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Research Article

Surface Tension and *In Vitro* Tyrosinase Inhibitory Activity of Tamanu Oil (*Calophyllum inophyllum* L.)

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ABSTRACT

Tamanu or *nyamplung* oil, derived from *Calophyllum inophyllum* L., is commonly used in traditional medicine and occasionally used as a moisturizer in skincare cosmetics. *In silico* predictions suggested that tamanu oil contained compounds capable of inhibiting tyrosinase activity. This study aimed to measure the surface tension of tamanu oil and evaluate its tyrosinase inhibitory activity *in vitro*. The surface tension of tamanu oil was measured using the du-Nouy ring method with a tensiometer, while tyrosinase inhibitory activity was assessed by spectrophotometry using a microplate reader. The study revealed that the surface tension of tamanu oil was measured at 41.83±0.76 mN/m, and the tyrosinase inhibitory activity of tamanu oil was determined to be 83.75±0.41%. Notably, the inhibitory activity of tamanu oil was comparable to that of the positive control, kojic acid, which exhibited a tyrosinase inhibition of 84.59±2.04%. We concluded that tamanu oil has potential as a natural surfactant raw material and may serve as an effective tyrosinase inhibitor.

Keywords: *Calophyllum inophyllum* L., *nyamplung*, surface tension, tamanu oil, tyrosinase inhibitor

Introduction

As the outermost organ enveloping the human body, the skin is crucial for maintaining health and protecting against external threats. One of its vital functions is to act as a natural shield against the harmful effects of ultraviolet (UV) radiation, which can induce skin hyperpigmentation (Aprilliani et al., 2018). Hyperpigmentation is a condition that arises when there are alterations in skin pigments, leading to an increased production of melanin, the pigment responsible for human skin, eye, and hair color. Melanin formation is guided by a pivotal enzyme known as tyrosinase (Carletti et al., 2014). Tyrosinase regulates a series of reactions that convert tyrosine to eumelanin (dark) or pheomelanin (light) pigments (Putri et al., 2018). The activity of the tyrosinase enzyme is significantly influenced by the level of exposure to UV radiation, with higher intensities of UV radiation activating tyrosinase, resulting in darker skin (Furi et al., 2021).

Conversely, many individuals aim to maintain fair radiant skin and seek to prevent skin darkening by inhibiting melanin production through tyrosinase inhibition. Since tyrosinase is a key enzyme in melanin production, inhibitors of this enzyme, such as ascorbic acid, hydroquinone, kojic acid, and mercury, are widely used in skin-brightening cosmetic products (Mustika et al., 2022). Although

effective, these compounds differ significantly in their safety profile. For instance, ascorbic acid, a natural antioxidant, is generally considered safe and well-tolerated, with minimal risk of adverse effects when used appropriately. In contrast, mercury poses significant health hazards, including kidney damage, neurological disorders, and systemic toxicity, and is banned in most countries for cosmetic use owing to its high toxicity (Sun et al., 2017). Similarly, hydroquinone and kojic acid, although effective, may cause skin irritation and allergic reactions with prolonged or excessive use (Puspitasari & Dari, 2022). Hence, the search for alternative tyrosinase inhibitors that are both safe and skin-friendly is of paramount importance.

One promising candidate for a safe tyrosinase inhibitor is tamanu or *nyamplung* oil extracted from *Calophyllum inophyllum* L., which is abundant in Indonesia, especially in sandy coastal areas. Although tamanu oil is commonly used for biodiesel production from its seeds, it also contains beneficial fatty acids for the skin, making it a natural moisturizer. Previous studies have demonstrated that tamanu oil pharmacological activities, including antibacterial and antioxidant properties, are attributed to its diverse secondary metabolites, such as flavonoids, alkaloids, steroids, tannins, and phenols (Hasibuan et al., 2013; Safrina et al., 2020).

In addition to its tyrosinase inhibitory potential, understanding the surface tension of tamanu oil is essential for its application in cosmetics. Surface tension influences the spreadability, absorption, and compatibility of the formulations with the skin barrier (Barnes et al., 2021). Therefore, evaluation of this property provides valuable insight for designing effective and skin-friendly cosmetic products.

