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Antioxidant Activity And Tirosinase Enzyme Inhibition Of Pandan Leaf Extract (Pandanus Amaryllifolius)

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Abstract

Continuous exposure to ultraviolet radiation triggers the formation of Reactive Oxygen Species (ROS), leading to oxidative stress and damage to cellular biological components. This condition is closely associated with increased activity of the tyrosinase enzyme, a key enzyme in the melanin biosynthesis process that can cause hyperpigmentation. This study aims to evaluate the antioxidant activity and tyrosinase enzyme inhibition potential of the ethanolic extract of pandan leaves (Pandanus amaryllifolius) in vitro. The research method began with the extraction of pandan leaf powder using 70% ethanol as a solvent. Subsequently, qualitative phytochemical screening was conducted to identify secondary metabolites. The antioxidant activity was evaluated using the DPPH method across various concentrations, with Vitamin C as the standard comparison. The tyrosinase enzyme inhibition assay was performed spectrophotometrically using L-DOPA as a substrate at a wavelength of 492 nm. Phytochemical screening revealed that the extract contains secondary metabolites, including flavonoids, alkaloids, saponins, and tannins. The antioxidant test using the DPPH method showed that at a concentration of 500 ppm, the extract achieved an inhibition percentage of 97.73%, which is comparable to the effectiveness of Vitamin C (99.36%). In the tyrosinase enzyme assay using L-DOPA as a substrate, the blank absorbance was 0.794, while the sample absorbance was 0.907. Although the sample absorbance was higher due to the extract's intrinsic color pigments, the calculation indicated a very strong enzyme inhibition effectiveness of 90.7%. In conclusion, the ethanolic extract of pandan leaves (Pandanus amaryllifolius) holds significant scientific potential as a natural therapeutic agent to counteract oxidative stress and inhibit melanogenesis in skin pigmentation disorders.

Keywords: Pandanus amaryllifolius, Antioxidant, DPPH, Tyrosinase Inhibitor, Melanin.

INTRODUCTION

The skin is the outermost and largest organ of the human body, serving as the primary protective barrier against various external environmental factors and acting as an indicator of an individual's health condition. Proper skin care and maintenance contribute to healthy, well-maintained skin and provide a fresh appearance. Biologically, the skin consists of complex epithelial tissues, possesses elastic and sensitive properties, and exhibits variations in type and color influenced by factors such as climate, race, gender, and age (Fatmawati et al., 2023).

Long-term exposure to ultraviolet (UV) radiation can cause various adverse effects on the skin, including accelerated aging, an increased risk of skin cancer, and reduced effectiveness of the immune response. These health problems are closely associated with the formation of Reactive Oxygen Species (ROS) resulting from UV radiation exposure (Ariani et al., 2022).

Skin aging is a complex process involving a decline in skin function and capacity, influenced by intrinsic factors (genetics, cellular metabolism, and hormonal changes) as well as extrinsic factors (UV radiation, air pollution, and infrared exposure). Skin aging leads to structural alterations in both the epidermis and dermis, including decreased basal cell proliferation, reduced numbers of mast cells and fibroblasts, and diminished production of collagen and elastin (Rosalinda, 2021).

Free radicals produced through metabolic processes in the body are known as Reactive Oxygen Species (ROS). When the production of these free radicals exceeds the body's antioxidant defense capacity, a condition known as oxidative stress occurs, which is associated with various health disorders. Free radicals can attack essential biological components such as lipids, proteins, and DNA,

thereby contributing to the development of numerous diseases. Therefore, the utilization of exogenous antioxidant sources plays an important role in controlling oxidative stress. Antioxidants are compounds that, even at low concentrations, can inhibit or prevent the oxidation of substrates. Their mechanism of action involves neutralizing free radicals, thereby reducing oxidative stress levels and protecting the body from cellular damage. Antioxidants may originate from endogenous defense systems or external dietary sources. Common antioxidants include vitamin C, vitamin E, carotenoids, glutathione, flavonoids, and other antioxidant compounds (Wibowo et al., 2022).

One plant recognized for its antioxidant potential is fragrant pandan leaf. *Pandanus amaryllifolius*, belonging to the family Pandanaceae, is widely distributed throughout Southeast Asia, including Indonesia. Fragrant pandan is a monocotyledonous plant characterized by its distinctive aroma and contains various phytochemical constituents, including alkaloids, flavonoids, saponins, tannins, and polyphenols, which contribute to its antioxidant properties. This study aims to evaluate the antioxidant potential of *Pandanus amaryllifolius* leaf extract and its inhibitory effect on tyrosinase enzyme activity.

