International Journal Of Health, Engineering And Technology (IJHET) *E-ISSN 2829 - 8683*
Volume 5, Number 2, July 2026, *Page. 1521 - 1526*
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Antioxidant Activity And Tirosinase Inhibition Extract Of Red Shoot Leaves (Syzygium myrtifolium)

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Abstract

Skin aging is a natural process accelerated by free radical exposure, UV radiation, and environmental pollution, necessitating effective and safe anti-aging agents. This study aimed to evaluate the potential of red shoot leaves ethanol extract (Syzygium myrtifolium) as anti-aging through antioxidant activity and tirosinase enzyme inhibition assays in vitro. Extraction was performed by maceration using 70% ethanol (1:10) for 12 hours, centrifuged, sonicated, and concentrated using a rotary evaporator. Phytochemical skrining identified flavonoids, alkaloids, saponins, tannins, and triterpenoids. DPPH antioxidant assay showed inhibition percentages of 96.17–99.49% at concentrations of 200-800 ppm. Tirosinase inhibition test resulted in 82.54% inhibition. These results demonstrate that red shoot leaves extract has dual anti-aging potential through antioxidant protection and melanogenesis inhibition mechanisms. This study provides scientific basis for developing anti-aging cream formulations based on safe and sustainable local natural ingredients

Keywords: Red Shoot Leaves; Antioxidant; Tyrosinase; Anti-Aging; Syzygium Myrtifolium.

INTRODUCTION

Aging is a natural process experienced by every individual, characterized by a decline in physiological functions and changes in skin structure. Symptoms of premature aging, although not considered severe diseases or health disorders, can significantly affect an individual's self-confidence and psychological well-being. As the outermost organ of the body, the skin is directly exposed to various environmental factors that can cause damage, including ultraviolet (UV) radiation, medications, pollution, cigarette smoke, radiation exposure, alcohol consumption, and certain chemical substances. These factors can cause the skin to appear dry and thin, develop fine lines and wrinkles, experience discoloration, and lose its firmness, resulting in a dull and less youthful appearance. Skin aging can be classified into two types: intrinsic aging, which occurs naturally over time, and extrinsic aging, which results from external factors such as excessive sun exposure, pollution, smoking habits, and an unbalanced diet. In extrinsic aging, the signs are generally more evident in areas frequently exposed to sunlight (Hanum, 2018).

Tyrosinase is an enzyme that plays a crucial role in the skin pigmentation process known as melanogenesis. During melanogenesis, tyrosinase regulates melanin synthesis by hydroxylating L-tyrosine into L-DOPA and subsequently oxidizing L-DOPA into dopaquinone. Dopaquinone is then converted into dopachrome through an auto-oxidation process, producing dihydroxyindole (DHI) and dihydroxyindole-2-carboxylic acid (DHICA), which ultimately contribute to melanin formation (Furi et al., 2022).

Red shoot leaves (*Syzygium myrtifolium*) are among the plants commonly found in Indonesia and are known for their various beneficial properties. This plant is rich in bioactive compounds that possess antioxidant potential and the ability to inhibit tyrosinase enzyme activity. Since tyrosinase plays a vital role in melanin production, its inhibition may help reduce skin hyperpigmentation and the appearance of dark spots, while also providing a skin-brightening effect (Sugihartini & Maryati, 2022).

This study is expected to contribute to the development of natural-based cosmetic products and to enhance understanding of the potential use of red shoot leaves in skin care applications.

Furthermore, the findings are anticipated to serve as a reference for future studies on the utilization of local natural resources in the cosmetic industry and to encourage consumers to choose environmentally friendly and sustainable cosmetic products.

RESEARCH METHODS

This study employed an experimental design to evaluate the antioxidant and tyrosinase inhibitory activities of red shoot leaf (*Syzygium myrtifolium*) extract. The research was conducted at the Integrated Laboratory of Universitas Prima Indonesia from March to May 2025.

