Original Research Article

GC-MS Analysis, Anti-Tyrosinase, and Antioxidant Activities of *Syzygium claviflorum* Fruits Extract

Muhammad Amirul Adli[1,3], Abdullah Haniff Abdul Halim Yap[1], Monporn Payaban[2], Rozaini Mohd Zohdi[1,3*]

[1]Faculty of Pharmacy, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia.
[2]Faculty of Science, Rangsit University, Muang-Ake, Paholyothin Rd., PathumThani 12000, Thailand.
[3]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia.

ABSTRACT

Excessive oxidative stress and tyrosinase activity are critical factors in the development of various dermatological issues, including hyperpigmentation and accelerated aging. Given the growing interest in natural products for their potential therapeutic benefits, this study aimed to determine the phytochemical compositions and evaluate the antioxidant and tyrosinase inhibitory activities of ethanolic fruit extracts from *Syzygium claviflorum*. Gas chromatography-mass spectrometry (GC-MS) was employed to analyze the phytochemical components of the extract. The tyrosinase inhibitory activity was evaluated using a mushroom tyrosinase inhibitory assay, while the antioxidant capacity of the extract was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP) assays. GC-MS analysis revealed that phenol was the major constituent, representing 17.71% of the extract, followed by carboxylic acid at 6.69%. The extract exhibited significant anti-tyrosinase activity, with an inhibition rate of 70.64±0.22% and an IC_{50} value of 41.82 ± 1.43 µg/mL. Additionally, the extract demonstrated a DPPH radical scavenging activity of 55.32 ± 1.32% with an IC_{50} value of 212.46 ± 1.73 µg/mL and a FRAP value of 296.72 ± 1.12 µM Fe^{2+}. These findings suggested that *S. claviflorum* ethanolic fruit extract demonstrated antioxidant and tyrosinase inhibitory activities, warranting further investigation for its potential applications in skincare.

Keywords: *Syzygium claviflorum*, GC-MS analysis, antioxidant, tyrosinase inhibition

Corresponding author:
Rozaini Mohd Zohdi
Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) Selangor Branch,
Puncak Alam Campus, 42300, Selangor, Malaysia.
Email: rozainizohdi@uitm.edu.my

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1.0 Introduction

Tyrosinase is a critical enzyme involved in the production of melanin, catalyzing both the hydroxylation of tyrosine and the oxidation of L-3,4-dihydroxyphenylalanine (L-Dopa) dopaquinone, which ultimately leads to melanin synthesis (1). While melanin plays a protective role against ultraviolet (UV) damage, excessive melanin production can result in conditions such as hyperpigmentation (2). Overactive tyrosinase, often triggered by environmental factors such as UV radiation, contributes significantly to pigmentation disorders, which are a common concern in dermatology (3). Conventional tyrosinase inhibitors such as hydroquinone and kojic acid are frequently used to treat hyperpigmentation but are associated with potential side effects, including skin irritation and contact dermatitis (4), leading to an increasing demand for safer, natural alternatives.

Oxidative stress, characterized by an overproduction of reactive oxygen species (ROS), further exacerbates tyrosinase activity (3). ROS not only oxidize tyrosinase directly but also activate pathways that upregulate its expression, accelerating melanin production (2,5). Antioxidants, known for their ability to neutralize ROS, offer a promising approach to mitigating oxidative damage and regulating melanin synthesis (5). Natural compounds, particularly those derived from plant extracts, are rich in antioxidants such as polyphenols and flavonoids, which exhibit both ROS-scavenging and anti-tyrosinase activities (6). Investigating the efficacy of natural compounds in skin care solutions aligns with current consumer trends and supports the development of safe, effective, and eco-friendly skin care products (7,8).

