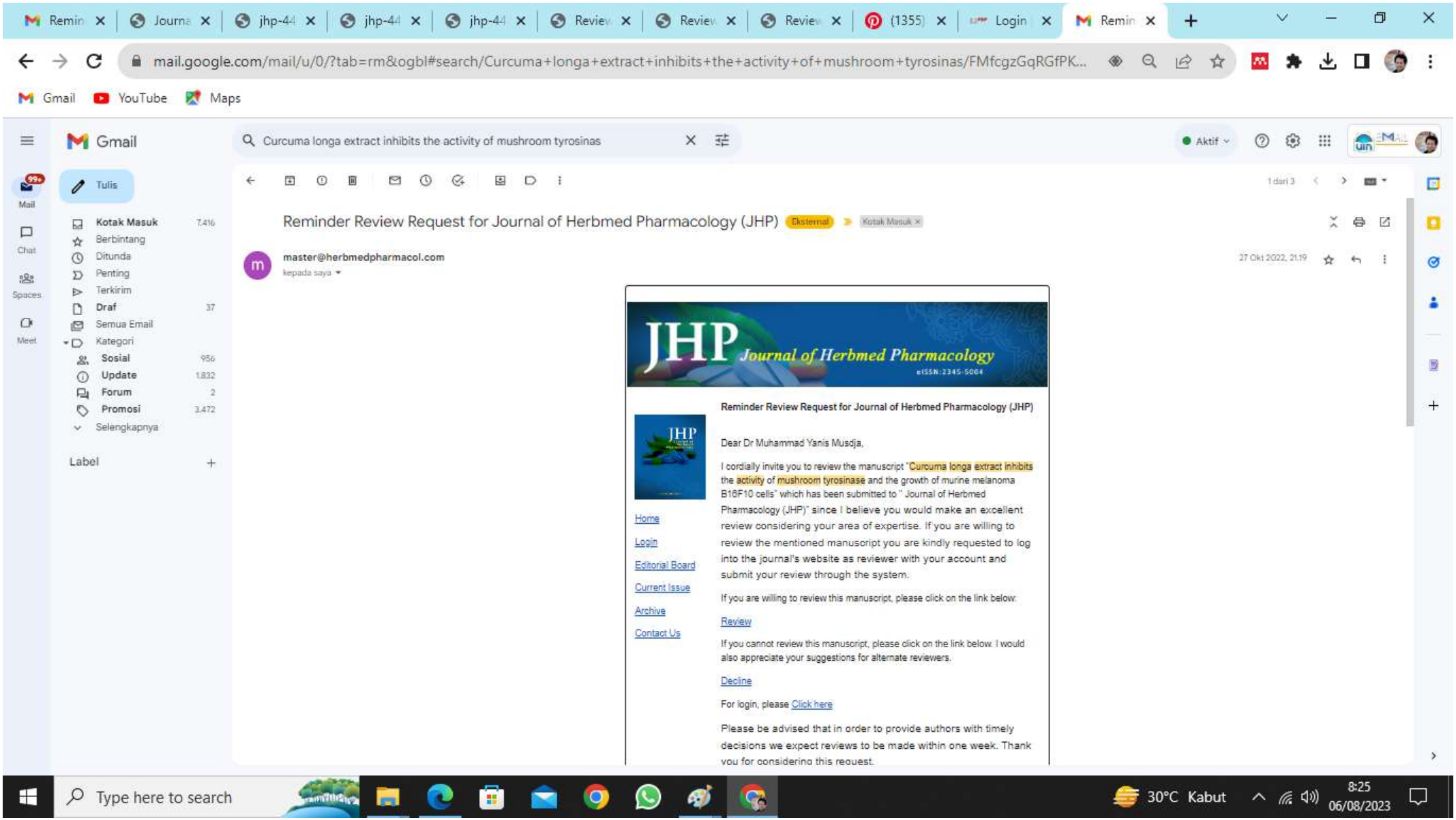
Curcuma longa extract inhibits the activity of mushroom tyrosinas
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Dear Dr Muhammad Yanis Musdja,
I cordially invite you to review the manuscript "Curcuma longa extract inhibits the activity of mushroom tyrosinase and the growth of murine melanoma B16F10 cells" which has been submitted to " Journal of Herbmed Pharmacology (JHP)" since I believe you would make an excellent review considering your area of expertise. If you are willing to review the mentioned manuscript you are kindly requested to log into the journal's website as reviewer with your account and submit your review through the system.
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Curcuma longa extract inhibits the activity of mushroom tyrosinase and the growth of murine skin cancer B16F10 cells

Reviewer Name:

Muhammad Yanis Musdja

Review Date:

2022-10-29

Revision:

--

Author's Response to Reviewer's Comments

Reviewer 1:

Comments to the Author:

There are several things that must be improved and explained in this manuscript: (Please see the results of the revision of this manuscript) including:

1. ON ABSTRACT:

This paragraph should be changed, because there are several ways to prevent pigmentation, namely:

1) the rate of synthesis and decay of tyrosinase; 2) the activity of tyrosinase in melanosomes; 3) the rate of synthesis and melanization of melanosomes; 4) melanosome size; 5) the efficiency of melanosome degradation of melanosomes in keratinocytes (https://www.sciencedirect.com/science/article/pii/S0022202X93903096)

Pigment content of cultured human melanocytes does not correlate with tyrosinase message level

https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2133.1991.tb14161.x (https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2133.1991.tb14161.x)

Author's Response:

Thank you for your comments. The first sentence in the Abstract discusses the process of skin pigmentation, not describe the prevention of pigmentation as suggested by the reviewer. Both references https://www.sciencedirect.com/science/article/pii/S0022202X93903096 and https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2133.1991.tb14161.x provided by the reviewer were published in 1991, which is considered old and outdated.