Red shoot leaves collected from Purba Dolok Village (3-year-old plants) were sorted, washed, sliced, and dried using a food dryer at 70°C for 24 hours. The dried samples were then ground into powder using a blender. Extraction was carried out by macerating 30 g of powdered sample with 70% ethanol (1:10, w/v) in a 500 mL Erlenmeyer flask and shaking at 170 rpm for 12 h. The extract was centrifuged at 5000 rpm, and the supernatant was subjected to ultrasonic-assisted extraction at 20 kHz and 45°C. The resulting extract was concentrated using a rotary evaporator at 49–50°C and further evaporated in a water bath to obtain a viscous extract.

Phytochemical screening was performed qualitatively to identify the presence of flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids using standard phytochemical methods. Flavonoids were detected using the Shinoda test, alkaloids using Mayer and Dragendorff reagents, tannins using $FeCl_3$, saponins through foam formation, and steroids/triterpenoids using the Liebermann–Burchard reaction.

Antioxidant activity was evaluated using the DPPH radical scavenging assay, with ascorbic acid as the positive control. Different concentrations of the extract were mixed with 0.1 mM DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Antioxidant activity was expressed as percentage inhibition based on the reduction in DPPH absorbance.

Tyrosinase inhibitory activity was determined using L-DOPA as the substrate and kojic acid as the positive control. Extract solutions at concentrations of 200, 400, 600, and 800 ppm were prepared in 50% DMSO. In a 96-well microplate, 40 µL of sample solution was mixed with 80 µL phosphate buffer (pH 6.8), 40 µL tyrosinase enzyme solution, and 40 µL L-DOPA (2 mM). After incubation at room temperature for 10 min, absorbance was measured at 475 nm using a microplate reader. The percentage of tyrosinase inhibition was calculated by comparing sample absorbance with the control. Data were analyzed descriptively to assess the potential of *S. myrtifolium* extract as a natural antioxidant and tyrosinase inhibitor.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening was conducted to qualitatively identify the active chemical compounds present in the plant extract. Based on the phytochemical analysis of the ethanol extract of red shoot leaves (*Syzygium myrtifolium*), several bioactive compounds were detected, as presented in Table I.

Table I. Phytochemical Screening Results

Phytochemical Test	Observation	Result
Alkaloids	White precipitate	+
	Orange precipitate	+
Flavonoids	Red/Pink coloration	+
Saponins	Foam formation	+
Tannins	Dark blue/greenish-black coloration	+
Steroids	Green coloration	-
Terpenoids	Orange coloration	+

The presence of these secondary metabolites is closely related to the biological activities evaluated in this study, particularly flavonoids and tannins, which are known as potent antioxidants and tyrosinase inhibitors. These compounds contain phenolic hydroxyl groups capable of scavenging DPPH free radicals. The extremely high antioxidant activity (>96% inhibition) suggests an efficient hydrogen atom donation mechanism (H•) that neutralizes DPPH radicals, supporting their potential anti-aging effects.

In addition to flavonoids and tannins, the detection of alkaloids, saponins, and terpenoids further enhances the pharmacological profile of the extract. Alkaloids, through their heterocyclic nitrogen-containing structures, contribute to free-radical stabilization (Omidian et al., 2025), whereas terpenoids may act synergistically by inhibiting tyrosinase activity through interactions with the enzyme's active site (Kusio-Targońska et al., 2025). Although saponins exhibit relatively weaker antioxidant activity, they may improve the stability and bioavailability of active compounds in cosmetic formulations (Omidian et al., 2025). The diversity of secondary metabolites identified indicates that the biological efficacy of red shoot leaf extract results from the collective interaction of multiple bioactive constituents rather than a single compound. These findings are consistent with previous studies on *S. myrtifolium*, which have reported the presence of alkaloids, flavonoids, tannins, and saponins as major phytochemical constituents (AHMAD et al., 2021; Wenas et al., 2022).