This study aimed to determine the surface tension of tamanu oil and concurrently assess its *in vitro* tyrosinase inhibitory activity. The outcomes of this study would provide a foundation for considering tamanu oil as a natural raw material in surfactant product formulations, such as facial cleansers, offering a safer and more natural alternative for maintaining bright and rejuvenated skin.

Materials and Methods

Materials

Tamanu oil was obtained from Madura, Indonesia, and processed using the cold-pressed method by CV Sahabat Atsiri Nusantara, Indonesia. Other ingredients were available in the market, such as tyrosinase enzyme (Sigma-Aldrich, Germany), L-tyrosine substrate (Sigma-Aldrich, Germany), dimethyl sulfoxide (DMSO; Merck, Germany), kojic acid (Beaute Lab, Indonesia), and phosphate buffer (Merck, Germany). The experiments used various instruments, including an analytical balance (FS-AR210; Fujitsu, Japan), a spectrophotometer (BMG Labtech, Germany), and a du-Nouy tensiometer.

Determination of Tamanu Oil's Surface Tension

The surface tension was measured using a du-Nouy tensiometer. Tamanu oil (20 ml) was carefully poured into a Petri dish. Subsequently, the Petri dish was positioned on the test table of the tensiometer. The test table was elevated until the ring was submerged in the oil. Subsequently, the table position was adjusted to ensure that the ring was parallel to the balance line. The scale needle was rotated until the ring was detached from the soil surface. The same procedure was performed using a blank consisting of distilled water. The oil surface tension was determined in triplicate.

Preparation of Tyrosinase Inhibition Activity Test Solutions

Tyrosinase enzyme, L-tyrosine substrate, kojic acid solution, and tamanu oil solutions were prepared based on Durai et al. (2021).

Tyrosinase enzyme solution preparation. An aliquot of 0.001 g of tyrosinase enzyme was accurately weighed and transferred into a volumetric flask, followed by dissolution in 5 ml of phosphate buffer solution. The prepared enzyme solution was stored in a freezer if not used immediately.

L-tyrosine substrate solution preparation. An aliquot of 0.01 g of L-tyrosine substrate was weighed, transferred into a volumetric flask, and dissolved in phosphate buffer solution to a final volume of 5 ml. The L-tyrosine substrate solution was stored in the freezer as stock if not used immediately.

Kojic acid solution preparation. A quantity of 0.1 g of kojic acid was accurately weighed, then dissolved in DMSO and titrated to a final volume of 10 ml in a volumetric flask. The solution was then homogenized and further diluted as required.

Tamanu oil solution preparation. An analytical balance was used to weigh 0.1 g of tamanu oil, which was then dissolved in 10 ml of DMSO, homogenized, and diluted to the appropriate concentration.

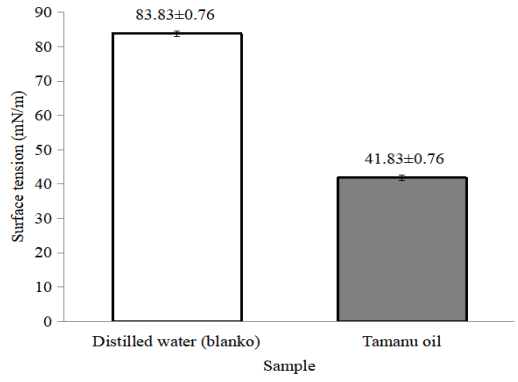

Fig. 1. Surface tension of tamanu oil compared to distilled water.

Determination of Maximum Wavelength and Optimum Incubation Time of Tyrosinase Enzyme

To find the best incubation time for the tyrosinase enzyme, a systematic approach was taken. A solution containing 40 µl of tyrosinase enzyme and 40 µl of L-tyrosine substrate was prepared with 120 µl of phosphate buffer. The resulting mixture was then placed into a microplate, and absorbance at the maximum wavelength was monitored. This systematic evaluation spanned an incubation duration from 0 to 60 min, with measurements taken every 10 min.

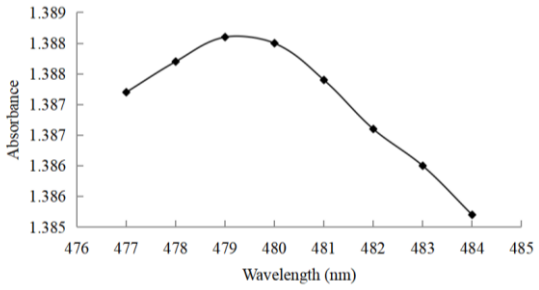

Fig. 2. Maximum wavelength of tyrosinase.

