

# Evaluation of Antioxidant, Antifungal, and Antibiofilm Activities of Four Malaysian *Syzygium* Species

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**Abstract:** The current work aims to evaluate the antioxidant, antifungal, and antibiofilm activities of four Malaysian *Syzygium* species (*S. dyerianum*, *S. zeylanicum*, *S. cinereum*, and *S. wrayi*). Evaluation of antioxidant activity was determined using DPPH, whereas antifungal activity was investigated by the microdilution method to determine MIC and MFC. Antibiofilm activity was determined against *Candida* spp. (*C. albicans*, *C. lusitanae*, and *C. auris*). The *S. dyerianum* extract showed the highest antioxidant activity in the DPPH assay (IC<sub>50</sub> value 67.08 µg/mL) and antifungal activity against *C. albicans* 4901 (MIC/MFC value 62.50 µg/mL). In antibiofilm activity, the highest inhibition was observed in *C. auris* biofilm after treatment with *S. cinereum* extract (*I*: 66.29%). These results provide new data on extracts obtained from a native plant, an edible species traditionally used in popular medicine, opening new perspectives for its possible therapeutic applications.

**Keywords:** antibiofilm; antifungal; antioxidant; *Candida* spp.; Myrtaceae; *Syzygium*.

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## 1. Introduction

The Myrtaceae family encompasses nearly 55,000 species, which are classified into two subfamilies, 17 tribes, and 142 genera. These species consist of evergreen shrubs or woody trees and are predominantly found in North Africa, South America, along the Mediterranean, and various regions of Asia [1]. Most plants in the family are easily recognized for their attractive, glossy green leaves and vibrant-colored flowers, which make them highly sought after as ornamental plants. The leaves of young plants are round and firmly attached to the branches, while mature plants have longer and narrower leaves. One distinguishing feature of this family is the presence of oil glands in the leaves that release a pleasant scent when crushed. The flowers of Myrtaceae are typically bisexual, have multiple petals, are often arranged in opposite pairs, and have a complete margin. The fruits of these plants can be in the form of

capsules, drupaceous berries, or drupes, and usually contain a large number of seeds. Additional characteristics include an ovary that is partially or fully inferior, numerous stamens with vibrant colors, internal phloem, and vestured pits on the xylem vessels [2]. Myrtaceae is widely recognized for its significant economic contributions and diverse range of applications, encompassing medicinal properties, nutritional fruits, perfumery, and spice production [3].

Numerous studies have been conducted to explore the therapeutic potential of *Syzygium* essential oil. Notably, these studies have revealed antimicrobial properties, demonstrating their efficacy against various bacteria and fungi. Additionally, *Syzygium* essential oil has shown promise as a natural analgesic and anti-inflammatory agent, thus suggesting its potential as a pain reliever. Moreover, the insecticidal properties make it a valuable tool for pest control. The collective findings from studies on *Syzygium* essential oil highlight its diverse range of health benefits and practical applications [4-7].

*Candida* species (*Candida* spp.) are fungi belonging to the yeast group. They are oval, elliptical, or cylindrical in shape, unicellular or bicellular, measuring  $2-3 \times 3-7 \mu\text{m}$ , with a double-layered cell wall [8]. *Candida* spp. are considered opportunistic fungal pathogens capable of causing infections in humans that range from mild conditions, such as oral or vaginal candidiasis, to severe, life-threatening infections, such as bloodstream infections (candidemia). These infections are common in immunocompromised individuals, including patients with HIV/AIDS, cancer, or those undergoing organ transplants. Additionally, *Candida* biofilms are particularly resistant to most antifungal therapies, complicating treatment and often leading to recurrent infections. The rising incidence of antifungal resistance has driven the search for new therapeutic agents, including those from natural sources [9]. Essential oils and plant extracts are increasingly being explored as potential solutions due to their promising antifungal properties.