In our Abstract, we refer to an article published in 2011, which stated:

Evidence reveals that L-tyrosine and L-DOPA, besides serving as substrates and intermediates of melanogenesis, are also bioregulatory agents acting not only as inducers and positive regulators of melanogenesis but also as regulators of other cellular functions https://doi.org/10.1111%2Fj.1755-148X.2011.00898.x

2. ON INTRODUCTION:

This paragraph should be exchanged for complete information; because pigmentation is affected by:

1) the rate of synthesis and decay of tyrosinase; 2) the activity of tyrosinase in melanosomes; 3) the rate of synthesis and melanization of melanosomes; 4) melanosome size; 5) the efficiency of melanosome degradation of melanosomes in keratinocytes

https://www.sciencedirect.com/science/article/pii/S0022202X93903096 (https://www.sciencedirect.com/science/article/pii/S0022202X93903096)

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Author's Response:

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3. ON MATERIALS AND METHODS

The concentration of curcuminoids in ECL greatly determines the pharmacological activity of ECL, why don't you determine the concentration of curcuminoids in ECL, because the content of curcuminoids variety, location, source, cultivation conditions, extraction, and storage processes?

Author's Response:

Thank you for your suggestion. We have repeated the HPLC analysis to determine the level of curcuminoids in ECL. The level of bisdemethoxycurcumin is 6.3306%, demethoxycurcumin is 3.1414%, and curcumin is 8.3754%.

4. ON DISCUSSION

1. Because there are 9 locations that have determined the content of curcuminoids, for your research what is the concentration of curcuminoids, because the content of curcuminoids is the main determinant.

Author's Response:

Thank you for your suggestion. We have repeated the HPLC analysis to determine the level of curcuminoids in ECL. The level of bisdemethoxycurcumin is 6.3306%, demethoxycurcumin is 3.1414%, and curcumin is 8.3754%.

2. Did the ECL you used for this study meet the standard requirements for the study? Because you did not determine the curcuminoid concentration of the ECL you used for this study.

Typically, the quality of commercial turmeric products can vary widely. While curcumin, demethoxycurcumin, and bisdemethoxycurcumin have been used as marker compounds for quality control of rhizomstudy these concentrations were not determined.

Author's Response:

Thank you for your suggestion. We have repeated the HPLC analysis to determine the level of curcuminoids in ECL. The level of bisdemethoxycurcumin is 6.3306%, demethoxycurcumin is 3.1414%, and curcumin is 8.3754%.

5. ON CONCLUSION

There are 3 main types of chemical compounds in ECL, namely: curcumin, demethoxycurcumin, and bisdemethoxycurcumin, please explain in the discussion so you can conclude that curcumin is efficacy, demethoxycurcumin, and bisdemethoxycurcumin are not efficacious, If you can't explain better in Conclusion You wrote that the curcuminoid compounds are efficacious and to determine which compounds curcuminoid compounds, further research is needed.

Author's Response:

Thank you for your suggestion. We have elaborated the discussion and conclusion as suggested.

Reviewer 2

Comments to the Author:

1. There are some grammatical mistakes.
   Author's Response:
   Grammatical mistakes have been corrected.

2. Introduction and discission should be improved.
   Author's Response:
   Introduction and Discussion have been corrected.

3. The sentence "The rhizome powder (1.0 kg) was extracted with ethanol 70% (10 l) at room temperature for 5 days at 25-26˚C" (line 80-81) should be can be rewritten as follows "The rhizome powder (1at room temperature (25-26˚C) for 5 days".
   Author's Response:
   The sentence has been corrected.

4. Line 82: "vacuum rotary evaporator at a temperature below 50˚C". The author needs to specify how many degrees Celsius.
   Author's Response:
   The temperature has been specified.

Editor comments:

1. Professional English checking and writing are recommended. Please note that this journal ONLY accepts articles written in good English for publication. Authors who are not native English speakers, should pa native English speaker or by a professional language editor for any grammatical, semantical/stylistic and typographical errors. Authors who are native English speakers should ensure that their article has been r(where appropriate). A section was edited as an example.
   Author's Response:
   Grammatical mistakes have been corrected using Grammarly.

2. All abbreviations should be presented in full and the abbreviation in parenthesis when appearing for the first time or in each table and figure.

   Author's Response:

   All abbreviations have been corrected and written in red color.

3. The similarity index of your manuscript should be less than 20% with no more than 3% similarity from a single source. Please check for plagiarism.
   Author's Response:
   Similarity index has been checked.