RESEARCH METHODS

The equipment used in this study included aluminum foil, a blender, food dryer, incubator shaker, mortar and pestle, Milwaukee MW802 PRO 3-in-1 combometer, rotary evaporator, glassware, centrifuge, UV-Visible spectrophotometer, analytical balance, ultrasonic bath, and water bath. The materials used were distilled water, acetic anhydride, chloroform, pandan leaves (*Pandanus amaryllifolius*), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Dragendorff reagent, tyrosinase enzyme, 70% ethanol, ferric chloride ($FeCl_3$), phosphate buffer, sulfuric acid (H_2SO_4), hydrochloric acid (HCl), L-DOPA, magnesium powder, Mayer reagent, methanol, and vitamin C.

Fresh pandan leaves (*Pandanus amaryllifolius*) weighing 3 kg were washed thoroughly under running water to remove dirt and impurities and then cut into small pieces to increase the surface area for extraction. The samples were dried using a food dryer at 70°C for 24 h to reduce moisture content while preserving thermolabile bioactive compounds. The dried leaves were ground into powder, and 30 g of the powder was extracted with 70% ethanol at a ratio of 1:10 (w/v) in an Erlenmeyer flask. The extraction process was carried out using an incubator shaker at 170 rpm for 24 h. The extract was then centrifuged at 4000 rpm for 15 min to separate the supernatant from the residue. The resulting supernatant was subjected to ultrasonic treatment at 40 kHz and 150 W for 30 min to enhance the release of bioactive compounds from the cellular matrix. Subsequently, the extract was concentrated using a rotary evaporator under reduced pressure at 45–50°C to remove the solvent without degrading thermolabile compounds. The concentrated extract was used for phytochemical screening, antioxidant activity analysis, and tyrosinase inhibition assays (Yosef Oeleu, 2022).

Phytochemical screening was conducted to identify the presence of secondary metabolites in the extract. Alkaloids were detected by treating the extract with Mayer and Dragendorff reagents after extraction with chloroform and acidification with concentrated sulfuric acid. The formation of a white precipitate with Mayer reagent or an orange-red precipitate with Dragendorff reagent indicated the presence of alkaloids. Flavonoids were identified by heating the extract in water, followed by the addition of magnesium powder and concentrated hydrochloric acid. The appearance of red, yellow, or orange coloration indicated a positive result. Steroids and triterpenoids were identified by adding glacial acetic acid and concentrated sulfuric acid to the extract. A blue or green color indicated steroids, whereas a red or purple color indicated triterpenoids. Tannins were detected by adding ferric chloride ($FeCl_3$) reagent to the filtrate obtained from the boiled extract. The formation of dark blue or greenish-black coloration indicated the presence of tannins or phenolic compounds. Saponins were identified by vigorously shaking the extract with distilled water followed by the addition of 1 N hydrochloric acid. The formation of stable foam that persisted for approximately 7 min indicated the presence of saponins (Handayani Santi Nur et al., 2020).

The antioxidant activity of the extract was evaluated using the DPPH free radical scavenging assay. Sample solutions at concentrations of 200, 300, 400, and 500 ppm were prepared, and 1 mL of each solution was mixed with 1 mL of DPPH solution (100 mg/L) and 2 mL of methanol. The mixtures were incubated at 37°C for 30 min and then analyzed using a UV-Visible spectrophotometer at a wavelength of 517 nm. All measurements were performed in duplicate. Antioxidant activity was indicated by the reduction of the purple DPPH radical to a yellow non-radical form. The percentage inhibition was calculated using the equation % inhibition = $[(A_0 - A_1)/A_0] \times 100$, where A_0 represents the absorbance of the blank solution and A_1 represents the absorbance of the sample solution (Himarniarwati, 2019).