Earlier reports demonstrated the antioxidant and antifungal activities of several *Syzygium* oils, including *S. cumini*, *S. alternifolium*, *S. samarangense*, *S. caryophyllatum*, *S. densiflorum*, and *S. paniculatum* [10]. The leaf oil of *S. cumini* showed significant DPPH radical scavenging ( $\text{IC}_{50}$ : 1.2 mg/mL) and strong antifungal properties against *Aspergillus flavus* (MIC: 0.1 mg/mL) [11]. Besides, *S. aromaticum* oil substantially reduced *Candida* growth kinetics and inhibited multispecies biofilm formation, with biofilm metabolic activity (91.4%) [12]. Furthermore, the alcoholic extracts of *S. zollingerianum*, *S. caryophyllatum*, and *S. caudatilimbum* were found to be radical scavengers with  $\text{IC}_{50}$  values ranging from 0.6  $\mu\text{g/mL}$  to 91.2  $\mu\text{g/mL}$  [13-15]. Moreover, the *S. jambos* butanol leaf extract showed activity against *Pseudomonas aeruginosa* and *Candida albicans* with inhibition zones of 17.5 and 19.0 mm, respectively [16]. Likewise, specific constituents, such as quinic acid isolated from *S. cumini*, have been found to possess antibiofilm activity against *C. tropicalis*, with 68–76% inhibition at 800  $\mu\text{g/mL}$  [17]. Additionally, studies have reported the maximum suppression of biofilm formation by *S. aromaticum* extract against *Streptococcus parasanguinis*, *S. oralis*, and *S. mutans*, with a percentage of 78.18%, 74.88%, and 72.87%, respectively [18]. Based on these findings, it is necessary to investigate other *Syzygium* species to develop standardized extracts for specific medicinal purposes in the future.

Hence, an effort has been made to establish the scientific validity by screening the antioxidant, antifungal, and antibiofilm activities of methanolic leaf extracts from four Malaysian *Syzygium* species (*S. dyerianum*, *S. zeylanicum*, *S. cinereum*, and *S. wrayi*). To the best of our knowledge, this is the first report describing these activities in the species.

## 2. Materials and Methods

### 2.1. Plant materials.

Four *Syzygium* species were collected from Fraser Hill, Pahang, Malaysia, in January 2023 and identified by Shamsul Khamis. The voucher specimens were deposited at the UKMB Herbarium, UKM. Table 1 shows the details of these species.

Table 1. List of *Syzygium* species.

Name	Local Name	Voucher no.	Yield (w/w)	Medicinal uses
<i>S. dyerianum</i>	Ubai Samak	SA-03-60	0.73	Traditionally used as an aphrodisiac herbal medicine [19].
<i>S. zeylanicum</i>	Kelat Nenasi	SA-03-50	0.65	Traditionally used to treat joint pain, headaches, arthritis, and fever [20].
<i>S. cinereum</i>	Jambu air gunung	SA-02-51	0.51	Agroforestry [21].
<i>S. wrayi</i>	Wray's satinash	SA-01-74	0.43	Ornamental plant [22].

### 2.2. Plant extraction.

The dried and powdered leaves (100 g) of the above-mentioned *Syzygium* species were subjected to cold extraction using methanol as the solvent. The extracts were filtered, and the solvent was removed under vacuum using a rotary evaporator (Eyela, Japan). The extracts were obtained and kept in the freezer until the experimental practices.

### 2.3. Solvents and chemicals.

Analytical grade methanol was purchased from Merck (Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), ascorbic acid, chlorohexidine, crystal violet solution, and phosphate buffer saline (PBS) were purchased from Sigma-Aldrich (USA). Sabouraud dextrose broth (SDB) and Sabouraud dextrose agar (SDA) were purchased from Difco (USA).

### 2.4. Antioxidant activity.

The free radical scavenging activity was evaluated using the DPPH assay with minor modifications [23]. Stock solutions of each sample (1 mg/mL in methanol) were serially diluted to obtain concentrations ranging from 25 to 200 µg/mL. A methanolic solution of DPPH (1.11 mg/50 mL) was freshly prepared, and its initial absorbance was measured immediately at 0 min as the control. Subsequently, 75 µL of the DPPH solution (0.1 µM) was mixed with 225 µL of each sample solution in a 96-well microplate, and the mixture was incubated in the dark at room temperature for 30 min. The absorbance was then recorded at 515 nm using an ELX-500 UV microplate reader (Bio-Tek, Winooski, VT). The percentage of DPPH radical scavenging was determined by comparing the absorbance of the control reaction (containing all reagents except the test sample) with that of the sample-treated reaction, and the reduction in absorbance was taken as an indicator of antioxidant activity. Ascorbic acid and butylated hydroxytoluene (BHT) were used as reference standards. The concentration required to inhibit 50% of the DPPH radicals (IC<sub>50</sub>) was obtained from the dose-response curve generated by plotting scavenging percentages against sample concentrations. All measurements were performed in triplicate, and results were expressed as mean ± SD.