Syzygium claviflorum, also known as ‘jambu arang’, is a member of the Myrtaceae family primarily found in tropical and subtropical regions, especially in Southeast Asia (4). Phytochemical investigations have revealed that *S. claviflorum* contains several bioactive compounds, including phenolics, alkaloids, flavonoids, steroids, terpenes, and tannins (9,10,11). Notably, the seeds of the plant are rich sources of phenolic compounds with excellent free radical-scavenging properties (9). Although extensive research has been conducted on other species within the Syzygium genus, such as *Syzygium jambos* (12), *Syzygium aqueum* (13), *Syzygium cumini* (14), and *Syzygium polyanthum* (15), highlighting their significant antioxidant and tyrosinase inhibitory activities, research on *S. claviflorum* remains limited. Hence, the objectives of this study are to explore the phytochemical composition of the ethanolic fruit extract of *S. claviflorum* and to assess its antioxidant and anti-tyrosinase activities using *in vitro* and enzyme-based approaches.

2.0 Materials and methods

2.1 Sample collection

The fruit of *S. claviflorum* were collected in July 2022 from Kedah, Malaysia. A botanist from Unversiti Kebangsaan Malaysia (UKM) in Bangi, Malaysia verified and authenticated the plant. A voucher specimen (AuRIns-20220322) was subsequently deposited in the Herbarium, Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns). Following harvesting, the fruits underwent multiple washes with distilled water to remove dirt. Then, the peel and seeds were manually separated, and the fruit pulp was freeze-dried, ground to powder, and stored at -20°C until further processing.

2.2 Sample extraction

The ethanolic extract was prepared by dissolving the powdered peel in an ethanolic

aqueous solvent (80:20, w/v), which was then shaken continuously for 24 hours at room temperature. Subsequently, the mixture was filtered using Whatman number 1 filter paper, and the residue was macerated again in the solvent to maximize the extraction. The extraction process was repeated three times with the same procedure, and the filtrates were combined. The ethanol was removed using a rotary evaporator and the remaining aqueous solution was freeze-dried. The sample was stored at -20°C for analysis.

2.3 GC-MS analysis

Prior to the GC-MS analysis, the extract was derivatized according to the method established by Bankova *et al*. (2016) (16). Briefly, 1 mg of the extract was combined with 75 µl of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and 50 uL of pyridine. The mixture was then heated at 80°C for 20 min and subsequently cooled to room temperature. The GC-MS analysis utilized an HP-5ms column (30 m x 250 µm, 0.25 µm film thickness), with an injection volume of 0.5 µL and a flow rate of 1.2 mL/min, respectively. The column temperature program was set as follows; 0 min: 80°C, 1 min: 80°C, 17 min: 160°C, 19 min: 160°C, 31 min: 280°C, 36 min: 280°C. The injection was carried out in splitless mode. The detector and injection port temperatures were maintained at 230°C and 250°C, respectively. Helium served as the gas carrier, and the identification of compounds was performed using the NIST 2014 library.

2.4 Determination of mushroom tyrosinase inhibitory activity

The mushroom tyrosinase inhibitory assay was conducted following a modified method described by Promden *et al*. (2018) (13). 20 µL of extract at various concentrations (125, 250, 500, and 1000 µg/mL) and 20 µL of tyrosinase (100 units/mL in 0.1 M sodium phosphate buffer, pH 6.8) were mixed with 140 µL of sodium phosphate buffer (20 M, pH 6.8) and pre-incubated at 30°C for 10 min. Subsequently, 20 µL of 2.5 mM L-Dopa was added to the mixture. The plate was then incubated in the dark at room temperature for 10 min before measuring the absorbance at 450 nm using a microplate reader. Kojic acid was utilized as the positive control. The percentage of tyrosinase inhibition was calculated using the following equation:

$$Tyrosinase\ inhibition\ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{sample}}\right) X\ 100\%$$

where, $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test sample. The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted between tyrosinase inhibition activity percentage and sample concentration.