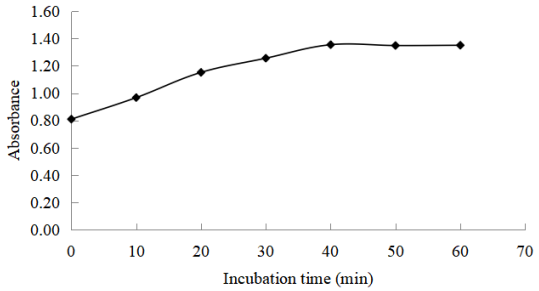

Fig. 3. Incubation time curve of tyrosinase.

Determination of Tyrosinase Inhibition Activity of Tamanu Oil

The tamanu oil concentration was precisely prepared by dissolving 0.1 g in DMSO to achieve a concentration of 10,000 µg/ml. The solution was carefully diluted with 50 mM phosphate buffer (pH 6.5) to yield extract concentrations of 2,000 µg/ml, 4,000 µg/ml, 6,000 µg/ml, 8,000 µg/ml, and 10,000 µg/ml. As a positive control, kojic acid was evaluated at concentrations of 5 µg/ml, 10 µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml.

To evaluate tyrosinase inhibition activity, 40 µl of the sample was mixed with 80 µl of phosphate buffer and 40 µl of tyrosinase (333 units/ml in phosphate buffer; Sigma-Aldrich, Germany). After

incubation at 37°C for 5 min, 40 µl of 2 mM L-tyrosine was added, and incubation was continued for an additional 40 min. The inhibition activity measurement for the blank followed the same method as the sample, with the only difference being that the well contained 120 µl of phosphate buffer, 40 µl of tyrosinase enzyme, and 40 µl of L-tyrosine substrate. The absorbance of the solution was measured at 479 nm using spectrophotometer (Charissa et al., 2016). The data are expressed as the absorbance values for each well. The absorbance values were then calculated as % inhibition using the following formula:

$$\% \text{ inhibition} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100\%$$

Data Analysis

The surface tension and tyrosinase inhibitory activity data were presented as mean ± standard deviation (SD). Data was analyzed using *t*-test with a confidence level of 95%.

Results and Discussion

Surface Tension of Tamanu Oil

The first step before surfactant manufacturing is to measure surface tension using a du-Nouy ring tensiometer. This method calculates the force necessary to lift a ring submerged in a liquid in proportion to surface tension, with results in mN/m (Ali et al., 2023). The procedure is simple and quick, measuring the effort of the ring emerging from the liquid surface.

Tamanu oil's surface tension was measured in triplicate, with water as the control. Tamanu oil had a surface tension of 41.83±0.76 mN/m, lower than water's (83.83±0.76 mN/m) (Figure 1). This significantly lower value suggests that tamanu oil could reduce the interfacial tension between the oil and water, which is a key property of surfactants (Dini et al., 2024). Specifically, tamanu oil demonstrates a natural ability to spread across the water surface rather than remain as isolated droplets, which enhances its potential as a raw surfactant. The data were presented as the average surface tension values along with SD to ensure the reliability of the measurements.

Measurement of surface tension is crucial in cosmetic formulations because it influences spreadability, absorption, and interaction with the skin (Barnes et al., 2021). Understanding this property allows the optimization of tamanu oil incorporation into water-based or emulsion systems, which are commonly used in cosmetics. Oils with lower surface tension tend to spread more easily and are simpler to apply, making them ideal for cosmetic applications. However, surface tension of tamanu oil (41.83 mN/m) was higher than that of oils commonly used as raw surfactant materials, such as olive oil (34.46 mN/m) (Dikko, 2015) and virgin coconut oil (30.90 mN/m) (Mustika et al., 2022). The moderately high surface tension of tamanu oil could be advantageous in stabilizing emulsions, contributing to consistent product performance during both storage and application.

Tamanu oil is rich in fatty acids, making it a potential candidate for surfactant production, such as soap manufacturing. The primary fatty acids in tamanu oil are oleic acid (41.27%), linoleic acid (33.63%), palmitic acid (12.23%), and stearic acid (11.02%). The high concentrations of palmitic and stearic acids are particularly advantageous as soap-making ingredients, mainly since tamanu oil is classified as a non-edible oil, which directs its use towards non-food products. Palmitic and stearic acids contribute to a solid soap texture and generate stable foam, while oleic and linoleic acids provide moisturizing properties to soap products (Hasibuan et al., 2013).