4. Please consult a recently published paper in JHP and add the followings:

   ORCID IDs of all the authors.

   Author's Response:

   ORCID ID of all authors has been added.

5. Running title.
   Author's Response:
   Running title: Firmansyah D et al., Curcuma longa inhibits tyrosinase. J Herbmed Pharmacol. x;x(x):x-x.

6. Implication for health policy/practice/research/medical education:
   Author's Response:
   This study may support the scientification and development of Curcuma longa rhizome as an active component of cosmetics.
   All other issues have been corrected accordingly.

7. **We choose 250 USD for fast-track publication.**

Abstract

Background: The pigmentation on the skin is influenced by tyrosinase, an enzyme that catalyzes the hydroxylation of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA), and the oxidation of L-DOPA to dopaquinone. In Indonesia, the rhizomes of *Curcuma longa* L. (synonym *C. domestica* Valeton) have been used as traditional cosmetics. This work aimed to explore the inhibitory activity of *C. longa* extracts on the activity of mushroom tyrosinase and the cytotoxicity of the extract towards murine skin cancer B16F10 cells.

Methods: In this study, the *C. longa* rhizomes were cold-extracted using ethanol 70% as the solvent and yielded 15.3% w/w of viscous *C. longa* extract (ECL). The presence of curcumin in ECL was also determined by reversed-phase high-performance liquid chromatography (RP-HPLC) using a gradient-elution system of water and acetonitrile. ECL was assessed for its inhibitory effect on mushroom tyrosinase activity using L-DOPA as substrate and kojic acid as the positive control drug. The cytotoxicity of ECL and curcumin, a known phytoconstituent contained in *C. longa* rhizome, was studied in murine skin cancer B16F10 cells.

Results: RP-HPLC chromatogram revealed that curcumin was positively contained in ECL as the major peak of a curcuminoid triplet with demethoxycurcumin and bisdemethoxycurcumin. The level of bisdemethoxycurcumin is 6.3306%, eluted at 12.646 min; demethoxycurcumin is 3.1414%, eluted at 13.675 min; and curcumin is 8.3754%, eluted at 14.802 min. ECL has a weak inhibitory activity towards mushroom tyrosinase (IC_{50} = 564.8 µg/ml) compared to that of kojic acid (IC_{50} = 55.70 µg/ml). Both ECL and kojic acid were of moderate toxicity to murine skin cancer B16F10 cells with IC_{50} of survival growth rates is 98.06 µg/ml and 65.54 µg/ml, respectively. In fact, curcumin is highly toxic to murin skin cancer B16F10 cells (IC_{50} = 14.42 µg/ml).

Conclusion: Taken together, ECL might be able to prevent melanogenesis via the inhibition of tyrosinase activity, and interestingly, it could inhibit the growth of murine skin cancer B16F10 cells. However, further studies are needed to verify its antimelanogenesis and anticancer properties.

Keywords: Curcumin; melanin; melanoma; skin pigmentation; Zingiberaceae

Implication for health policy/practice/research/medical education:

This study may support the scientification and development of *Curcuma longa* rhizome as an active component of cosmetics.

Please cite this paper as: Firmansyah D et al., Curcuma longa inhibits tyrosinase. J Herbmed Pharmacol. x;x(x):x-x.

Introduction

The pigmentation on the skin is influenced by tyrosinase, a copper-containing enzyme, which catalyzes the hydroxylation of L-tyrosine (the enzyme's substrate) to L-DOPA, and eventually, the oxidation of L-DOPA to dopaquinone. Polymerization of these quinones results in the formation of melanin (1,2). It was evidenced that L-tyrosine and L-DOPA function as substrates and intermediates of melanin production (3). Alteration in melanin biosynthesis can lead to various skin diseases in humans (4). It has been known that plants with antioxidant properties might be utilized for the treatment of skin disorders.

In Indonesia, the rhizomes of turmeric or *Curcuma longa* L. (synonym *C. domestica* Valeton), local name *kunyit,* have traditionally been used as *lulur*, to cleanse, soften, and whiten the skins of Javanese princesses. The major active compounds contained in *C. longa* rhizomes are curcumin, demethoxycurcumin, and bisdemethoxycurcumin (5). *In vitro* studies on curcumin, a known polyphenol compound contained in *C. domestica*, reported its activity in reducing the melanin content and tyrosinase activity and blocking the expression of melanogenesis-related proteins in human melanocytes. Curcumin also activated phosphatidylinositol 3-kinase/Akt/glycogen synthase kinase-3β (PI3K/Akt/GSK-3β), extracellular-signal-regulated kinase (ERK), and p38 mitogen-activated protein kinase (p38 MAPK) pathway (6). Curcumin decreased melanin levels and blocked the tyrosinase activity in alpha-melanocyte stimulating hormone-stimulated B16F10 cells (7). Nonetheless, curcumin is notable for its antioxidant property indicating high reactivity in scavenging peroxyl radicals (8).

In fact, clinical trials in several countries have reported the safety of (mostly) curcumin prepared as a pharmaceutical dosage form in healthy subjects.

