RESEARCH ARTICLE

REVISED A bioactive compound isolated from Duku (*Lansium domesticum* Corr) fruit peels exhibits cytotoxicity against T47D cell line [version 2; peer review: 2 approved]

Khusnul Fadhilah [1], Subagus Wahyuono[2], Puji Astuti[2]

[1]Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[2]Departement of Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyaka, 55281, Indonesia

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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 IC_{50} 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

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
version 2 (revision) 23 Apr 2021	✓ report	
version 1 06 Jan 2020	? report	✓ report

1. **Ratana Banjerdpongchai**, Chiang Mai University, Chiang Mai, Thailand

2. **Deden Derajat Matra**, Bogor Agricultural University, Bogor, Indonesia

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Subagus Wahyuono (subagusw_fa@ugm.ac.id)

Author roles: Fadhilah K: Investigation, Writing – Original Draft Preparation; **Wahyuono S**: Conceptualization, Data Curation, Methodology, Supervision, Writing – Review & Editing; **Astuti P**: Conceptualization, Data Curation, Methodology, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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First published: 06 Jan 2020, **9**:3 https://doi.org/10.12688/f1000research.21072.1

REVISED **Amendments from Version 1**

In this 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.

Any further responses from the reviewers can be found at the end of the article

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[1]. 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[2]. Based on previous studies, chloroform and methanol extracts of *L. domesticum* displayed cytotoxic activity on murine melanoma ($B_{16}F_{10}$) and colon cancer (HT29) cells[3]. 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_2O_2 exposure[4]. Onoceranoid-type of triterpenoids have been isolated from twigs and leaves of *L. domesticum*, and these compounds showed antibacterial and antimutagenic activities[5,6]. 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 (KBr) spectrum was obtained from spectrophotometer (Shimadzu) using a method previously described by Ashokkumar and Ramaswamy[7]. Ultraviolet spectrum ($CHCl_3$) was obtained from UV spectrophotometer (Hitachi UH 5300). Sample (1 mg) were diluted in 1 mL $CHCl_3$ and was run between 200–400 nm. Spectra of ^{1}H- and ^{13}C- NMR in $CDCl_3$ solvent were measured using JEOL JNM-ECZ 500R/S1 at 500 MHz.

Extraction and fractionation

The peel was separated from the fruit, dried in oven 50°C for 24 hours and powdered using a blender. Powdered *L. domesticum* fruit peel (200 g) was macerated with ethyl acetate (EtOAc; 2 L) overnight. This solution was filtrated (0.45µm) and the filtrate was evaporated to dryness with a rotary evaporator set at 50°C, to give dried EtOAc extract (A, 50.13 g). In order to separate the extract into non-polar and polar fractions, 6 g of extract A was diluted 5 times using n-hexane (20 mL) to give soluble n-hexane fraction B (supernatant; 3.03 g) and insoluble n-hexane fraction C (residue; 2.83 g).

Fraction C was the most active among other fractions (see *Results*), and was therefore further fractionated using vacuum liquid chromatography as described by Mae Sri Hartati *et al.*[8]. In brief, using silica gel preparation grade (15 g) as stationary phase this was eluted with n-hexane and increasing amounts of ethyl acetate. Six subfractions were obtained and subfraction 2 contained a major compound which appeared as white crystals (referred to as compound 1). Compound 1 was obtained as a single compound from subfraction 2, while the other subfractions still contained various compounds. Compound 1 (142.6 mg), had a melting point at 140–150°C (Figure 1). Compound 1 was identified using spectroscopy data such as ultraviolet (UV), infrared (IR), ^{13}C-NMR and ^{1}H-NMR (see section *Chemicals and equipment*).

Cytotoxic assay

The bioassay followed the methodology described by Bahuguna *et al.*[9] with modifications. In brief, 100 µl T47D and Vero cell line (in RPMI mediaFaculty of Medicine, Universitas Gadjah Mada) were placed in each well of a 96 well micr oplates, 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,^{13}C-NMR: see Table 1; m/z 502; (Calculated for $C_{31}H_{50}O_5$)