Determination of the Maximum Wavelength and Optimum Incubation Time of Tyrosinase Enzyme

The maximum wavelength and optimum incubation time were 479 nm and 40 min, respectively. Based on the maximum wavelength curve (Figure 2),

479 nm was selected as it exhibited the highest absorbance value, indicating the best dopachrome formation. The 40-min optimum incubation time was chosen because, at this point, the production of dopachrome was maximized, and the absorbance remained stable. As shown in the optimum incubation time curve (Figure 3), incubation periods longer than 40 min led to a stagnation point where the additional substrate concentration no longer increased enzyme activity. Furthermore, prolonged incubation results in decreased enzyme activity over time.

The optimal conditions for tyrosinase activity were determined by identifying the maximum wavelength and the optimum incubation time. The wavelength that yielded the highest absorbance was determined by determining the maximum wavelength. The maximum wavelength determination is a critical factor in chemical analysis, as measurements at this wavelength produce the most considerable absorbance change for each concentration level. The optimum incubation time was determined to establish the ideal time for measuring tyrosinase enzyme activity and to assess when the absorbance reached its maximum stability. The optimum incubation time was determined by measuring the relationship between the incubation time and absorbance of the solution. Identifying the maximum wavelength and incubation time is essential for minimizing measurement errors (Suharyanto & Prima, 2020). The maximum wavelength and incubation time were determined based on the maximum wavelength of the positive control blank solution containing kojic acid.

The maximum wavelength and optimum incubation time of tyrosinase used in this study differed slightly from those reported in the literature. Some studies have used a wavelength of 492 nm with an optimum incubation time of 30 min (Furi et al., 2021). Several factors such as the equipment conditions and differences in the instruments used may cause this wavelength and incubation time to shift. Although the literature contains valuable information, it is necessary to re-evaluate the maximum wavelength and incubation time to test, validate, or change them into unique scenarios, conditions, and requirements.

Inhibitory Activity of Tyrosinase Enzyme by Tamanu Oil

Tamanu oil was tested for its ability to inhibit tyrosinase activity. The findings of this study indicate that tamanu oil could suppress tyrosinase enzyme activity. The inhibition rates at extract concentrations of 2,000 µg/ml, 4,000 µg/ml, 6,000 µg/ml, 8,000 µg/ml, and 10,000 µg/ml were 75.32±0.24%, 80.27±0.74%, 81.38±0.35%, 82.34±0.18%, and 83.75±0.41%, respectively (Figure 4). In contrast, the inhibition rates for kojic acid, as a positive control, at extract concentrations of 5 µg/ml, 10 µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml were 10.26±5.78%, 48.57±4.09%, 67.84±5.47%, 78.48±8.59%, and 84.59±2.04%, respectively (Figure 5). These data indicate that higher concentrations resulted in better inhibition activity. The inhibition percentages are presented as mean values ± SD.

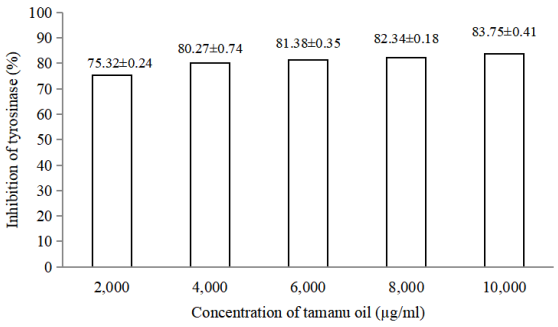

Fig. 4. Tyrosinase inhibition rate of tamanu oil.

Inhibition of tyrosinase activity in this study was assessed using L-tyrosine as the substrate, which acts on the monophenolase pathway, with kojic acid as a positive control. The basic principle of tyrosinase inhibitor activity testing involves the interaction between the L-tyrosine substrate and tyrosinase enzyme, resulting

in dopachrome-forming melanin. Brown color indicates dopachrome formation. The presence of a tyrosinase inhibitor hinders the reaction between the substrate and enzyme, thereby preventing dopachrome formation, which is reflected by a decrease in the intensity of the measured brown color.