An open-label, prospective clinical study of a standardized *C. longa* extract has been performed on 12 healthy adult Indian ethnicity participants for 90 days and revealed that the liver function and other hematological parameters were not significantly altered, which proved the safety of the extract (9). Another clinical study of a low dose of curcumin on healthy middle-aged males and females in Ohio, USA, resulted in a significant decrease in plasma triglyceride, plasma beta-amyloid, plasma alanine aminotransferase, salivary amylase, and an increase in plasma catalase, plasma myeloperoxidase, and plasma nitric oxide (10). A randomized-control double-blind prospective trial on 59 healthy non-smoking adults in Texas, USA, treated with 200 mg oral curcumin for 8 weeks resulted in a clinically significant rectification in endothelial function thus reducing the risk of cardiovascular diseases (11). An hour after treatment with 400 mg oral curcumin, healthy elderly

participants (n=60, aged 60-85 years) significantly improved performance on sustained attention and working memory tasks (12).

Previous studies had reported the advantage of curcumin in maintaining human health and its mechanism in blocking the melanogenesis process. However, there is limited study on the activity of the plant extract in melanogenesis, thus, our work aimed to explore the inhibitory activity of *C. longa* extract towards mushroom tyrosinase as well as its effect on the survival rate of murine skin cancer B16F10 cells.

Materials and Methods

Plant materials and identification. Turmeric rhizomes were obtained from the Experimental Plantation of the Research Institute for Spices and Medicinal Plants (BALITTRO) Manoko, Cikahuripan, Lembang, West Java, Indonesia. The plants were taxonomy identified by a certified botanist at the Laboratory of Identification and Determination, School of Life Sciences and Technology, Bandung Institute of Technology, and based on characteristics described by Newman et al. (2004) (13) and two older references, the sample was confirmed as *Curcuma longa* L. (Letter No. 5727/I1.CO2.2/PL/2019).

The rhizomes were washed under tap water to remove soil and dirt and oven-dried at a temperature of a maximum of 40 ^{o}C for 2 d. The dried rhizomes were ground to powder and sieved using a sieve mesh-16.

Preparation of the extract. The rhizome powder (1.0 kg) was extracted with ethanol 70% (10 l) for 5 days at 25-26˚C. The extract was filtered the solvent was removed by a vacuum rotary evaporator at 45-50 ˚C. The yield of the viscous ethanol extract of *C. longa* (ECL) was 15.3% w/w.

HPLC analysis to determine curcumin in ECL. Determination of curcumin in ECL was carried out by employing reversed-phase high-performance liquid chromatography (RP-HPLC) (Waters Alliance 2695) following a procedure described elsewhere (14), with a few modifications. Samples (20 µl) were injected into the C18 column (Merck LiChroCART 250 mm x 4.6 mm) and were eluted in a mixture of water-acetonitrile in a gradient-elution mobile phase, with an initial of 60% acetonitrile increased to 80% acetonitrile in 12 min, maintained for 5 min, increased to 90% acetonitrile in 17 min. with a flow rate of 0.5 ml/min. Detection (Waters 2489 UV-visible detector) was set at 425 nm. The chromatogram of ECL was compared to that of standard curcumin.

Chemicals, cells, and cell culture. Tyrosinase inhibitor screening kit (colorimetric) (Sigma-Aldrich product number MAK257), kojic acid K3125 (Sigma-Aldrich CAS number 501-30-4), murine melanoma cell lines B16F10 (ATCC® CRL-6475™) (a collection of the Cell and Molecular Biology Laboratory, Faculty of Pharmacy, Universitas Padjadjaran). B16F10 is a cell line exhibiting a morphology of spindle-shaped and epithelial-like cells that were isolated from the skin tissue of a mouse with melanoma (https://www.atcc.org/products/crl-6475).

The cells were grown at 37°C with 5% CO2 in the Dulbecco's Modified Eagle Medium (DMEM) produced by ATCC, added with 10% fetal bovine serum (FBS) (GIBCO), and 1% of penicillin-streptomycin (10,000 U penicillin and 10 mg streptomycin/ml) (Sigma-Aldrich). After the cells reached a confluency of 80%, they were subcultured in 0.05% of TrypLE enzymes (GIBCO).

Cytotoxicity assay. The cytotoxicity of ECL against the B16F10 cells (8,000 cells/well) was assessed using the water-soluble WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium/Cell Counting Kit-8. ECL solutions were prepared by dissolving the extract in 1% DMSO in a culture medium. The solution was serial-diluted to final concentrations of 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, and 500 µg/ml, and incubated at 37ºC for 48 h. The absorbance of the formazan product was measured using a microplate reader (Infinite M200 Pro, Nano Quant TECAN). The IC_{50} was calculated using GraphPad Prism 8.4.2. Furthermore, the cytotoxicity of kojic acid was also assessed using the same procedure. Kojic acid solutions were prepared to final concentrations of 7.813 µg/ml, 15.625 µg/ml, 31.25 µg/ml, 62.5 µg/ml, and 125 µg/ml. The morphology of the cells was observed by using ZEISS Axio Vert A1 Bio inverted microscope in 100x magnification.