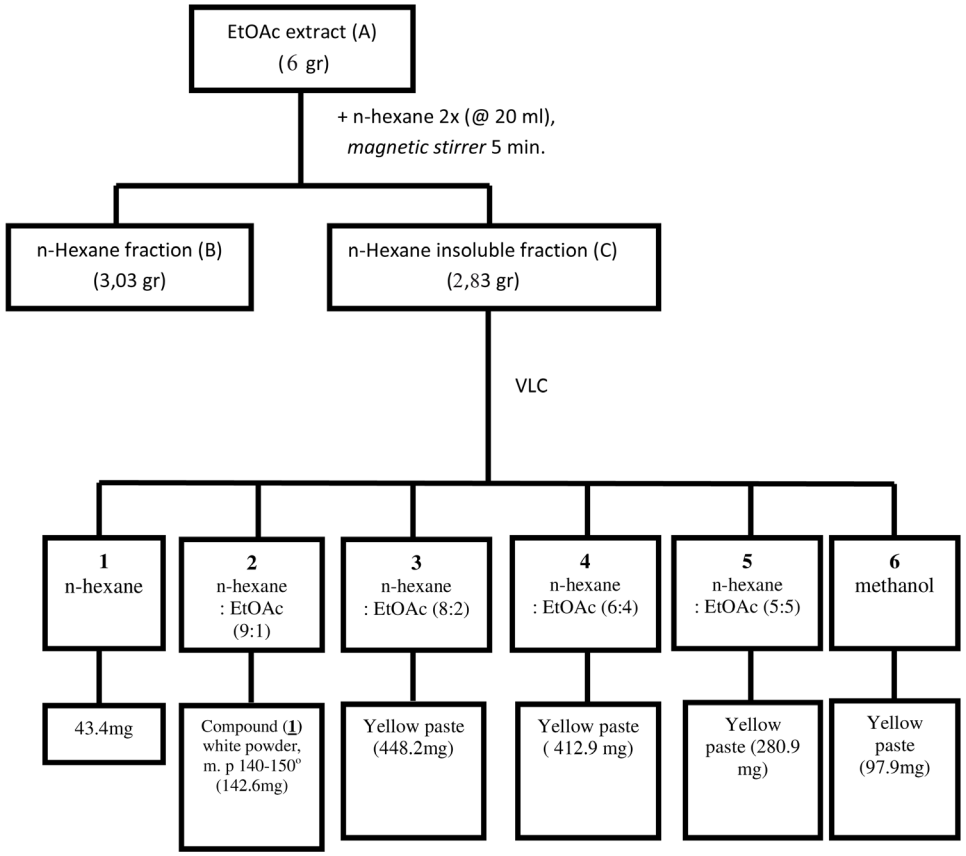

Figure 1. Isolation process of cytotoxic compound 1 (subfraction 2) from *Lansium domesticum* fruit peels.

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

Position	^{1}H-NMR (J, Hz)		^{13}C-NMR
	δ (ppm)	Multiplicity	δ (ppm)
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
16	1.7	2H, dd (7.0, 6.8)	30.59
17	1.1	1H, triplet	31.81
18	-	-	38.85
19	1.2	2H, multiplet	32.09
20	2.1	2H, multiplet	33.15
21	-	-	148.10
22	-	-	147.20
23	1.7	3H, singlet	23.37
24	1.9	3H, singlet	23.76
25	0.8	3H, singlet	16.16
26	4.8	2H, dublet (9.1)	107.4
27	1.7	3H, Singlet	17.91
28	0.7	3H, singlet	14.37
29	4.6	2H, dublet (9.2)	114.23
30	1.6	3H, singlet	22.88

^{1}H- and ^{13}C- NMR ($CDCl_3$) spectra were obtained from JEOL JNM-ECZ 500R/S1, 500 MHz

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 ^{13}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 ^{13}C-NMR and ^{1}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[5].

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[10]. Based on National Cancer Institute guidelines, a natural compound has potent anticancer activity if it has IC_{50} <4 µg/ml or 10 µM[11].

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[12]. Lansiosida A and Dukunolida A has been isolated from n-hexane extract of *L. domesticum* fruit peel[13,14]. 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[5]. Another onoceranoid-type triterpenoid

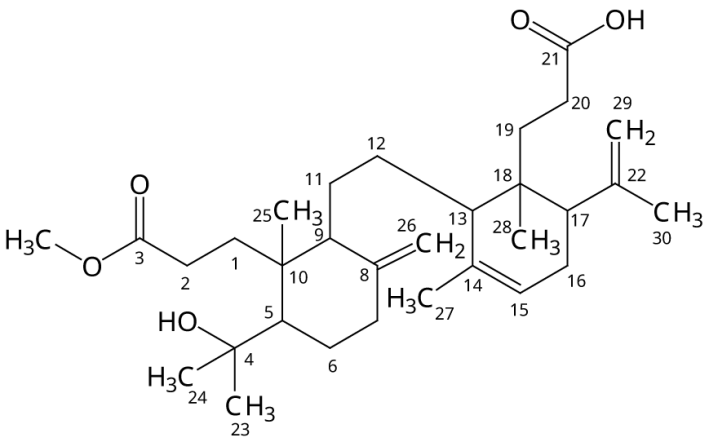

Figure 2. Isolated compound 1, Lamesticumin A.

Table 2. IC_{50} values of extract, fractions and isolated compound 1 against T47D breast cancer cell line and normal cell line (Vero).