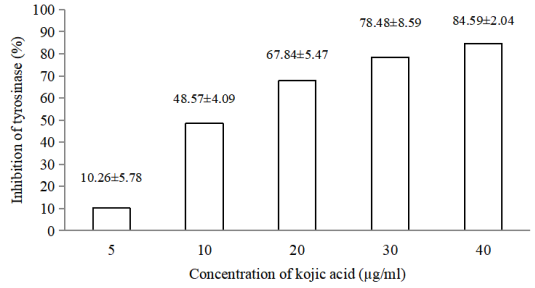

Fig. 5. Tyrosinase inhibition rate of kojic acid.

Kojic acid was used as a positive control to compare the inhibitory strength of the test sample to that of tyrosinase enzyme activity. Kojic acid was chosen as the positive control because it is commonly used as a whitening agent in cosmetic products and has been proven to prevent premature aging caused by sun exposure. Additionally, kojic acid is frequently used to discover new compounds or materials with potential tyrosinase inhibitors. In addition to its inhibitory activity on tyrosinase, kojic acid also exhibits a relatively high level of stability (Ripaldo & Sagala, 2020). Kojic acid is recommended for use at concentrations of ≤1%, as within this range, it demonstrates both effective and safe properties. However, using kojic acid at concentrations higher than 1% could lead to adverse skin effects such as dermatitis, inflammation, pain, rashes, and itching. Prolonged use of kojic acid over time also increases the risk of skin cancer (Saeedi et al., 2019).

The tyrosinase inhibition activity of tamanu oil was demonstrated by the percentage of inhibition or the inhibitory capacity it exhibited. The percentage inhibition reflects the degree of suppression of tyrosinase enzyme activity at each sample concentration. The higher the inhibition percentage, the greater the inhibitory effect of the sample on tyrosinase enzyme activity (Furi et al., 2021). The results, presented as percentages, provide comparative information on the inhibitory activity of the enzyme at various sample concentrations. This approach was employed to evaluate and compare the effectiveness of enzyme inhibitors at each concentration used.

Tamanu oil inhibited tyrosinase enzyme activity at the highest extract concentration of 10,000 µg/ml, with an inhibition percentage of 83.75±0.41%. In contrast, kojic acid was inhibited at the highest extract concentration of 40 µg/ml, with an inhibition percentage of 84.59±2.04%. Based on these data, tamanu oil has potential as a tyrosinase inhibitor, as evidenced by its relatively high inhibition percentage, which is comparable to that of the positive control, kojic acid, at the highest concentration. This result supported the findings of Widyodhari (2022), who identified three tyrosinase inhibitor compounds with more significant activity than kojic acid: 2,4-diamino-6-[[4-phenyl-2-thiazolyl]thio]quinazoline, 1,5-dihydroxyxanthone, and cinnamic acid.

The tyrosinase inhibition activity of tamanu oil observed in this study was probably related to the compounds it contained. According to Safrina et al. (2020), phytochemical screening tests of tamanu oil revealed that it contains various compounds, including flavonoids. Flavonoids can act as tyrosinase inhibitors in enzymatic reactions because they are known to bind free radicals and chelate copper (Cu) ions at the active site of the tyrosinase enzyme (Jayantie et al., 2022). The position of the hydroxyl groups attached to the benzene ring and the number of hydroxyl groups in a flavonoid are essential for inhibiting tyrosinase enzyme activity (Mustika et al., 2022).

Flavonoid compounds exhibit strong interactions with enzymes, thereby preventing dopachrome formation. If dopachrome production increased,