The tyrosinase inhibitory activity assay was performed to evaluate the ability of the extract to inhibit melanin formation catalyzed by tyrosinase. The assay was carried out spectrophotometrically by mixing 40 µL of 5 mM L-DOPA, 40 µL of sample solution, and 80 µL of phosphate buffer (pH 6.5), followed by the addition of 40 µL of tyrosinase enzyme solution (250 U/mL). The reaction mixture was homogenized and incubated at 30°C. Absorbance was measured at 492 nm using a microplate reader, and the percentage inhibition was calculated based on the absorbance values of the control, sample, and blank solutions (Sholikha & Puspitasari, 2023). The blank solution (B1) consisted of phosphate buffer, tyrosinase enzyme, and L-DOPA substrate and was used to determine the normal enzymatic activity. The control blank (B0) was prepared by replacing the substrate with phosphate buffer to correct background absorbance unrelated to enzymatic reactions. The sample solution (S1) contained extract, enzyme, and substrate, while the sample control (S0) was prepared without substrate to correct for absorbance derived from the natural color of the extract rather than enzyme activity (Eden et al., 2024).

Kojic acid was used as a positive control because it is a well-established tyrosinase inhibitor. Kojic acid inhibits tyrosinase activity by chelating Cu^{2+} ions at the enzyme active site, thereby preventing substrate binding and subsequent melanin synthesis. The positive control was tested using the same procedure as the extract samples at various concentrations, and absorbance was measured at 492 nm (Syahputra et al., 2022).

RESULTS AND DISCUSSION

Phytochemical Screening Results

Phytochemical screening was conducted on pandan leaves, extracts, and fractions to obtain a qualitative overview of the secondary metabolite compounds contained in each sample. The phytochemical screening included qualitative tests for alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids. The screening procedure followed the phytochemical screening method reported by Santi Nur et al. (2020).

The results of the phytochemical screening of pandan leaf extract are presented in Table 1.

Table I. Phytochemical Screening Results of Pandan Leaf Extract

Phytochemical Compound	Result	Observation
Flavonoids	+	Orange, red, or yellow coloration
Steroids	−	Blue coloration
Alkaloids	+	Brown precipitate (Dragendorff reagent)
Saponins	+	Formation of stable foam
Tannins	+	Dark blue or greenish-black coloration

Note:
(+) Presence of Chemical Compounds
(−) Absence of Chemical Compounds

Based on the phytochemical screening results of pandan leaf extract, the test produced an orange color, indicating the presence of flavonoids in the sample. This finding is consistent with the characteristics of flavonoids, which possess hydroxyl groups and aromatic rings, making them water-

soluble and capable of producing positive reactions with flavonoid reagents. The presence of flavonoids is relevant because these compounds are known to exhibit strong antioxidant activity and therefore have the potential to provide protection against free radicals (Juanda et al., 2023).

The addition of Dragendorff reagent to the pandan extract produced an orange-red precipitate, indicating the presence of alkaloid compounds. This reaction occurs because the basic nitrogen groups of alkaloids interact with heavy metal ions in the Dragendorff reagent, forming an insoluble orange-red complex. This positive result indicates that pandan extract contains alkaloids, which are known to possess various biological activities, including antioxidant and antimicrobial potential (Anggreni et al., 2023).

The saponin test showed positive results, indicated by the formation of stable foam approximately 1 cm in height that persisted for 10 minutes and remained stable after the addition of 2 N HCl. This observation meets the criteria for a positive saponin test, namely foam with a height of 1–10 cm that remains stable for at least 10 minutes after acid addition. The addition of HCl increases the polarity of the solution, thereby enhancing the stability of the hydrophilic groups of saponins and maintaining foam formation. The presence of saponins suggests potential biological activities such as antioxidant and antimicrobial effects (Fauzia & Kartika, 2023).

The tannin test showed positive results, as indicated by a color change to blue-green after the addition of $FeCl_3$ to the pandan leaf extract. This reaction occurs because tannins react with Fe^{3+} ions to form colored complexes, which are characteristic of phenolic compounds such as tannins. The presence of tannins contributes to the antioxidant activity and other biological potentials of pandan leaf extract (Qomaliyah et al., 2023).

The steroid test showed negative results, which may be attributed to variations in the secondary metabolite content of pandan leaves (*Pandanus amaryllifolius*). Internal factors such as plant age, growth stage, and physiological processes, as well as external factors including environmental conditions, solvent type, and extraction method, may influence the presence of steroids in the extract. Therefore, these negative results do not necessarily exclude the possibility of steroid occurrence under different conditions or in other parts of the plant (Lina et al., 2020).