### 2.5. Antifungal activity.

Four strains of *Candida* species were used: *C. albicans* 4901, *C. albicans* ECE1 mutant, *C. lusitanae*, and *C. auris*. All strains were kept in glycerol (25%) in a -80°C freezer. The pathogens were grown in Sabouraud dextrose broth (SDB) and incubated at 37°C for 24 h. Then, the turbidity of the suspension was verified by measuring the optical density (OD) at 620 nm with the spectrophotometer. Proper dilutions were performed to obtain an absorbance of 0.008-0.10, corresponding to a 0.5 McFarland standard. To determine the minimal inhibitory concentration (MIC), the microdilution method was used in this study. Serial double-fold dilutions were carried out in a 96-well plate. The wells are filled with 120 µL of SDB broth containing *Candida* spp. in one well plate. Then, 60 µL of extract was transferred to the first well. A three-fold serial dilution was formed. The microplate was incubated at 37°C for 24 h. The growth in each well was compared with that of the growth control well. Chlorohexidine 0.12% was used as a positive control, and wells without antifungal agents served as the negative control. MICs were visually determined as the lowest extract concentration that produced no visible growth. Each experiment was performed in triplicate. The minimum fungicidal concentration (MFC) was determined immediately after the MIC test. MFC test was performed by culturing 10 µL (in triplicate) from the wells showing no visible growth onto Sabouraud dextrose agar (SDA) plates in order to reconfirm the inhibition of fungal growth. MFC was considered the lowest concentration of the test substance in which no microbial growth was observed after the incubation period (37°C for 24 h) [24].

### 2.6. Antibiofilm activity.

The antibiofilm of *Syzygium* extracts was analysed at a concentration of 500 µg/mL in 96-well plates. Firstly, streak diluted cultures of four *Candida* spp. (*C. albicans* 4901, *C. albicans* ECE1 mutant, *C. lusitanae*, and *C. auris*) were grown on SDB for 24 h at 37°C, and several single colonies were resuspended in RPMI-1640 and standardized to a final cell density of 10<sup>6</sup> cells/mL in separate sterile 2 mL Eppendorf tubes, resulting in an absorbance of 0.5 at 620 nm (OD<sub>620nm</sub>). The suspensions were mixed thoroughly using a vortex mixer for 30 seconds. Subsequently, 200 µL of each suspension containing 2×10<sup>5</sup> cells (*Candida* spp.) of the initial inoculum were pipetted into each well of a sterile 96-well plate (Nunc, Denmark) without and with *Syzygium* extract. Chlorohexidine 0.12% and antifungal agent-free wells were used as positive and negative controls, respectively. Finally, the 96-well plate was incubated aerobically at 37°C for 72 h, and the medium was replenished aseptically every 24 h. After incubation, biofilm biomass was quantified using crystal violet (CV) staining as previously reported [25]. Initially, the wells containing biofilms were washed twice with sterile phosphate-buffered saline (PBS) to remove non-adherent cells. Then, the biofilms were fixed by adding 200 µL of methanol and incubating for 15 min at 25°C. After the supernatant was discarded, the plate was air-dried for 45 min. Next, 200 µL of 0.1% (w/v) CV solution was added to each well and incubated for 20 min at 25°C. The plate was washed gently twice using sterile distilled water to remove the unbound stain. The biofilms were then destained with 200 µL of 33% (v/v) acetic acid for 5 min at room temperature. Finally, 100 µL of the acetic acid solution was transferred to a new sterile 96-well plate, and the absorbance was measured at OD<sub>620nm</sub> using a microtiter plate reader (Tecan NanoQuant Infinite M200, CA). Each assay was performed three times, and the mean absorbance values were used to assess biofilm inhibition.

2.7. Statistical analysis.

Data obtained from the biological activities are expressed as mean  $\pm$ SD of triplicate. The statistical analyses were carried out by employing one-way ANOVA. A statistical package (SPSS version 11.0) was used for the data analysis.