Mushroom tyrosinase inhibition assay. Tyrosinase inhibition assays were carried out by strictly following the protocol in the kit that employs mushroom tyrosinase. Mushroom tyrosinase was chosen because it possesses high similarity and homology to human tyrosinase (15). L-DOPA was used as the enzyme's substrate. The reaction mixture (1000 µl) contained 685 µL of phosphate buffer (50 mM, pH 6.5), 50 µL of mushroom tyrosinase (333 U/ml in phosphate buffer), 20 µL of ECL dissolved in DMSO, and 100 µl of 5 mM L-DOPA. After the addition of L-DOPA, the reaction was immediately measured at 510 nm for the formation of the product (dopachrome). Kojic acid (concentration range of 12.5-200 µg/ml) was used as a positive control. The concentration range of ECL used for the mushroom tyrosinase inhibition assay was 125-2000 µg/ml. Each measurement was made in triplicate. The IC_{50} value, a concentration giving 50 % inhibition of tyrosinase activity, was determined by interpolating the logarithmic concentration against the % inhibition of tyrosinase activity (16):

The inhibition of tyrosinase activity (%) = [(A - B)/A] × 100 %

where A is the absorbance of the control with the enzyme at 510 nm; B is the absorbance of the test sample with the enzyme at 510 nm

---

Results

Determination of curcumin in ECL. RP-HPLC chromatogram revealed that curcumin was positively contained in ECL as a curcuminoid triplet with demethoxycurcumin and bisdemethoxycurcumin. The level of bisdemethoxycurcumin is 6.3306%, eluted at 12.646 min; demethoxycurcumin is 3.1414%, eluted at 13.675 min; and curcumin is 8.3754%, eluted at 14.802 min (Figure 1).

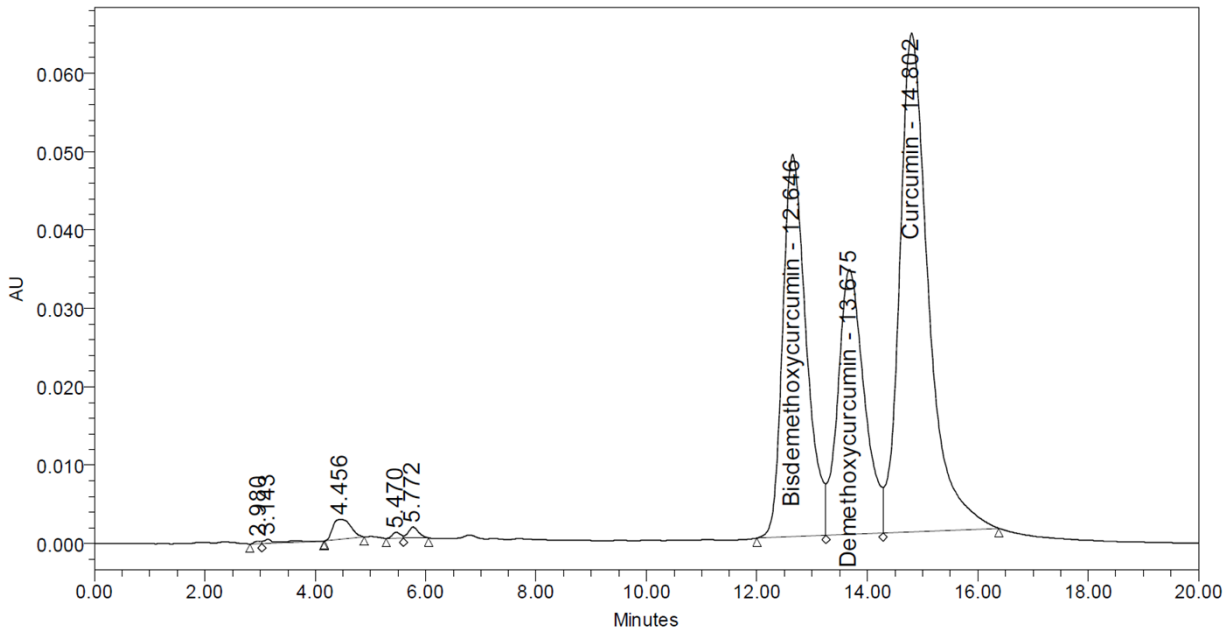


Figure 1. Reversed-phase high-performance liquid chromatography chromatogram of ECL using a gradient-elution system of water and acetonitrile revealing triplet peaks of bisdemethoxycurcumin at retention time 12.646 min; demethoxycurcumin at retention time 13.675 min; and curcumin at retention time 14.802 min.

The effect of ECL on mushroom tyrosinase activity was depicted as a plot between the logarithmic concentration of ECL or kojic acid against % tyrosinase activity. ECL has a weak inhibitory activity towards mushroom tyrosinase (IC_{50} = 564.8 µg/ml) (Figure 2A) compared to that of kojic acid (IC_{50} = 55.70 µg/ml) (Figure 2B). By increasing the concentration of ECL or kojic acid, the % inhibition of mushroom tyrosinase activity is also elevated in a sigmoidal pattern curve.