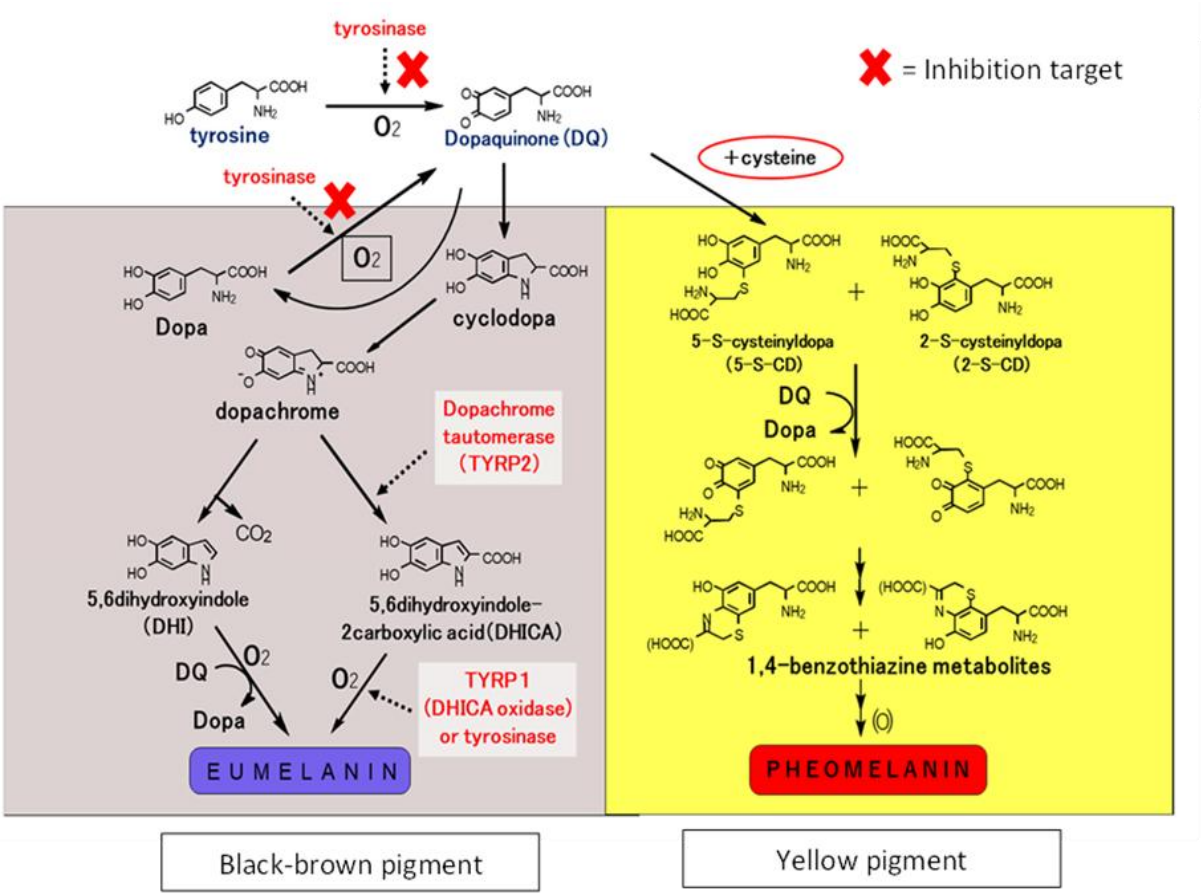

Fig. 6. Biosynthetic pathway of melanin (Hida et al., 2020).

tyrosinase inhibition did not occur. Conversely, if dopachrome formation is prevented, maximum inhibition of tyrosinase enzyme activity can be achieved (Mustika et al., 2022). The inhibition mechanism involves the competitive inhibition of L-tyrosine oxidation by tyrosinase. The flavonoid structure contains a 3-hydroxy-4-keto group, which acts as a copper chelator (Cu) for the tyrosinase enzyme. Typically, one molecule of the tyrosinase enzyme contains two Cu atoms, Cu^{+} and Cu^{2+}, bound to the histidine amino acid. Copper acts as an essential cofactor for tyrosinase activity. When flavonoids bind copper, the catalysis of tyrosinase is disrupted and dopachrome is not formed. As a result, the catalytic ability of the tyrosinase enzyme is diminished due to the loss of copper from the enzyme active site (Sagala & Telaumbanua, 2020).

As shown in Figure 6, tyrosinase is a crucial enzyme involved in melanin (eumelanin and pheomelanin) biosynthesis. It catalyzes the conversion of tyrosine and dihydroxy phenylalanine (L-Dopa) into dopaquinone, a key intermediate in this pathway. Additionally, tyrosinase functions alongside two related proteins, TYRP1 and TYRP2 (also known as dopachrome tautomerase or DCT) to facilitate the production of eumelanin, a brown-black pigment. Owing to its central role in melanin production, tyrosinase has emerged as a primary target for the development of active compounds used in cosmetics and skin-lightening products. Tyrosinase inhibitors effectively suppress melanin synthesis by blocking the conversion of tyrosine to L-Dopa or by inhibiting the oxidation of L-Dopa to dopaquinone (Hida et al., 2020). Furthermore, many natural and synthetic inhibitors not only reduce tyrosinase activity but also alleviate oxidative stress, offering additional benefits for skin health and aesthetic improvement.

