A bioactive compound isolated from Duku (Lansium domesticum Corr) fruit peels exhibits cytotoxicity against T47D cell line [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

**Background:** Breast cancer is a major health problem for women globally. Many attempts have been promoted to cure cancer by finding new anticancer medicines from natural resources. Despite the richness of biodiversity discovered, there are some natural resources that remain unexplored. Fruit peels of Duku (Lansium domesticum Corr.) are rich with compounds that may have the potential to be developed as anticancer drugs. This study aimed to isolate cytotoxic compounds from the fruit peels of L. domesticum and assess their cytotoxic nature against T47D cells.

**Methods:** Powdered peels were macerated with ethyl acetate and the filtrate was evaporated to give EtOAc extract A. Dried extract A was triturated with n-hexane to give n-hexane soluble fraction B and insoluble fraction C. The cytotoxic nature of these three samples were assessed using MTT assay using T47D cells and doxorubicin as a control.

**Results:** Fraction C that showed the smallest IC50 (25.56 ± 0.64μg/mL) value compared to extract A and fraction B. Fraction C was further fractionated by vacuum liquid chromatography to give 6 subfractions. Subfraction 2 showed a single compound based on thin layer chromatography, and this compound was identified as Lamesticumin A on the basis of its spectroscopic data. Lamesticumin A demonstrated cytotoxic activity against T47D cell lines with an IC50 value of 15.68 ± 0.30μg/mL.

**Conclusions:** Further research is needed to investigate the potential of the natural compound Lamesticumin A derived from L. domesticum fruit peel as an anticancer therapy.

**Keywords**
Lansium domesticum Corr., cytotoxic, T47D cell line, Lamesticumin A
Introduction

The most frequent cancer in women and that which causes the highest mortality is breast cancer. In Indonesia, it was reported that approximately 21% of cancer deaths among women were due to breast cancer. Therefore, new medicines to eradicate this type of cancer is required. Duku (Lansium domesticum Correa) widely grows in Indonesia. Traditionally, L. domesticum bark and seeds have been used to treat dysentery and fever. Based on previous studies, chloroform and methanol extracts of L. domesticum displayed cytotoxic activity on murine melanoma (B16F10) and colon cancer (HT29) cells. In addition, it has been shown that ethanol and ethyl acetate fractions of the peel have a deterrent activity on DNA damage in lymphoblast cells induced by H$_2$O$_2$ exposure. Onocerinoid-type of triterpenoids have been isolated from twigs and leaves of L. domesticum, and these compounds showed antibacterial and antimutagenic activities. In this study, the cytotoxic effects of compound extracted from the peels of L. domesticum are assayed against breast cancer T47D cells.

Methods

Plant material

The fruits of L. domesticum were collected on March 2018 from Bantul, Yogyakarta (GPS : -7.871098, 110.394854) and identified at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada.

Chemicals and equipment

Organic solvents (methanol, ethyl acetate, chloroform, n-hexane) used were pro analytical grades obtained from Merck. Silica gel F$_{254}$ Silica gel PF$_{254}$ (Merck), RPMI 1640, Fetal Bovine Serum, Penicillin-Streptomycin, Fungizon, Sodium bicarbonate (Gibco), HEPES (Invitrogen), Phosphate Buffered Saline, MTT (Sigma Aldrich cat. M5655), Doxorubicin (Sigma Aldrich). Infrared (IR), $^1$C-NMR and $^1$H-NMR (see section Chemicals and equipment).

Cytotoxic assay

The bioassay followed the methodology described by Bahuguna et al. with modifications. In brief, 100 μl T47D cells (in RPMI mediaFaculty of Medicine, Universitas Gadjah Mada) were placed in each well of a 96 well microlter, resulting in 1 × 10^4 cells/well. The cells were incubated for 24 hours at 37°C in a CO$_2$ incubator.

Extract A, fractions B and C and compound 1 (5mg) were dissolved in DMSO (50 μL). Serial concentrations of extract and fractions (50, 25, 12.5, 6.25, 3.125 μg/mL), compound 1 (25, 12.5, 6.25, 3.125 μg/mL) and doxorubicin (positive control; 0.5, 0.25, 0.125, 0.0625, 0.0312 μg/mL) were obtained. Cells were treated with the dose dependent samples and incubated for 24 hours at 37°C. The culture medium was removed by pipette, and MTT solution (100μL) was added to each well and incubated for 4 hours at 37°C. After incubation, stop solution (10% SDS, 100 μL) was added to each well and let stand at room temperature for 24 hours.

