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 T47D, 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 T47D cell lines (IC₅₀=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 (IC₅₀ 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 H₂O₂ exposure (Klungsupya et al., 2015). In addition, several compounds have been isolated from this plant. Dukunolide, tetrannotriterpenoid 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 ¹³C-NMR and ¹H-NMR data, this compound was identified as Lamesticumin A and displayed...
cytotoxic properties against T-47D cell line with IC\textsubscript{50} 15.68±0.30\,\mu g/mL (Fadhilah et al., 2020). Lamesticumin A has been isolated from \textit{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 \textit{F\textsubscript{254}} was used for thin-layer chromatography dan silica gel \textit{PF\textsubscript{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 (Hitachi UH 5300), mass spectra were obtained from UV spectrophotometer (CHCl\textsubscript{3}), mass spectra were obtained from JEOL JNM-ECZ 500R/S1, 500MHz.

**Separation method**

\textit{Lansium domesticum} rind was obtained from Purbalingga, Central Java. The fruit peels were separated from the fruit and dried in an oven at 50°C. 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 \textit{PF\textsubscript{254}}, \textit{I\textsubscript{n}th} mobile phase (n-hexane: acetone 6:1) and \textit{2\textsuperscript{nd}} 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\textsuperscript{4} cells / well and incubated for 24h (temperature 37°C and 5% CO\textsubscript{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 4h. 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, 0.03125µg/mL. The data generated were used to plot a dose-response curve and IC\textsubscript{50} of compound 1 was determined. The IC\textsubscript{50} values were analyzed by one-way ANOVA with statistical significance \textit{P}<0.05 using IBM SPSS ver.23.

**RESULTS AND DISCUSSION**

Compound 1 appeared as a colorless oil, displayed maximum absorption (UV \textsubscript{\lambda\textsubscript{max}}) at 260nm indicating the presence of a chromophoric group. The IR spectrum showed absorption bands at 3426, 3000, 1679, 1464cm\textsuperscript{-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 \textsubscript{1\textsuperscript{H}} and \textsubscript{13\textsuperscript{C}} NMR (CDCl\textsubscript{3}) spectra were obtained from JEOL JNM-ECZ 500R/S1, 500 MHz (Figure 2, Table I). The \textsubscript{13\textsuperscript{C}}NMR (CDCl\textsubscript{3}) (Figure 2a) spectrum of compound 1 showed the presence of
15 carbon atoms, the spectrum displayed carbon signals attributed to four methyl groups ($\delta$ 15.4 (C-1)), 21.5 (C-10), 21.5 (C-9'), 26.4 (C-7'), three methylene groups ($\delta$ 22.4 (C-5'), 22.2 (C-4), 42 (C-6'), four alkene carbons, also the presence of $\alpha,\beta$-unsaturated carbonyl (194.8 ppm) two methines ($\delta$ 45.7 (C-8'), 49.8 (C-4')) and one tertiary carbon attached to an oxygen atom ($\delta$ 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 ($\delta$ 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 ($\delta$ 1.31 (H-5'), 1.42-1.67 (H-6'), 2.40-2.47 (H-4), two signals of methine groups ($\delta$ 1.82-1.85 (H-4'), 1.94-2.21 (H-8'), two singlet signals of vinyl protons that also indicated the presence of $\alpha,\beta$-unsaturated protons at $\delta$ 6.69 (H-2'), 6.841 (H-3) and one singlet signal of aldehyde hydrogen ($\delta$ 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 $-\text{CH}_2$ at C1' at $\beta$ position while isopropyl at C4' could be at $\alpha$ or $\beta$ position, in some cases could be a mixture of both (Moss, 1996).

Based on the results of the cytotoxicity test, compound 1 was cytotoxic against T47D cells with an IC$_{50}$ value of 39.18 $\pm$ 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, vielain K and P for example, were reported to increase doxorubicin activity. Vielain K increased apoptosis induction of MCF-7 and MCF-7/MDR cells through activation of IRE1α-TRAF2-JNK signaling (Zhang et al., 2020). Meanwhile, Vielain P increased cytotoxicity by inhibiting PI3K / Nrf2-stimulated MRPI 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 CCR-CEM leukemia cancer cells and GaCo-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.

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