Antioxidant Activity Test Results

Free radicals are reactive molecules that can damage healthy cells by attacking cellular components such as lipids, proteins, and DNA, thereby triggering degenerative diseases. Antioxidants play an important role in neutralizing free radicals, preventing cellular damage, and reducing the risk of diseases such as cancer and diabetes. In the DPPH assay, pandan leaf extract demonstrated strong antioxidant activity by converting DPPH radicals (purple) into a stable form (yellow). This result indicates that pandan leaf extract effectively scavenges free radicals and therefore has potential as a natural antioxidant agent capable of protecting cells from oxidative damage (Fitokimia et al., 2021). The antioxidant mechanism in the DPPH method involves the transfer of hydrogen atoms from antioxidants to DPPH radicals, reducing DPPH to DPPH-H. This process causes the color change of the solution from purple to yellow (Mamay et al., 2022).

The percentage inhibition of DPPH free radicals at various concentrations of pandan leaf extract is presented in Table II.

Table II. Percentage Inhibition of DPPH by Pandan Leaf Extract and Vitamin C

Concentration (ppm)	Pandan Leaf Extract (% inhibition ± SD)	Vitamin C (% inhibition ± SD)	p-value
200	96.22 ± 0.01	91.85 ± 0.01	<0.05
300	95.95 ± 0.01	94.84 ± 0.01	<0.05
400	96.52 ± 0.01	98.28 ± 0.01	<0.05
500	97.73 ± 0.01	99.36 ± 0.01	<0.05

The antioxidant activity test demonstrated that pandan leaf extract (*Pandanus amaryllifolius*) possesses a strong ability to scavenge DPPH radicals. As the sample concentration increased, DPPH absorbance decreased, indicating that a greater amount of antioxidant compounds donated hydrogen

atoms or electrons to DPPH radicals, converting the purple DPPH solution into a yellow form. At a concentration of 500 ppm, pandan leaf extract achieved 97.73% inhibition, approaching the value of Vitamin C (99.36%), indicating excellent free radical scavenging capacity. The DPPH method was selected because it is simple, rapid, sensitive, and reproducible, making it suitable for polar samples while requiring only a small amount of sample. These findings indicate that pandan leaf extract has strong potential as a natural antioxidant source (Muslihin & Salam, 2025).

The percentage inhibition values indicate the ability of the sample to inhibit tyrosinase enzyme activity at each tested concentration. Higher percentage inhibition values correspond to greater inhibitory activity against the tyrosinase enzyme. This suggests that the sample has promising potential to suppress melanin formation. Statistical analysis using One-Way ANOVA demonstrated that variations in the concentrations of pandan leaf extract and Vitamin C significantly affected DPPH inhibition percentages ($p < 0.05$), indicating that increasing sample concentration directly enhanced antioxidant activity. These results confirm that the observed differences in antioxidant activity among concentrations were not due to chance but rather resulted from the concentration treatments applied. This test demonstrates that sample concentration is an important factor in determining antioxidant potential (Moch Hamdan, 2022).

Comparison of Antioxidant Activity Between Pandan Leaf Extract (*Pandanus amaryllifolius*) and Vitamin C

The data presented in Figure 1 show that increasing sample concentration was directly proportional to the increase in antioxidant percentage and inversely proportional to absorbance values. This phenomenon can be explained by the higher concentration of antioxidant compounds present in pandan leaf extract at increased concentrations, resulting in enhanced DPPH free radical scavenging activity. Vitamin C (ascorbic acid) was used as a positive control because it is a natural antioxidant that is relatively safe, stable, and non-toxic, making it a commonly used standard in antioxidant activity testing. The use of Vitamin C as a reference standard allows for a more accurate comparison between the antioxidant activity of the sample and a well-established antioxidant standard (Gusungi et al., 2020).