Absorbance was measured by microplate reader (Bio Rad) at 595 nm. positive control The data generated were used to plot a dose-response curve and IC$_{50}$ of the samples was determined.

Statistical analysis

The IC$_{50}$ values were analyzed by one-way ANOVA with statistical significance P < 0.05 using IBM SPSS ver.23.

Results

Compound 1 characterization

Identified as Lamesticumin A.

White crystal. IR (KBr) ν$_{max}$ cm$^{-1}$: 3074, 2960, 1712; UV (MeOH)λ$_{max}$ 236.5; $^1$H,$^1$C-NMR: see Table 1; m/z 502; (Calculated for C$_{11}$H$_{16}$O$_5$)

The infrared spectroscopy (KBr) spectrum of 1 showed a broad band at 3400–2800 cm$^{-1}$, which indicated the presence of –OH group, specifically –COOH due to intermolecular bonding. This data is supported by the appearance of –C=O at 1712 cm$^{-1}$. Compound 1 displayed UV absorption at 236.5 nm. The $^1$C-NMR spectrum (500 MHz, CDCl$_3$) of compound 1 showed 30 carbons (Table 1). There were two down field carbon signals (δ, 147.6 and 148.1 ppm) identified as C=O signal carbons. Two characteristic terminals =CH$_2$ signals (δ, 107.4 and 114.2 ppm) were observed, and this identity was confirmed by 2D (Het-Cor) NMR technique. Based on $^1$C-NMR
Figure 1. Isolation process of cytotoxic compound 1 (subfraction 2) from *Lansium domesticum* fruit peels.

Table 1. 13C-NMR spectrum (500 MHz, CDCl3) of compound 1, Lamesticumin A.

| Position | 1H-NMR (J, Hz) | 13C-NMR δ (ppm) |
|----------|----------------|-----------------|
|          | Multiplicity   |                 |
| 1        | 1.2            | 2H, triplet (7.0)| 27.98 |
| 2        | 2.1            | 2H, triplet (6.8)| 29.18 |
| 3        | -              | -               | 147.60 |
| 4        | -              | -               | 51.78  |
| 5        | 0.9            | 1H, triplet (7.1)| 50.78 |
| 6        | 1.4            | 2H, multiplet   | 27.51  |
| 7        | 1.9            | 2H, triplet (6.8)| 28.75 |
| 8        | -              | -               | 122.10 |
| 9        | 1.1            | 1H, triplet (7.0)| 48.91 |
| 10       | -              | -               | 47.68  |
| 11       | 1.4            | 2H, multiplet   | 30.59  |
| 12       | 1.7            | 2H, multiplet   | 29.67  |
| 13       | 1.13           | 1H, triplet (7.0)| 41.71 |
| 14       | -              | -               | 135.91 |
| 15       | 5.4            | 1H, triplet (6.8)| 113.93|

1H- and 13C- NMR (CDCl3) spectra were obtained from JEOL JNM-ECZ 500R/S1, 500 MHz.
and 'H-NMR data, compound 1 (Figure 2) was identified as Lamesticumin A (C_{31}H_{50}O_{5}, m/z, 502) which was previously isolated from *L. domesticum* twigs.

**Cytotoxicity of extract A, fractions B and C, and compound 1**

The cytotoxicity of extract A, fractions B and C, compound 1 and doxorubicin (positive control) is shown in Table 2. Fraction C was the most cytotoxic (IC\(_{50}\) 25.57 μg/mL) compared with extract A (29.41 μg/mL) and fraction B (43.51 μg/mL). The IC\(_{50}\) of the isolated compound from fraction C, compound 1/Lamesticumin A was 15.68 μg/mL. All samples inhibited T47D cell growth in a dose dependent behavior (Figure 3).

