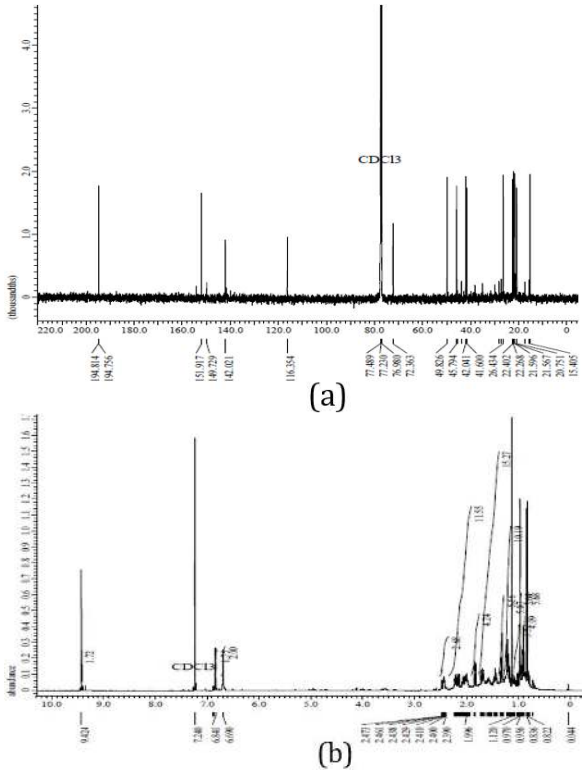


Figure 2. ^{13}C-NMR spectra and H-NMR spectra ($CDCl_3$, 500 MHz) of compound 1

The ^{1}H-NMR spectrum (Figure 2b) of compound 1 showed four signals of terminal methyl groups (δ_H 0.82-0.83-(H-10'), 0.95-0.97 (H-9'), 1.12 (H-7'), 1.21-1.23 (H-5) three signals of methylene groups (δ_H 1.31-(H-5'), 1,42-1,67 (H-6'), 2.40-2.47 (H-4), two signals of methine groups (δ_H 1.82-1.85 (H-4'), 1.94-2.21 (H-8') , two singlet signals of vinyl protons that also indicated the presence of α,β-unsaturated protons at δ_H 6.69 (H-2'), 6.841 (H-3) and one singlet signal of aldehyde hydrogen (δ_H 9.42 (H-1).

Based on the spectroscopic data above, the structure of compound 1 was deduced as 2-ethyl,3-(1'-hydroxy-2'-menthene) propenal ($C_{15}H_{24}O_2$, m/z 236) (Figure 3). There is still no report found about this compound; however, the configuration of carbons number 1' and 4' were not assigned yet as additional 2D NMR data was needed. However, according to literature, mostly $-CH_3$ at C1' at β position while isopropyl at C4' could be at α or β position, in some cases could be a mixture of both (Moss, 1996).

Table I. ^{1}H-NMR and ^{13} C-NMR chemical shifts of compound 1

No	**^{1}H-NMR** δ (ppm), J=Hz	**^{13}C-NMR** δ (ppm)
1	9.42 (1H, s)	194.8
2	-	151.9
3	6.84 (1H, s)	142.0
4	2.40-2.47 (2H,q)	22.2
5	1.20-1.22(3H, m)	15.4
1'	-	72.3
2'	6.69 (1H, s)	116.3
3'	-	149.7
4'	1.82-1.84 (1H,m)	49.8
5'	1.31 (2H, m)	22.4
6'	1,42-1,67 (2H, m)	42.0
7'	1.12 (1H,s)	26.4
8'	1.93-2.21 (1H,m)	45.7
9'	0.95-0.97 (3H,d. J=7 Hz)	21.5
10'	0.82-0.83 (3H,d, J=7 Hz)	21.5

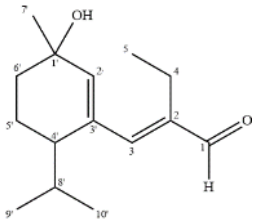


Figure 3. Structure of compound 1

Table II. IC_{50} values of the isolated compound

Sample	**IC_{50} (μg/mL)**		
	T-47D	WiDr	Hep G2
Compound 1	39.53+1.82	>50	>50
Doxorubicin	0.21+0.02	0.59+0.03	0.86+0.08

Based on the results of the cytotoxicity test, compound 1 was cytotoxic against T47D cells with an IC_{50} value of 39.18 ± 1.54 μg/ml. On the other hand, the compound was less cytotoxic against HepG2 and WiDr cells, with IC_{50} values> 50 μg/ml (Table 2). The cytotoxic activity of this compound was stronger in T-47D cells compared to WiDr and Hep G2 cells. Based on NCI standards, pure compounds were considered to have potent activity if they have an IC_{50} value <4 μg / ml (Barros *et al*., 2013).

Further research is needed to evaluate the potential of compound 1 in combination with other chemotherapy agents. Based on several studies, several terpenoid compounds had been reported to have the potential to increase the sensitivity of chemotherapy agents. Sesquiterpene compounds, vielanin K and P for example, were reported to increase doxorubicin activity. Vielanin K increased apoptosis induction of MCF-7 and MCF-7/MDR cells through activation of IRE1α-TRAF2-JNK signaling (Zhang *et al*., 2020). Meanwhile, Vielanin P increased cytotoxicity by inhibiting PI3K / Nrf2-stimulated MRP1 expression on doxorubicin-resistant MCF-7 and K562 cells(Gao *et al*., 2019). Geraniol monoterpene compounds were also capable of increasing the apoptosis induction of 5-FU in Caco-2 colon cancer cells *in vitro* (Carnesecchi *et al*., 2004). Protopanaxadiol, a triterpenoid, also synergized the effect of 5-FU on HCT116 colon cancer cells and induced cell cycle arrest in G1 (Wang *et al*., 2015). Limonin triterpenoid compounds were reported to increase doxorubicin cytotoxicity by inhibiting P-gp efflux in CCRR-CEM leukemia cancer cells and CaCo-2 resistant drug colon cancer (El-Readi *et al*., 2010).

CONCLUSION

Compound 1 was identified as a sesquiterpene aldehyde, 2-ethyl,3-(1'-hydroxy-2'-menthene) propenal. This compound showed weak cytotoxicity and still needs further investigation for its combination with other chemotherapeutic agents.

ACKNOWLEDGEMENTS

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