2.5 Determination of DPPH free radical scavenging activity

The DPPH free radical scavenging assay was conducted based on the method described by Adli *et al*. (2023) (11). The extract and quercetin were serially diluted to produce a range of concentrations (7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/mL). Amounts of 25 µL of sample solution or standard, 200 µL of 1 mM DPPH solution were mixed in a 96-well microplate. The plate was incubated for 30 min at room temperature, protected from light. The absorbance was measured at 517 nm with Trolox as a positive control. The experiment was performed in triplicate, and the percentage of inhibition was calculated using the following equation:

$$DPPH\ inhibition\ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{sample}}\right) X\ 100\%$$

where, $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test sample. Sample concentrations providing 50% scavenging activity (IC_{50}) were calculated from the graph plotted between free radical scavenging inhibition percentage and sample concentration.

2.6 Determination of ferric reducing antioxidant power (FRAP) activity

The FRAP assay was performed according to the protocol outlined by Idris *et al.* (2023) (12). The FRAP reagent was prepared by mixing acetate buffer, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) dissolved in hydrochloric acid, and ferric chloride. Standard solutions were prepared using ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) at various concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.63, and 7.81 µg/mL). Trolox was used as a positive control. Samples, blanks, positive control, and standards were added to a 96-well plate, followed by the addition of the FRAP reagent. The plate was incubated in the dark at 37°C for 30 min. Absorbance was measured at 593 nm using a microplate spectrophotometer, and results were calculated using a linear regression plot.

2.7 Statistical analysis

Results were expressed as mean ± standard deviation (SD) of three independent experiments. Statistical analyses were performed using GraphPad Prism version 7.0, including t-tests for comparative analyses. Differences between experimental groups were considered significant if $p < 0.05$.

3.0 Results and discussion

3.1 GC-MS analysis

GC-MS was utilized to analyze the phytochemical constituents of the ethanolic extract of *S. claviflorum*. This technique was chosen due to its high sensitivity and specificity in separating, identifying, and quantifying volatile and semi-volatile compounds, making it ideal for phytochemical analysis of plant extracts (17). The chromatogram of the ethanolic extract of *S. claviflorum* is presented in Figure 1, while the identified compounds are listed in Table 1. Only compounds with a mass spectral match quality score exceeding 80% were reported, ensuring a reliable match to reference spectra in the mass spectrometry library. The compound's identification was confirmed based on a high-quality spectral match and distinct fragmentation peaks corresponding to those reported in reference databases.

The analysis revealed that the most abundant volatile compound in the extract was 2,4-di-tert-butylphenol, constituting 17.71% of the total composition. This was followed by 1,2-cyclopentanedione, which accounted for 6.69%. Other minor constituents included 4-ethyl-benzaldehyde (1.59%) and methoxyphenyl-oxime (1.52%). These compounds have been associated with various bioactive properties, supporting the potential therapeutic applications of the extract. 2,4-di-tert-butylphenol is particularly noteworthy for its broad spectrum of biological activities, including strong antibacterial effects against pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (14). Additionally, it has demonstrated significant antioxidant and antifungal activities, being effective against fungi such as *Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium chrysogenum* (15). The presence of 1,2-cyclopentanedione, known for its antioxidant properties found in the leaves and fruits of