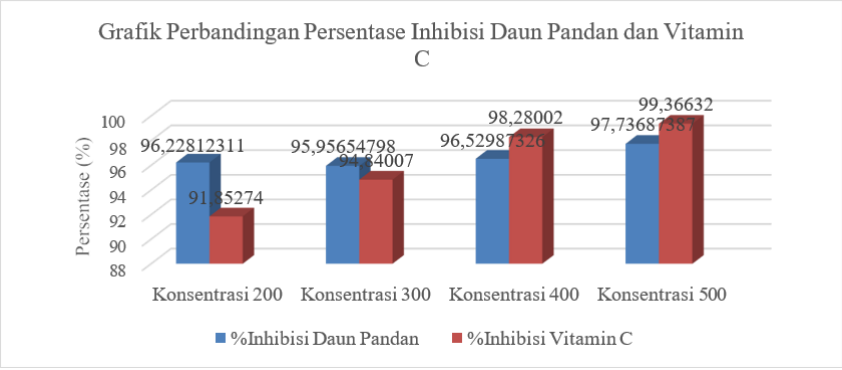

Figure 1. Antioxidant Activity of Papaya Leaf Extract and Vitamin C

Pandan leaf extract demonstrated optimal performance as a natural antioxidant agent, with an inhibition value of 91.85% at a concentration of 200 ppm, which increased to 97.73% at a concentration of 500 ppm. Although this value remained slightly lower than that of Vitamin C (99.36%), the difference was relatively small, indicating the high bioactivity potential of secondary metabolites present in pandan leaves, particularly phenolic and flavonoid compounds (Dyah, 2021).

Vitamin C contains free hydroxyl groups that function as free radical scavengers, while its polyhydroxyl structure enhances its antioxidant capacity. When interacting with DPPH free radicals, Vitamin C donates hydrogen atoms (H) to neutralize the radicals, converting them into stable forms. This process generates relatively stable and non-reactive antioxidant radicals, thereby terminating free radical chain reactions. Furthermore, these antioxidant radicals may react with other radicals to form

harmless non-radical products. Through this mechanism, Vitamin C effectively scavenges free radicals and protects cells from oxidative damage (Lina et al., 2020).

Tyrosinase Enzyme Inhibition Results

Tyrosinase inhibition was evaluated using L-DOPA as the substrate because the reaction between L-DOPA and tyrosinase produces dopachrome, which subsequently forms melanin. In the presence of tyrosinase inhibitory compounds, melanin formation is suppressed. Therefore, a reduction in melanin production can be used as an indicator of tyrosinase inhibitory activity in the tested extract (Alchemy et al., 2023).

Table III. Tyrosinase Enzyme Inhibition Test Results

Sample	Positive Control (+)	Blank	Result
0.907	0.982	0.794	90.7%

The results showed that the blank absorbance was 0.794, representing the maximum activity of the tyrosinase enzyme. The sample absorbance was 0.907, which was higher than that of the blank, possibly due to the intrinsic color of the sample. However, the calculated result indicated that the sample exhibited 90.7% tyrosinase inhibition, demonstrating a very strong inhibitory effect (Muslihin & Salam, 2025). These findings are consistent with those reported by Sholikha and Puspitasari (2023), who observed tyrosinase inhibitory activity of up to 96%, which was categorized as very strong inhibition. Enzyme activity is influenced by various factors, including enzyme concentration, substrate concentration, product concentration, inhibitors, activators, pH, solvent type, ionic strength, and temperature. These factors can alter enzyme structure and consequently affect its catalytic activity. In this study, pandan leaf extract contained bioactive compounds that acted as tyrosinase inhibitors, thereby suppressing melanin formation (Imanullah et al., 2024).

CONCLUSIONS

Based on the results of this study, it can be concluded that the ethanol extract of fragrant pandan leaves (*Pandanus amaryllifolius*) contains secondary metabolites, including flavonoids, alkaloids, saponins, and tannins. The presence of these phenolic and flavonoid compounds contributed significantly to the strong antioxidant activity of the extract, which was capable of scavenging DPPH free radicals by up to 97.73% at a concentration of 500 ppm. This effectiveness was comparable to that of the positive control, Vitamin C, which exhibited an inhibition value of 99.36%. Statistical analysis demonstrated that increasing extract concentration was significantly associated with an increase in the percentage of free radical inhibition. In addition to its antioxidant potential, the fragrant pandan leaf extract also exhibited effective tyrosinase inhibitory activity, achieving an inhibition percentage of 90.7%. Although the sample absorbance value (0.907) was higher than that of the blank (0.794), this result was influenced by the intrinsic color or natural pigments present in the extract. Functionally, however, the extract remained capable of effectively inhibiting the oxidation of L-DOPA to dopachrome. Therefore, fragrant pandan leaves possess considerable scientific potential for further development as a natural therapeutic agent for inhibiting melanogenesis and protecting cells against oxidative stress-induced damage.

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