Sample	IC_{50} (µg/ml), mean ± SD	
	T47D	Vero
Extract A	29.41 + 0.67	66.10 ± 3.26
Fraction B	43.51 ± 1.77	77.53 ± 5.03
Fraction C	25.56 ± 0.64	81.12 ± 1.12
Lamesticumin A	15.68 ± 0.30	47.46 ± 2.63
Doxorubicin	0.18 ± 0.01	11.02 ± 3.63

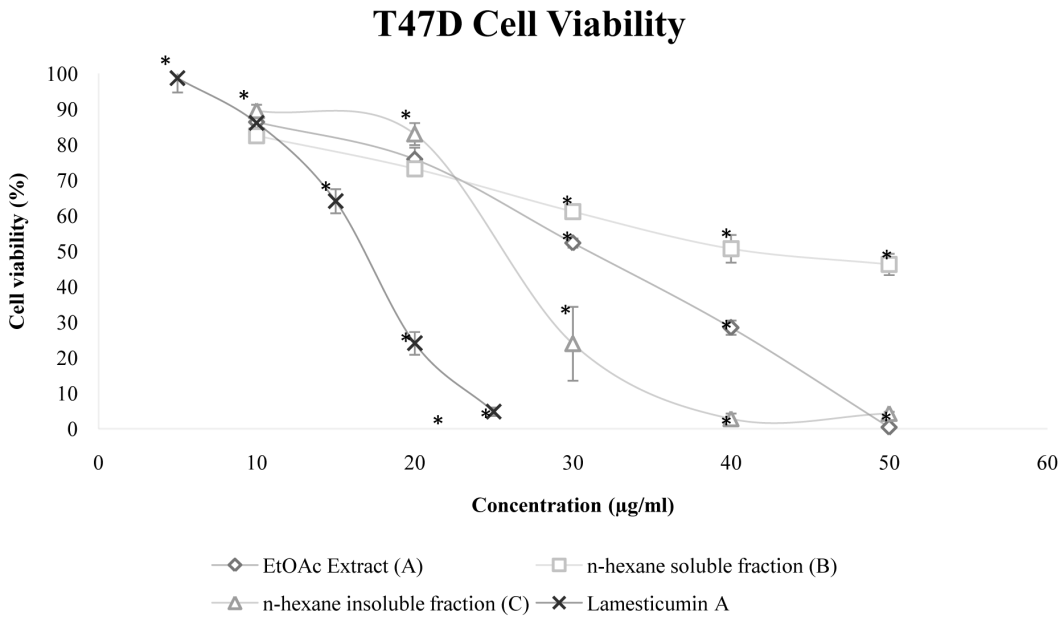

Figure 3. T47D breast cancer cell line viability after treatments with extracts from *Lansium domesticum* fruit peels. Error bar shows standar deviation (SD, n=3). symbol (*) indicates statistical significance ($p<0.05$).

Lansium acid I-IX were isolated from *L. domesticum* leaves, which was reported to have antimutagenic activity[6].

Based on several *in vitro* tests, some terpenoid compounds had anticancer activity. Sesquiterpene lactone compounds are known to inhibit Nf-kB, thereby inducing apoptosis[15]. Celastrol has anticancer properties by regulating various transcription factors, angiogenesis processes, cell cycle arrest and induction of apoptosis[16]. 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)[17]. Clematangoticosides D and F from *Clematis tangutica* are known to have cytotoxic activity against human gastric cancer cell line (SGC-7901) with IC_{50} 24.22 and 21.35 µM, respectively[18]. Cycloartane-type and oleanane-type triterpenoids from *Ligularia przewalskii* show cytotoxicity in Hela, HEPG2, SGC7901, MDA-MB-231, HL-60, and Lewis cell lines with IC_{50} 8.40–24.39 µM[19].

It has been reported that natural compounds combined with low doses of antineoplastics can increase effectiveness and reduce toxic effects[20]. Betulinic acid can induce apoptosis when combined with 5-fluorouracil, irinotecan and oxaliplatin[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 chemo-sensitizing agent to increase the effect of conventional anticancer drugs[21], and to increase the effect of doxorubicin by increasing the cellular amount of the drug in the MCF-7 cell line[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[23].

This project contains the following underlying data:

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

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http://www.doi.org/10.5281/zenodo.3539670

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 07 June 2021

https://doi.org/10.5256/f1000research.52564.r83806

✔ **Ratana Banjerdpongchai**
Department of Biochemistry, Chiang Mai University, Chiang Mai, Thailand

The manuscript is improved according to the comments of the reviewer' major comments. However, the incidence of breast cancer in female should be updated to be in year 2000 of GLOBOCAN.

For minor points: The scientific names of plants should be all in italic. Sometimes the authors used g and sometimes as gr, it should be consistent with each other throughout the manuscript, including the diagram of extraction in Figure 1: Isolation process of cytotoxic compound 1 (subfraction 2) from *Lansium domesticum* fruit peels. There are still typo and grammar errors, which have been highlighted here. For examples, ml vs mL. et al sometimes in italic and sometimes was not. In References, there is an et al. not in italic form. The usage of IC50, the number should be in subscript form.

After correction of the points mentioned above, the manuscript can be indexed under the publisher's supervision and handling.

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.

Version 1

Reviewer Report 17 September 2020

https://doi.org/10.5256/f1000research.23191.r64744

Deden Derajat Matra
Department of Agronomy, Bogor Agricultural University, Bogor, Indonesia

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.

Reviewer Report 26 August 2020

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

?

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 chemosensitizing 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

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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.

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.