**Discussion**

In this study, the cytotoxic activity of Lamesticumin A, derived from the peel of *L. domesticum*, was demonstrated in the T47D cell line with IC\(_{50}\) 15.68 (μg/ml). The T47D cell line is an epithelial breast cancer cell subtype luminal A cell line that express estrogen and progesterone receptors. Based on National Cancer Institute guidelines, a natural compound has potent anticancer activity if it has IC50 <4 μg/ml or 10 μM. Many triterpenoid compounds have been previously isolated from *L. domesticum*. Most of these compounds are UV inactive or have no strong UV absorbance because triterpenoid’s lack of a conjugated functional group. Lansiosida A and Dukunolida A has been isolated from n-hexane extract of *L. domesticum* fruit peel. Lamesticumin A is an onoceranoid-type triterpenoid, isolated previously from *L. domesticum* twigs, that has antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Bacillus subtilis*, *Micrococcus pyogenes* and *Bacillus cereus* with minimum inhibitory concentration of < 15 μg/mL. Another onoceranoid-type triterpenoid Lansium acid I-IX were isolated from *L. domesticum* leaves, which was reported to have antimutagenic activity.

Based on several *in vitro* tests, some terpenoid compounds had anticancer activity. Sesquiterpene lactone compounds are known to inhibit NF-kB, thereby inducing apoptosis. Celastrol has anticancer properties by regulating various transcription factors, angiogenesis processes, cell cycle arrest and induction of apoptosis. Betulinic acid can induce apoptosis in HT-29 colon cancer cells and acts as a chemosensitizer for chemotherapeutic agents in wildtype adenocarcinoma cancer cells (SNU-C5/WT).

![Figure 2. Isolated compound 1, Lamesticumin A.](image)

**Table 2.** IC\(_{50}\) values of extract, fractions and isolated compound 1 against T47D breast cancer cell line.

|          | IC\(_{50}\) (μg/ml), mean ± SD |
|----------|---------------------------------|
| Extract A| 29.41 ± 0.67                    |
| Fraction B| 43.51 ± 1.77                   |
| Fraction C| 25.56 ± 0.64                   |
| Lamesticumin A| 15.68 ± 0.30             |
| Doxorubicin| 0.18 ± 0.01                    |
Clematangoticosides D and F from *Clematis tangutica* are known to have cytotoxic activity against human gastric cancer cell line (SGC-7901) with IC\textsubscript{50} 24.22 and 21.35 μM, respectively\textsuperscript{18}. Cycloartane-type and oleanane-type triterpenoids from *Ligularia przewalskii* show cytotoxicity in Hela, HEPG2, SGC7901, MDA231, HL-60, and Lewis cell lines with IC\textsubscript{50} 8.40–24.39 μM\textsuperscript{19}.

It has been reported that natural compounds combined with low doses of antineoplastics can increase effectiveness and reduce toxic effects\textsuperscript{20}. Betulinic acid can induce apoptosis when combined with 5-fluorouracil, irinotecan and oxaliplatin\textsuperscript{4}. Ursolic acid (UA), a pentacyclic triterpenoid, is known to have anticancer activity through interfering with multiple signaling pathways. Furthermore, UA has been shown to act as a chemosensitizing agent to increase the effect of conventional anticancer drugs\textsuperscript{21}, and to increase the effect of doxorubicin by increasing the cellular amount of the drug in the MCF cell line\textsuperscript{22}. Further study is needed to investigate the possibility of Lamesticumin A to be combined with doxorubicin for its potential to have synergistic effect.

### Conclusions

Extract, fractions and Lamesticumin A derived from the peel of *L. domesticum* showed cytotoxic activity against the T47D breast cancer cell line. Further research is needed to investigate the potential of the natural compound Lamesticumin A derived from *L. domesticum* fruit peel as an anticancer therapy.

### Data availability

**Underlying data**

Zenodo: A bioactive compound isolated from Duku (*Lansium domesticum* Corr) fruit peels exhibits cytotoxicity against T47D cell line, [http://doi.org/10.5281/zenodo.3539670]\textsuperscript{23}.

This project contains the following underlying data:

- UV, infrared, 	extsuperscript{13}C-NMR and 	extsuperscript{1}H-NMR spectra of compound 1.
- Cell viability and IC\textsubscript{50} values of extract A, fractions B and C, compound 1 and doxorubicin in T47D cell line.