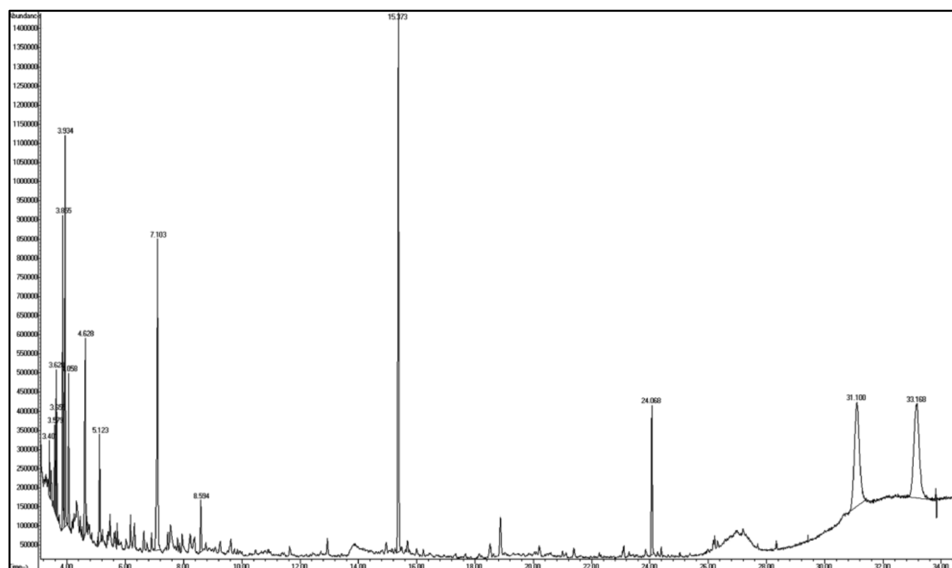

Figure 1: GC-MS chromatogram of *S. claviflorum* ethanolic extract.

Table 1: The volatile compounds identified from the GC-MS chromatogram of *S. claviflorum* ethanolic extract

RT (min)	Compounds	Compound group	Area (%)
3.6548	Methoxyphenyl-oxime	Alkaloid	1.517
3.9335	1,2-cyclopentanedione	Ketone	6.693
8.5938	4-ethylbenzaldehyde	Aldehyde	1.591
15.3735	2,4-di-tert-butylphenol	Phenol	17.706

Trichosanthesis dioica, and wood vinegars of various woody plants, further underscores the extract's antioxidant potential (18,19). Moreover, methoxyphenyl-oxime has been identified for its antibacterial activity against several Gram-negative bacteria, including *Escherichia coli*, *P. aeruginosa*, and *Bacillus subtilis* (20,21).

3.2 Mushroom tyrosinase inhibitory activity

Tyrosinase is a key enzyme involved in melanin production, catalyzing two critical reactions: the hydroxylation of L-tyrosine to L-Dopa, and the subsequent oxidation of L-Dopa to dopaquinone (21). Dopaquinone then undergoes further reactions to produce melanin, the pigment responsible for skin colour (5). Overactivity of tyrosinase leads to excessive melanin production, contributing to conditions such as hyperpigmentation, melasma, and age spots (3). Thus, tyrosinase inhibitors are commonly explored for therapeutic and cosmetic applications aimed at managing pigmentation issues. To assess the potential of *S. claviflorum* ethanolic extract as a tyrosinase inhibitor, the mushroom tyrosinase inhibitory assay was employed. This assay is widely used due to its simplicity, cost-effectiveness, and ability to simulate the inhibition of human

tyrosinase (21). While the enzyme used in the assay is derived from mushrooms, it closely mimics human tyrosinase in its catalytic activity, making it a reliable model for screening potential inhibitors (6).

The results, as shown in Table 2, demonstrate that *S. claviflorum* ethanolic extract exhibited notable tyrosinase inhibitory activity, with an inhibition rate of 70.64 ± 0.22% and an IC_{50} value of 41.82 ± 1.43 μg/mL. Interestingly, this activity is comparable to that of kojic acid, a well-known synthetic tyrosinase inhibitor, which showed 72.23 ± 0.13% inhibition and an IC_{50} of 38.06 ± 1.02 μg/mL. This close similarity in performance suggests that the ethanolic extract of *S. claviflorum* holds potential as a natural alternative to kojic acid for treating hyperpigmentation. In the broader context of *Syzygium* species, the tyrosinase inhibitory activity of *S. claviflorum* ethanolic extract was less potent than that reported for methanolic extracts of *Syzygium petrinense* (IC_{50}: 25.5 μg/mL) and *Syzygium latifolium* (IC_{50}: 32.2 μg/mL) (22). However, it was significantly more effective than *Syzygium glomeratum* methanolic extract, which had an IC_{50} of 459.7 μg/mL (23). This variability among Syzygium species highlights the influence of different extraction methods and phytochemical profiles on tyrosinase inhibition.