3. Results and Discussion

Free radicals are reactive molecules involved in many physiological processes and have been associated with many diseases, such as cancer, arthritis, and liver injury. The DPPH assay aims to evaluate the hydrogen-atom or electron-donating capacity of extracts toward the stable radical DPPH in solution. This assay measures the capacity of the extract to scavenge free radicals in solution. The DPPH radical solution exhibits a deep violet due to a strong absorption band at 516 nm. Upon reduction with a hydrogen donor, the solution changes from deep violet to pale yellow or colourless [26]. The results for antioxidant activity are summarized in Table 2. Figure 1 illustrates a significant ( $p < 0.05$ ) decrease in the concentration of DPPH radicals.

Table 2. Antioxidant activity of *Syzygium* extracts.

Conc. ( $\mu\text{g/mL}$ )	% DPPH Inhibition					
	SDL	SZL	SCL	SWL	AA	BHT
200	89.28 $\pm$ 0.01	88.33 $\pm$ 0.01	88.33 $\pm$ 0.01	28.94 $\pm$ 0.08	84.90 $\pm$ 0.54	83.70 $\pm$ 0.03
150	88.22 $\pm$ 0.03	77.50 $\pm$ 0.05	84.27 $\pm$ 0.02	27.31 $\pm$ 0.03	79.98 $\pm$ 0.09	59.68 $\pm$ 0.01
100	75.58 $\pm$ 0.03	58.74 $\pm$ 0.01	62.25 $\pm$ 0.04	23.39 $\pm$ 0.04	68.54 $\pm$ 0.24	54.96 $\pm$ 0.01
50	41.18 $\pm$ 0.02	32.51 $\pm$ 0.02	37.78 $\pm$ 0.01	15.70 $\pm$ 0.03	56.33 $\pm$ 0.66	34.72 $\pm$ 0.13
25	26.71 $\pm$ 0.01	21.76 $\pm$ 0.02	24.85 $\pm$ 0.12	6.61 $\pm$ 0.06	37.37 $\pm$ 0.22	30.43 $\pm$ 0.25
IC <sub>50</sub>	67.08	90.32	80.07	350.69	44.58	95.82

Data represent mean  $\pm$ SD of three independent experiments; SDL – *S. dyerianum*; SZL – *S. zeylanicum*; SCL – *S. cinereum*; SWL – *S. wrayi*; AA – Ascorbic acid; BHT - butylated hydroxytoluene

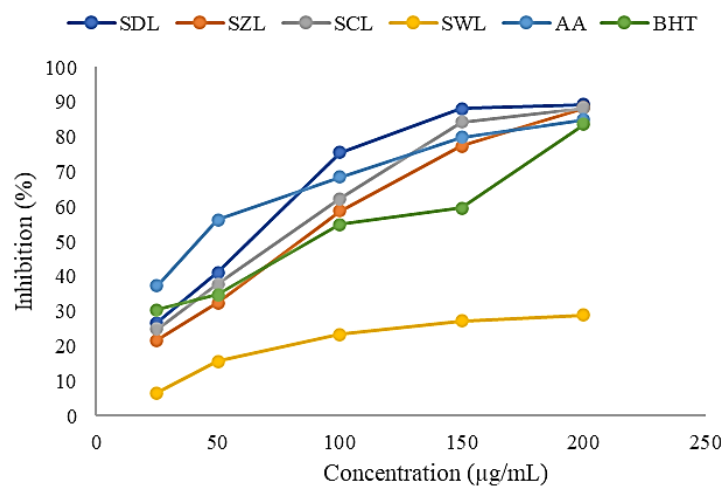


Figure 1. DPPH radical scavenging of *Syzygium* extracts.

The maximum scavenging activity was observed for *S. dyerianum* extract ( $I$ : 89.28%) at 200  $\mu\text{g/mL}$ , whereas the control, ascorbic acid, and BHT demonstrated scavenging activities of 84.90% and 83.70% at the same concentration. Meanwhile, the concentration of the extract, determined as the initial DPPH concentration at 50% ( $\text{IC}_{50}$ ), ranged from 67.08 to 350.69  $\mu\text{g/mL}$ . In addition, the *S. dyerianum* and *S. cinereum* extracts were found to be good radical scavengers with  $\text{IC}_{50}$  values of 67.08 and 80.07  $\mu\text{g/mL}$ , respectively. However, the values were lower than those of the positive control, ascorbic acid (44.58  $\mu\text{g/mL}$ ) and BHT (95.82  $\mu\text{g/mL}$ ).