Conclusion

Tamanu oil generated a surface

tension of 41.83±0.76 mN/m, which was lower than water's, making it a potential raw material for surfactant production. Additionally, tamanu oil exhibited significant tyrosinase inhibition activity, with the highest extract concentration in this study (10,000 µg/ml) showing an inhibition percentage of 83.75±0.41%. The inhibitory activity of tamanu oil was notably comparable to that of kojic acid, the positive control, which achieved a tyrosinase inhibition rate of 84.59±2.04%. These findings suggest the potential of tamanu oil as a natural ingredient in cosmetic formulations, particularly skin-brightening products. Further research should include toxicity testing and *in vivo* studies to confirm its safety and efficacy as well as investigations into its stability and long-term effects in formulations.

Conflict of Interest

We declare that we have no conflicts of interest, whether financial, personal, or otherwise, with any individuals or organizations connected to the subject matter presented in this manuscript.

Acknowledgement

N/A.

References

Ali, M., Ikbal, M. S., & Jusman (2023). Determining the surface tension of a liquid and the drop comparison method. *Jurnal Pendidikan Fisika*, *11*(1), 143–150. https://doi.org/10.24252/jpf.v11i1.34113

Aprilliani, A., Suganda, A. G., & Hartati, R. (2018). Uji inhibisi aktivitas enzim tirosinase beberapa jenis tumbuhan Zingiberaceae. *Jurnal Ilmiah Farmasi*, *14*(1), 46–58. https://doi.org/10.20885/jif.vol14.iss1.art05

Barnes, T. M., Mijaljica, D., Townley, J. P., Spada, F., & Harrison, I. P. (2021). Vehicles for drug delivery and cosmetic moisturizers: Review and comparison. *Pharmaceutics*, *13*(12), 2012. https://doi.org/10.3390/pharmaceutics13122012

Carletti, G., Nervo, G., & Cattivelli, G. (2014). Flavonoids and melanins: A common strategy across two kingdoms. *International Journal of Biological Sciences*, *10*(10), 1159–1170. https://doi.org/10.7150/ijbs.9672

Charissa, M., Djajadisastra, J., & Elya, B. (2016). Uji aktivitas antioksidan dan penghambatan tirosinase serta uji manfaat gel ekstrak kulit batang taya (*Nauclea subdita*) terhadap kulit. *Jurnal Kefarmasian Indonesia*, *6*(2), 98–107. https://doi.org/10.22435/jki.v6i2.6224.98-107

Dikko, A. B. (2015). Density and surface tension relationship of olive oil and carrot oil at different temperatures. *World Wide Journal of Multidisciplinary Research and Development*, *1*(2), 15–17. Retrieved from https://wwjmrd.com/upload/density-and-surface-tension-relationship-of-olive-oil-and-carrot-oil-at-different-temperatures.pdf

Dini, S., Bekhit, A. E. A., Roohinejad, S., Vale, J. M., & Agyei, D. (2024). The physicochemical and functional properties of biosurfactants: A review. *Molecules*, *29*(11), 2544. https://doi.org/10.3390/molecules29112544

Durai, P., Ko, Y. J., Kim, J. C., Pan, C. H., & Park, K. (2021). Identification of tyrosinase inhibitors and their structure-activity relationships via evolutionary chemical binding similarity and structure-based methods. *Molecules*, *26*(3), 566. https://doi.org/10.3390/molecules26030566

Furi, M., Alfatma, A., Dona, R., Fernando, A., Aryani, F., Utami, R., Muharni, S., Husnawati, Suhery, W. N., & Octaviani, M. (2021). Uji inhibitor

enzim tirosinase ekstrak dan fraksi daun kedabu (*Sonneratia ovata* Backer) secara *in vitro*. *Jurnal Ilmiah Manuntung*, *8*(2), 201–214. https://doi.org/10.51352/jim.v8i2.529