3.3 Antioxidant activities

The analysis of the antioxidant properties of the *S. claviflorum* ethanolic extract was outlined in Table 3. The DPPH free radical scavenging assay, a commonly used method for assessing the free radical scavenging capacities of antioxidants and their ability as hydrogen donors (25), was conducted. Furthermore, the FRAP assay, which is based on the ability of antioxidants to donate electrons, resulting in the reduction of the Fe^{3+} TPTZ complex to the blue-coloured Fe^{2+} form (26), was also performed. The study indicated that the *S. claviflorum* ethanolic extract demonstrated a DPPH free radical scavenging activity of 55.32 ± 1.32% with an IC_{50} of 212.46 ± 1.73 μg/mL. Additionally, the extract showed a FRAP value of 296.72 ± 1.12 μM Fe^{2+}. These results indicated that the extract had noticeable antioxidant activity against DPPH radicals and ferric antioxidant reducing power. However, the extract exhibited a lower antioxidant activity compared to Trolox.

Table 2: Anti-tyrosinase activity of *S. claviflorum* ethanolic extract

Samples	Tyrosinase inhibition (%)	IC_{50} values (μg/mL)
S. claviflorum	70.64 ± 0.22[a]	41.82 ± 1.43[a]
Kojic acid	72.23 ± 0.13[a]	38.06 ± 1.02[a]

Values are expressed as mean ± standard deviation of three replicates. No significant differences were observed between treatments, as indicated by the same superscript letter ([a]) in the same column ($p > 0.05$).

Table 3: Antioxidant activities of S. claviflorum ethanolic extract as measured by DPPH and FRAP assays.

Samples	DPPH inhibition (%)	IC_{50} DPPH (μg/mL)	FRAP values (μM Fe^{2+})
S. claviflorum	55.32 ± 1.32[a]	212.46 ± 1.73[b]	296.72 ± 1.12[a]
Trolox	92.18 ± 1.17[b]	33.86 ± 1.52[a]	6715.42 ± 0.18[b]

Values are expressed as mean ± standard deviation of three replicates. The mean in the same column with different superscript letters ([a,b]) differ significantly ($p < 0.05$).

Trolox displayed significantly higher ($p < 0.05$) radical scavenging activity, with an IC_{50} of 33.86 ± 1.52 μg/mL and a FRAP value of 6715.42 ± 0.18 μM Fe^{2+}. These findings are consistent with previous research by Ahmed *et al.* (2021) (27), which reported lower antioxidant activity of *S. claviflorum* fruit extract compared to its seed extract.

4.0 Conclusion

The research findings revealed the presence of various volatile compounds in the ethanolic fruit extract of *S. claviflorum*, including 2,4-di-tert-butylphenol, 1,2-cyclopentanedione, 4-ethylbenzaldehyde and methoxyphenyl-oxime. Although the extract exhibited moderate antioxidant capacity, it demonstrated significant tyrosinase inhibitory activity, similar to that of kojic acid, a well-known tyrosinase inhibitor. These results indicate the potential of the extract as a natural alternative for managing hyperpigmentation and related skin conditions. Nonetheless, further research is needed to identify and understand the specific bioactive components responsible for this activity.

Authorship contribution statement

MAA: Data analysis, Methodology, Formal analysis, Writing – original draft. **AHAHY**: Data analysis, Methodology. **MP**: Data analysis, Methodology. **RMZ**: Supervision, Funding acquisition, Writing – review & editing.

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Conflict of Interest

The authors declared that they have no conflicts of interest to disclose.

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