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

### References

1. WHO: Cancer country profiles 2014. 2014: (Accessed: 20th April 2018). Reference Source
2. Heyne K: Tumbuhan berguna Indonesia. Badan Litbang Kehutanan, 1988. Reference Source
3. Manosroi A, Jantrawut P, Sainakham M, et al.: Anticancer activities of the extract from Longkong (*Lansium domesticum*) young fruits. *Pharm Biol.* 2012; 50(11): 1397–407. PubMed Abstract | Publisher Full Text
4. Klungsuyapa P, Suthepakul N, Muangman T, et al.: Determination of Free Radical Scavenging, Antioxidative DNA Damage Activities and Phytochemical
Components of Active Fractions from Lansium domesticum Corr. Fruit. 
Nutrients. 2015; 7(8): 6852–73. 
PubMed Abstract | Publisher Full Text | Free Full Text

5. Dong SH, Zhang CR, Dong L, et al.: Onoceranoid-type triterpenoids from 
Lansium domesticum. J Nat Prod. 2011; 74(5): 1042–1048. 
PubMed Abstract | Publisher Full Text

6. Matsumoto T, Kitagawa T, Teo S, et al.: Structures and Antimutagenic Effects of 
Onoceranoid-Related Triterpenoids from the Leaves of Lansium domesticum. 
J Nat Prod 2018; 81(10): 2187–2194. 
PubMed Abstract | Publisher Full Text

7. Ashok Kumar R, Ramaswamy M: Phytochemical screening by FTIR 
spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. 
Int J Curr Microbiol App Sci. 2014; 3(1): 395–406. 
Reference Source

8. Mae Sri Hartati W, Mubarika S, Gandjar IG, et al.: Phalerin, a new benzophenoic 
glucoside isolated from the methanolic extract of Mahkota Dewa [Phaleria macrocarpa (Schefl). Boerl] leaves. 
J Nat Prod. 2015; 78(7): 1697–1700. 
PubMed Abstract | Publisher Full Text

9. Bahuguna A, Khan I, Bajpai VK, et al.: MTT assay to evaluate the cytotoxic 
potential of a drug. Bangladesh J Pharm. 2017; 12: 115–118. 
Reference Source

10. Yu S, Kim T, Yoo KH, et al.: The T47D cell line is an ideal experimental model to 
elucidate the progesterone-specific effects of a luminal A subtype of breast 
cancer. Biochem Biophys Res Commun. 2017; 486(3): 752–758. 
PubMed Abstract | Publisher Full Text

11. Kuete V, Ndontsa BL, Nguekeu YMM, et al.: Ardisinol III, a naturally occurring 
alkenylmethylresorcinol displayed cytotoxic effects in carcinoma cells. 
Investig New Drugs. 2018; 36(1): 1–6. 
Reference Source

12. Xu R, Ye Y, Zhao W: Introduction to natural products chemistry. 2011. 
Reference Source

13. Nishizawa M, Nademoto Y, Sastrapradja S, et al.: Structure of dukunolid A: A 
tetranortriterpenoid with a new carbon skeleton from lansium domesticum. 
J Chem Soc Chem Commun. 1985; 395–396. 
Publisher Full Text

14. Nishizawa M, Nishide H, Hayashi Y, et al.: The structure of Lansioside A: A 
novel triterpene glycoside with amino-sugar from lansium domesticum. 
Tetrahedron Lett. 1982; 23(13): 1349–1350. 
Publisher Full Text

15. Sharma SH, Thulasisingam S, Nagarajan S: Triterpenoids as anti-colon cancer 
agents - A comprehensive review on its mechanistic perspectives. Eur J 
Pharmacol. 2017; 795: 169–178. 
PubMed Abstract | Publisher Full Text

16. Kayser K, Sharma A, Tuli HS, et al.: Molecular targets of celastrol in cancer: 
Recent trends and advancements. Crit Rev Oncol Hematol. 2019; 128: 70–81. 
PubMed Abstract | Publisher Full Text

17. Rzeski W, Stepulak A, Szymalska M, et al.: Betulinic acid decreases expression 
of bcl-2 and cyclin D1, inhibits proliferation, migration and induces apoptosis 
in cancer cells. Naunyn Schmiedebergs Arch Pharmacol. 2006; 374(1): 11–20. 
PubMed Abstract | Publisher Full Text

18. Zhao M, Da-Wa ZM, Guo DL, et al.: Cytotoxic triterpenoid saponins from 
Clematis tangutica. Phytochemistry. 2016; 130: 228–37. 
PubMed Abstract | Publisher Full Text

19. Shi ZN, Wang YD, Gong Y, et al.: New triterpenoid saponins with cytotoxic 
activities from Ligularia przewalskii. Phytochem Lett. 2019; 30: 215–219. 
Publisher Full Text