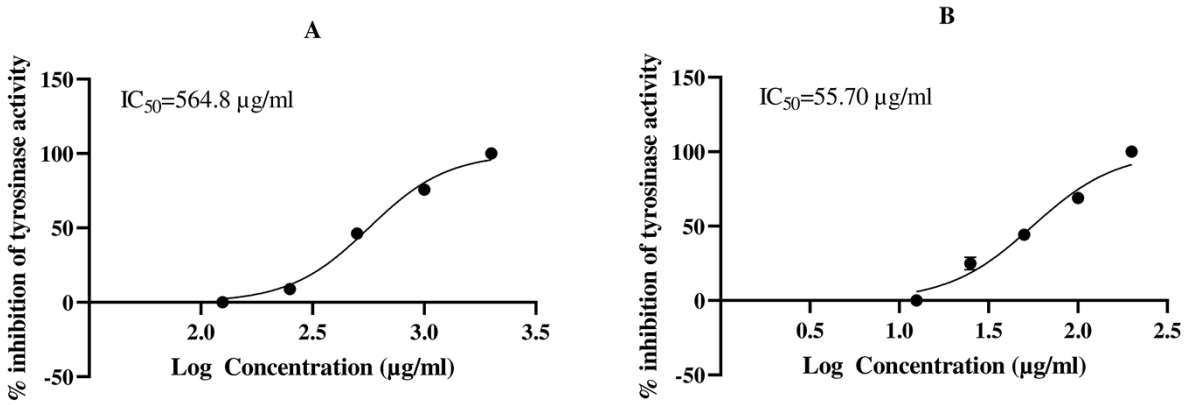


Figure 2. Effect of (A) ECL (IC_{50} = 564.8 µg/ml) and (B) kojic acid (IC_{50} = 55.70 µg/ml) on mushroom tyrosinase activity

The effect of ECL on murine skin cancer B16F10 cells. The ECL exhibited a weak inhibitory on mushroom tyrosinase activity, therefore its ability to influence the survival growth rate of murine skin cancer B16F10 cells was also assayed. The cells were treated with ECL at concentrations ranging from 31.25 µg/ml to 500 µg/ml for 48 h at 37 °C and were examined using WST-8/CCK-8. The results revealed that ECL started inhibiting the survival growth rate of the murine skin cancer cells at 62.5 µg/mL with a survival growth rate of 60.13 %, while concentrations of 125, 250, and 500 µg/ml resulted in a survival growth rate of 31.56 %, 1.69 %, and 0.65 % respectively. The cytotoxicity is depicted as a plot between the logarithmic concentration of ECL against the % survival growth rate of the cells. ECL is confirmed as moderate cytotoxicity to murine skin cancer B16F10 cells with IC_{50} of 98.06 µg/ml (Figure 3A), whereas curcumin is categorized as a compound with high cytotoxicity with IC_{50} of 14.42 µg/ml (Figure 3B). Kojic acid is similar to ECL of moderate cytotoxicity with IC_{50} of 65.54 µg/ml (Figure 3C).