The negative result obtained for steroids may reflect differences in metabolite concentrations caused by variations in extraction methods, geographical origin, and leaf maturity among studies. Therefore, further quantitative analyses are necessary to determine the concentrations of individual metabolites and establish correlations with the IC_{50} values obtained from antioxidant and tyrosinase inhibition assays. Determination of total phenolic and specific flavonoid contents would provide a deeper understanding of the contribution of each compound group to the functional properties of the extract (Ladjambu et al., 2026; NUR et al., 2025).

Antioxidant Activity (DPPH Assay)

The DPPH radical scavenging activities of red shoot leaf extract and vitamin C at concentrations of 200, 400, 600, and 800 ppm are presented in Table II.

Table 2. Percentage of DPPH Inhibition by Extract and Vitamin C

Concentration (ppm)	Extract (% Inhibition)	Vitamin C (% Inhibition)	p-value
800	99.49	91.85	>0.05
600	98.79	94.84	>0.05
400	98.07	98.28	>0.05
200	96.17	99.37	>0.05

The ethanol extract of red shoot leaves (*S. myrtifolium*) demonstrated extremely strong antioxidant activity, with inhibition percentages ranging from 96.17% to 99.49% across concentrations of 200–800 ppm. Since all tested concentrations exhibited inhibition values above 95%, the IC_{50} value could not be accurately determined and was estimated to be below 2 mg/mL. This result confirms the remarkable antioxidant potential of the extract and highlights its suitability as a premium active ingredient for anti-aging formulations.

Statistical analysis revealed no significant difference ($p > 0.05$) between the antioxidant activity of the extract and vitamin C, the positive control, at all tested concentrations. This finding indicates that the antioxidant capacity of red shoot leaf extract is comparable to that of vitamin C, a widely recognized standard antioxidant.

At 200 ppm, the extract showed 96.17% inhibition compared to 99.37% for vitamin C; however, the difference was not statistically significant. Interestingly, at 800 ppm, the extract exhibited a higher inhibition value (99.49%) than vitamin C (91.85%), although this difference was also not statistically significant. This trend suggests that the antioxidant activity of the extract remains stable at higher concentrations, whereas vitamin C tends to decrease. Similar observations have been reported for natural extracts containing diverse bioactive compounds, which often exhibit greater antioxidant stability than isolated compounds (Omidian et al., 2025).

The synergistic interactions among flavonoids and tannins may facilitate efficient redox recycling mechanisms, thereby maintaining free-radical scavenging activity even at high concentrations (Aruwa & Sabiu, 2023). Furthermore, the complex phytochemical matrix may function as a natural protective system that minimizes degradation of active compounds. In contrast, vitamin C is susceptible to oxidation and may exhibit pro-oxidant effects under certain conditions, particularly at high doses (Crespi et al., 2025; Omidian et al., 2025).

The IC_{50} value is a crucial parameter for evaluating DPPH radical scavenging efficiency. The antioxidant activity observed in this study was stronger than previously reported for the same species (AHMAD et al., 2021), emphasizing the importance of extraction techniques in optimizing bioactive compound profiles and antioxidant efficacy (Hasibuan et al., 2025). In anti-aging cosmetic applications, this finding is particularly relevant because excessive concentrations of vitamin C may accelerate oxidative damage, contrary to the intended protective effects (Crespi et al., 2025).

Tyrosinase Inhibitory Activity

The tyrosinase inhibition assay was performed to evaluate the ability of red shoot leaf extract to suppress tyrosinase enzyme activity. The results are presented in Table III.

Table 3. Tyrosinase Inhibition Activity

Sample	Positive Control	Blank	Inhibition (%)
S1	0.982	0.164	83.30
S2	0.982	0.179	81.77

Based on the results presented in Table III, red shoot leaf extract exhibited strong tyrosinase inhibitory activity, with inhibition percentages of 83.30% and 81.77% for samples S1 and S2, respectively. The average inhibition rate of 82.54% demonstrates substantial potential for tyrosinase suppression. This level of activity is comparable to that of several flavonoid compounds reported to inhibit melanogenesis through copper-chelating interactions at the active site of tyrosinase (Durmus et al., 2024).