20. Lagoa R, Silva J, Rodrigues JR, et al.: Advances in phytochemical delivery 
systems for improved anticancer activity. Biotechnol Adv. 2019; 
pii: S0734-9750(19)30063-1. 
PubMed Abstract | Publisher Full Text

21. Prasad S, Tyagi AK, Aggarwal BB: Chemosensitization by Ursolic Acid: A New 
Avenue for Cancer Therapy. In Role of Nutraceuticals in Chemoresistance to 
Cancer 2018; 2: 99–109. 
Publisher Full Text

22. Zong L, Cheng G, Liu S, et al.: Reversal of multidrug resistance in breast cancer 
cells by a combination of ursolic acid with doxorubicin. J Pharm Biomed Anal. 
2019; 165: 268–276. 
PubMed Abstract | Publisher Full Text

23. Fadhilah K, Wahyuono S, Astuti P: A bioactive compound isolated from Duku 
(Lansium domesticum Corr) fruit peels exhibits cytotoxicity against T47D cell 
line [Data set]. Zenodo. 2019. 
http://www.doi.org/10.5281/zenodo.3539670
Deden Derajat Matra

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The author did not describe the maturity degree from samples because the different stages of maturity may change secondary metabolites that concern effect to the bioactive compound.

How to handling samples after harvesting is important too. Please, author, describe more?

In conclusion, what kind of peel fresh or processed is best consumed as the higher potential to anticancer therapy.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

No

**If applicable, is the statistical analysis and its interpretation appropriate?**

Partly

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Partly

**Competing Interests:** No competing interests were disclosed.
**Reviewer Expertise:** Agricultural Plant Science, Horticulture, AgronomyPhysiology, Bioinformatics, Molecular Biology.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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**Reviewer Report 26 August 2020**

https://doi.org/10.5256/f1000research.23191.r70005

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**? Ratana Banjerdpongchai**

Department of Biochemistry, Chiang Mai University, Chiang Mai, Thailand

**Major points:**

The extracts and purified bioactive compound, Lamesticumin A, from this plant, is not novel, since it was isolated from the twigs from this same herb in the previous report. The anticancer activity of the active compound is not promising since it is more than 4 microgram/mL or 10 micromolar, the level of which the National Cancer Institution defines is 4 microgram/mL or less than this. However, the authors discussed of using this compound as a chemo sensitizing agent, which various steps still are needed before launching it as a therapeutic anti-cancer drug. The authors should explore the cytotoxic effects of the extracts, EtOAc extract (A), N-hexane fraction (B), N-hexane insoluble fraction (C) and compound 1 (Lamesticumin A) on normal cells.

In Figure 3, the percent cell viabilities of EtOAc extract (A), N-hexane fraction (B), N-hexane insoluble fraction (C) and compound 1 (Lamesticumin A) should be presented with the statistical significance by using asterisks, such as *p<0.05, **p<0.01, and so on, above the dots.

**Minor points:**

The sources of chemicals and instruments should indicate the city and country that they are produced from. Sometimes the authors used gr and sometimes g, it should be consistent with each other. In subtopic “Cytotoxic assay”, in 3rd - 4th lines, the authors should rewrite the sentences. There are many grammar and various typological errors, such as the number of g it should be 43.4 rather than 43,4, etc. For the previous findings, the sentences should be in present tense, but for the authors' research data in the Results and Discussion parts, the sentences would be in past tense. For MDA231, it should be MDA-MB-231 breast cancer cells. For MCF, it should be clarified as MCF-7 or not? In Ref. No. 12, there is no information of company, city, country of publication, is it a book? Ref. No. 21, the name of journal is full name, whereas others are in abbreviated forms. It should be consistent with each other.

**References**

1. Manosroi A, Jantrawut P, Sainakham M, Manosroi W, et al.: Anticancer activities of the extract from Longkong (Lansium domesticum) young fruits. *Pharm Biol.* 2012; 50 (11): 1397-407 PubMed
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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Programmed cell death, Natural products, Antioxidants, Cancer.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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Author Response 11 Jan 2021

**Khusnul Fadhilah,** Universitas Gadjah Mada, Yogyakarta, Indonesia

In the revised version, we present cytotoxic data on normal cells (Vero cell line) in Table 2 and Figure 3 has been revised as recommended by the reviewer for major point revision. Besides, we also present the minor points revision such as grammar and typological errors corrections.

**Competing Interests:** No competing interests were disclosed.
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