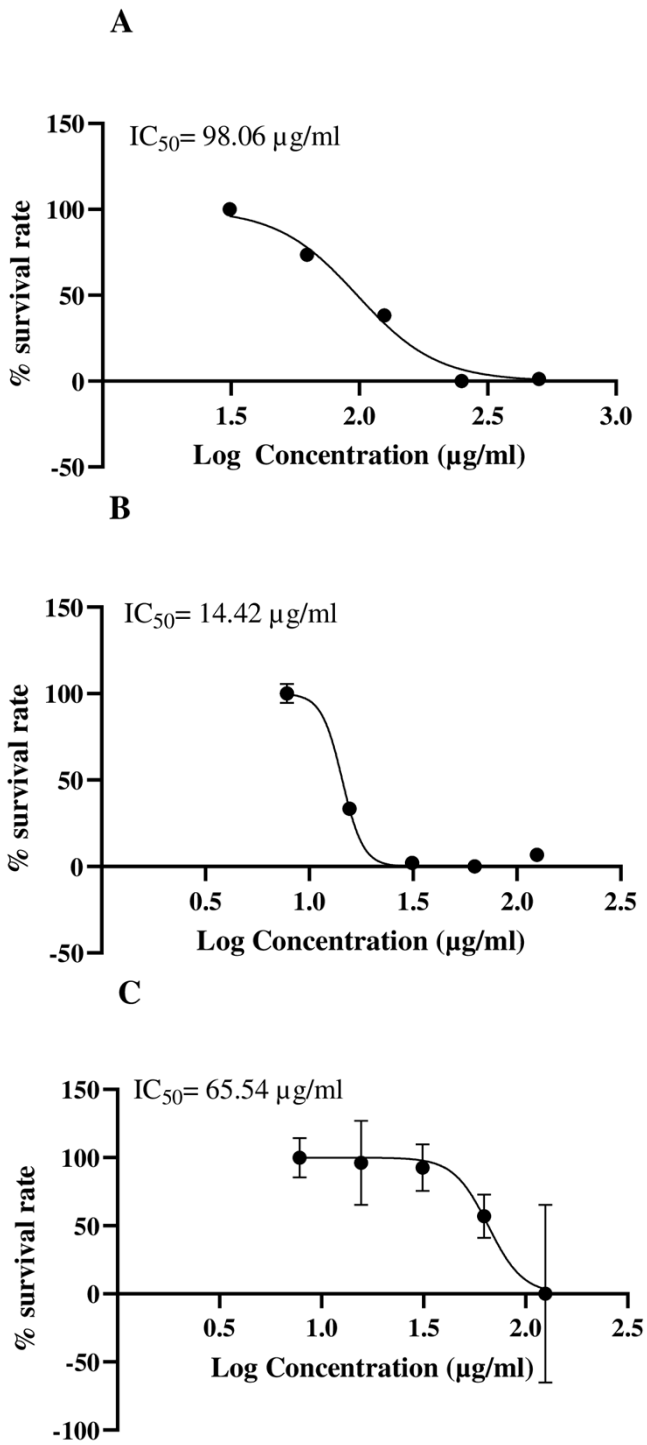
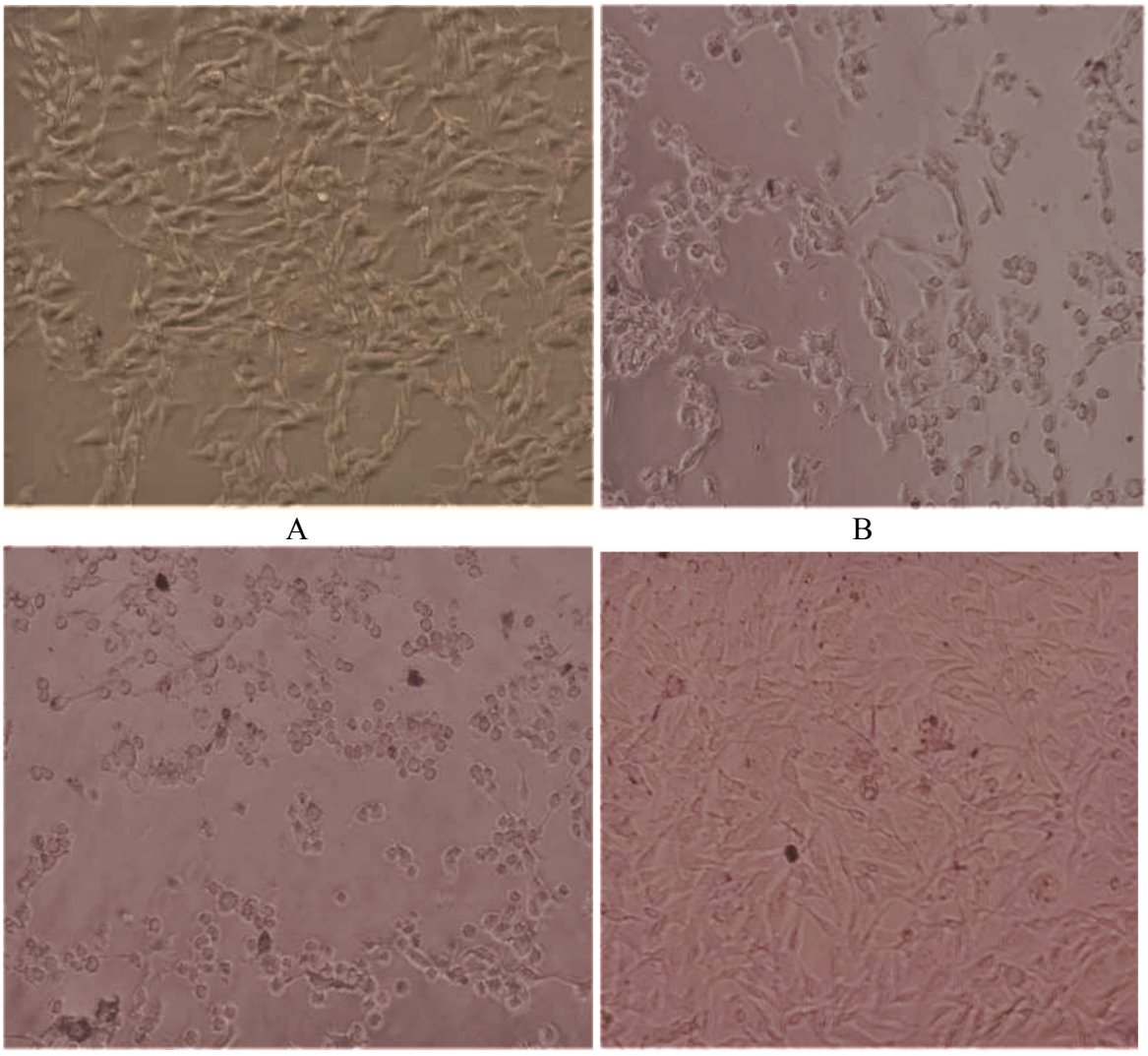


Figure 3. The effect of (A) ECL, (B) curcumin, and (C) kojic acid on the survival growth rate of B16F10 cells.