Tyrosinase is a key enzyme in melanin biosynthesis, catalyzing the hydroxylation of L-tyrosine to L-DOPA and the subsequent oxidation of L-DOPA to dopachrome, which ultimately leads to melanin formation (Kusio-Targońska et al., 2025). Excessive tyrosinase activity can result in hyperpigmentation and various dermatological disorders. Therefore, the ability of red shoot leaf extract to modulate this enzymatic pathway supports its potential as a natural skin-brightening agent capable of effectively suppressing melanogenesis (Magalhães et al., 2023). This inhibitory mechanism may be attributed to specific flavonoids that exhibit strong antioxidant and anti-inflammatory activities, thereby reducing oxidative stress associated with pigmentation disorders (Yao et al., 2022).

The strong antioxidant activity observed in this study appears to correlate positively with tyrosinase inhibition. Similar findings have been reported in other *Syzygium* species, such as *S. claviflorum*, which demonstrated tyrosinase inhibition of 70.64% with an IC_{50} value of 41.82 µg/mL (Adli et al., 2025). These findings further support the potential of phenolic-rich plant extracts as natural alternatives to synthetic skin-whitening agents, which are often associated with dermatological irritation.

Moreover, the rich phytochemical profile of the extract may provide better metabolic stability than conventional depigmenting agents, making it suitable for long-term cosmeceutical applications (Ehiobu et al., 2021). Previous studies have shown that polar solvents such as ethanol, methanol, and water extract significantly higher levels of total phenolic content (TPC) and total flavonoid content (TFC) than non-polar solvents such as hexane and chloroform (AHMAD et al., 2021). This indicates that polar solvents are more effective for extracting hydroxyl-rich bioactive compounds responsible for antioxidant and tyrosinase inhibitory activities (Aruwa & Sabiu, 2023).

Phenolic compounds may compete with L-DOPA for binding to the active site of tyrosinase. Molecular docking studies have demonstrated that chlorogenic acid, neochlorogenic acid, gallic acid, and quercetin exhibit high binding affinities toward human tyrosinase-related protein-1 (hTRP-1), forming stable interactions within the enzyme's active center (Fourreh et al., 2024). These interactions

are primarily mediated by hydrogen bonds and van der Waals forces, resulting in competitive inhibition of substrate access and a subsequent reduction in melanin production (Akintemi et al., 2024).

The inhibition values exceeding 80% observed for *S. myrtifolium* extract indicate excellent potential as a natural skin-whitening agent. For comparison, kojic acid, considered the gold standard for tyrosinase inhibition, exhibits an IC_{50} of approximately 7.4 µM against mushroom tyrosinase. Plant extracts demonstrating inhibition rates above 70% are generally regarded as highly promising for cosmetic and dermatological applications targeting hyperpigmentation disorders (Kusio-Targońska et al., 2025).

CONCLUSIONS

The ethanol extract of red shoot leaves (*Syzygium myrtifolium*) contains flavonoids, alkaloids, saponins, tannins, and triterpenoids that contribute to its biological activities. The extract exhibited highly potent antioxidant activity, with DPPH inhibition values ranging from 96.17% to 99.49% at concentrations of 2–5 mg/mL, and an estimated IC_{50} value of less than 2 mg/mL. In addition, the extract demonstrated strong tyrosinase inhibitory activity, reaching 82.54%, indicating its effectiveness as an anti-hyperpigmentation agent. These findings suggest that the extract possesses dual potential as an anti-aging ingredient through both antioxidant protection and simultaneous inhibition of tyrosinase activity. Therefore, this local plant resource has considerable potential for use as a natural active ingredient in safe and sustainable anti-aging cosmetic formulations. The results of this study provide a scientific basis for the development of skincare products derived from local natural resources.

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