Hasibuan, S., Sahirman, & Yudawati, N. M. A. (2013). Karakteristik fisikokimia dan antibakteri hasil purifikasi minyak biji nyamplung (*Calophyllum inophyllum* L.). *agriITECH*, *33*(3), 311–319. https://doi.org/10.22146/agritech.9553

Hida, T., Kamiya, T., Kawakami, A., Ogino, J., Sohma, H., Uhara, H., & Jimbow, K. (2020). Elucidation of melanogenesis cascade for identifying pathophysiology and therapeutic approach of pigmentary disorders and melanoma. *International Journal of Molecular Sciences*, *21*(17), 6129. https://doi.org/10.3390/ijms21176129

Jayantie, D. W., Farida, Y., & Taurhesia, S. (2022). Aktivitas antioksidan dan inhibisi enzim tirosinase ekstrak etanol buah gandaria (*Bouea macrophylla* Griff.) secara *in vitro*. *Pharmacoscript*, *5*(1), 63–70. https://doi.org/10.36423/pharmacoscript.v5i1.856

Mustika, R., Hindun, S., & Auliasari, N. (2022). Potensi tanaman sebagai pencerah wajah alami. *Jurnal Sains dan Kesehatan*, *2*(4), 558–562. https://doi.org/10.25026/jsk.v2i4.233

Puspitasari, L., & Dari, N. P. D. R. W. (2022). Uji aktivitas *inhibitor* enzim tirosinase dan antioksidan *Tagetes erecta* L. sebagai *whitening agent* formulasi losio pencerah kulit. *Jurnal Mandala Pharmacon Indonesia*, *8*(2), 318–331. https://doi.org/10.35311/jmpi.v8i2.248

Putri, W. E., Kurniawati, Y., & Djauhari, T. (2018). *Depigmenting agent* melanotoksik pada pengobatan melasma. *Medical and Health Science Journal*, *2*(2), 23–31. https://doi.org/10.33086/mhsj.v2i2.584

Ripaldo, F., & Sagala, Z. (2020). Uji aktivitas *inhibitor* enzim tirosinase dan uji antioksidan ekstrak etanol buah harendong (*Melastoma malabathricum* L.) secara *in vitro*. *Indonesia Natural Research Pharmaceutical Journal*, *5*(1), 1–16. https://doi.org/10.52447/inspj.v5i1.1800

Saeedi, M., Eslamifar, M., & Khezri, K. (2019). Kojic acid applications in cosmetic and pharmaceutical preparations. *Biomedicine & Pharmacotherapy*, *110*, 582–593. https://doi.org/10.1016/j.biopha.2018.12.006

Safrina, U., Wardiyah, & Murtini, G. (2020). Phytochemical screening and antioxidant activity of nyamplung seed oils (*Calophyllum inophyllum* L.). *SANITAS: Jurnal Teknologi dan Seni Kesehatan*, *11*(2), 256–268. https://doi.org/10.36525/sanitas.2020.24

Sagala, Z., & Telaumbanua, K. (2020). Formulasi uji stabilitas dan aktivitas inhibitor enzim tirosinase sediaan krim dari ekstrak buah harendong (*Melastoma affine* D. Don). *Indonesia Natural Research Pharmaceutical Journal*, *5*(2), 149–173. Retrieved from https://media.neliti.com/media/publications/341656-formulation-stabilty-test-and-enzyme-act-6932960e.pdf

Suharyanto, & Prima, D. A. N. (2020). Penetapan kadar flavonoid total pada juice daun ubi jalar ungu (*Ipomoea batatas* L.) yang berpotensi sebagai hepatoprotektor dengan metode spektrofotometri UV-Vis. *Cendekia Journal of Pharmacy*, *4*(2), 110–

119. https://doi.org/10.31596/cjp.v4i2.89

Sun, G. F., Hu, W. T., Yuan, Z. H., Zhang, B. A., & Lu, H. (2017). Characteristics of mercury intoxication induced by skin-lightening products. *Chinese Medical Journal*, *130*(24), 3003–3004. https://doi.org/10.4103/0366-6999.220312

Widyodhari, S. M. (2022). Prediksi *inhibitor* tirosinase dalam tanaman nyamplung (*Calophyllum inophyllum*) dan minyak tamanu lokal asal Kebumen (Jawa Tengah). [Thesis] Bogor, Indonesia: IPB University.