The microscopic examination of the cells treated with ECL or curcumin or kojic acid is depicted in Figure 4. Normal murine skin cancer B16F10 cells (Figure 4A) reveal high-density cells while treating

189 the cells with ECL (Figure 4B) and/or curcumin (Figure 4C) and/or kojic acid (Figure 4D) shows
190 shrunken cells and low-density.
191



192
193
194 Figure 4. The morphology of (A) normal B16F10 cells, (B) ECL-treated B16F10 cells, (C) curcumin-
195 treated B16F10 cells, and (D) kojic acid-treated B16F10 cells
196
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201 **Discussion**
202 Discovering a novel plant-based antimelanogenesis is challenging. In particular, our country was
203 gifted with mega biodiversities of plants and inherited *jamu* as indigenous medicines, thus, the chance
204 to further explore these herbs is very fascinating.

In an earlier study, curcuminoids extracted from nine places were determined by UV spectrophotometry and resulted in a maximum absorbance at the wavelength of 420 nm (17). In our work, curcumin was analyzed using an RP-HPLC using a gradient-elution system of water–acetonitrile as a mobile phase. The RP-HPLC chromatogram revealed that curcumin was positively contained in ECL as a curcuminoid triplet with demethoxycurcumin and bisdemethoxycurcumin. The level of bisdemethoxycurcumin is 6.3306%, eluted at 12.646 min; demethoxycurcumin is 3.1414%, eluted at 13.675 min; and curcumin is 8.3754%, eluted at 14.802 min (Figure 1). The level of curcumin is the highest of the three curcuminoids. The presence of triplets in the HPLC chromatogram was also reported by previous studies (14,18).

Our work revealed that ECL weakly inhibits the activity of mushroom tyrosinase (IC_{50} = 564.8 µg/ml) (Figure 2A). The methanol extract, instead of ethanol extract, of *C. longa* rhizome, demonstrated an inhibition towards tyrosinase activity (19). However, a previous study on curcumin by Athipornchai and colleagues also reported similar results to ours. In their work, it was confirmed that curcumin exhibited moderate inhibition against the monophenolase activity of tyrosinase with an IC_{50} of 326.5 µM (20). The activity of curcumin is attributed to its two phenyl structures, each with -OH and -OCH3 groups attached in the orto position (21), which the other two curcuminoids lack.

The cytotoxicity of drugs or compounds is categorized as high if IC_{50} is less than 20 µg/mL, moderate if IC_{50} ranges between 21-200 µg/mL, weak if IC_{50} ranges between 201-500 µg/mL, and no cytotoxicity if IC_{50} exceeds 500 µg/mL (22). ECL is confirmed as moderate cytotoxicity to murine skin cancer B16F10 cells and curcumin exhibits high cytotoxicity. These findings suggest that ECL and curcumin might possess anticancer properties on murine skin cancer B16F10 cells.

Kojic acid inhibits the activity of mushroom tyrosinase (IC_{50} = 55.70 µg/ml) (Figure 2B). Phenolic compounds, e.g., kojic acid and curcumin, may be utilized as antimelanogenesis due to their similarity in structure to tyrosine, the substrate of tyrosinase (19). A previous study reported that kojic acid was observed bound at the opening of the active site of tyrosinase, implicating a competitive inhibition to the substrate (tyrosine). Two residues, Arg209 and Val218, located in the second outer layer of the active site, function a role in the binding of tyrosine (23). Our preliminary *in silico* study visualized that curcumin and other phenolic constituents of *C. longa* bind to tyrosinase and tyrosinase-related protein-1 with binding modes similar to those of kojic acid (24).

Conclusion

The inhibitory activity of the ethanol extract of *C. longa* (ECL) on the activity of mushroom tyrosinase and the cytotoxicity of the extract towards murine skin cancer B16F10 cells have been evaluated. ECL weakly inhibits the activity of mushroom tyrosinase and is of moderate cytotoxicity to murine skin

cancer B16F10 cells as proven by its ability to reduce the survival growth rate of the cells. Curcumin, a major constituent contained in *C. longa* rhizome, has shown high cytotoxicity. Taken together, ECL which positively contains curcumin, bisdemethoxycurcumin, and demethoxycurcumin, might be able to prevent melanogenesis via the inhibition of tyrosinase activity, and interestingly, it could inhibit the growth of murine skin cancer B16F10 cells. Considering this, ECL is prospective to be developed as an active component in cosmetics. However, further studies are needed to verify its antimelanogenesis and anticancer properties.

Acknowledgements

The authors thank the Rector of Universitas Padjadjaran via the Directorate of Research and Community Engagement for funding both the research project and the publication fee in the scheme of Academic-Leadership Research Grant No. 1959/UN6.3.1/PT.00/2021 and 1959/UN6.3.1/PT.00/2022.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Conflict of interest

None.

Funding

Academic-Leadership Research Grant No. 1959/UN6.3.1/PT.00/2021 and 1959/UN6.3.1/PT.00/2022.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the first author upon reasonable request.

Author contribution

JL was responsible for the study design and methodology. SAS and JL contributed to the data interpretation. DF contributed to the investigation, data collection, data validity, and statistical analysis. JL, SAS, and NMS contributed equally to the supervision of the project. JL and DF

contributed to manuscript preparation and revision. All authors have read and approved the final manuscript.

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