Meanwhile, *S. wrayi* extract showed four times the scavenging activity of the control, with an IC<sub>50</sub> value of 350.69 µg/mL.

Antioxidants are electron donors with low molecular weights that can prevent oxidation reactions from progressing by scavenging radicals. Herbal plant extract contains flavonoid phenolic compounds, which are known as plant antioxidant agents due to their ability as reducing agents, hydrogen donors, and singlet oxygen scavengers. The effectiveness of antioxidants in suppressing the formation of free radicals is directly proportional to the combined value of phenols and flavonoids contained in the plant extract, as it reflects their ability to donate electrons [27]. Previous studies have demonstrated that *Syzygium* extracts, such as those from *S. cumini* (IC<sub>50</sub> value of 81.40 µg/mL for the fruit extract) and *S. samarangense* (IC<sub>50</sub> values of 72.90 µg/mL for the pulp extract and 78.40 µg/mL for the seed extract), exhibited significant activity in the DPPH assay [28,29]. Additionally, the stem bark extract of *S. filiforme* showed an IC<sub>50</sub> value of 44.7 µg/mL in the DPPH radical scavenging assay [30].

*Candida* is a type of microorganism that naturally exists in the human body, particularly in the mouth, digestive system, and vagina. *Candida* can cause infections in individuals with weakened immune systems, such as those with HIV. One notable feature of *Candida* is its ability to invade oral tissues by forming hyphae, which can cause inflammation [31]. Most *Candida* species are opportunistic pathogens that can form biofilms, which increases their resistance to antifungal therapies and to the host immune response [32]. Medicinal plants and their chemical components offer an alternative for developing new antifungal drugs, given their broad effects on cellular viability, cell communication, and metabolism. In this work, the evaluation of antifungal and antibiofilm potential towards four *Candida* spp., which are *C. albicans* 4901, *C. albicans* ECE1 mutant, *C. lusitanae*, and *C. auris*. Concentrations ranging from 7.8 to 1,000 µg/mL, obtained by dilution, were used to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Table 3 shows the MIC and MFC results obtained from the leaf extracts of *Syzygium* species.

**Table 3.** Antifungal activity of *Syzygium* extracts.

Sample	Fungal strains							
	<i>C. albicans</i> 4901		<i>C. albicans</i> ECE1 mutant		<i>C. lusitanae</i>		<i>C. auris</i>	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
SDL	NA	NA	NA	NA	NA	NA	NA	NA
SZL	NA	NA	NA	NA	NA	NA	NA	NA
SCL	62.5	62.5	125	125	250	250	125	125
SWL	250	250	500	500	250	250	250	250

SDL – *S. dyerianum*; SZL – *S. zeylanicum*; SCL – *S. cinereum*; SWL – *S. wrayi*; NA – not active; MIC - minimal inhibitory concentration; MFC - minimum fungicidal concentration; MIC and MFC in µg/mL

The *S. dyerianum* extract showed strong activity against *C. albicans* 4901 with MIC and MFC values of 62.5 µg/mL. However, other extracts displayed moderate activity, with MICs and MFCs ranging from 125 to 500 µg/mL against *Candida* sp. The antifungal activity of plant extracts against *Candida* is attributed to various active compounds, including phenolic compounds, hydrolysable tannins, phenolic amides, and flavonoids. These compounds interact with the fungal cell membrane, potentially disrupting it or integrating into the cell wall and DNA [33]. In another study on *Syzygium* extracts, the methanolic clove extract of *S. zeylanicum* exhibited noble activity against *C. albicans* (MIC value of 31.0 µg/mL) [34], while the dichloromethane stem bark extract of *S. samarangense* showed activity against *C. albicans* with an inhibition zone of 15 mm [35].

Antibiofilm activity is the ability of extracts to reduce biofilm-forming capacity at subinhibitory concentrations. In this study, the antibiofilm effect of the *Syzygium* extracts was observed at 500 µg/mL. The effects of *Syzygium* extracts on the percentage of inhibition of biofilm formation are tabulated in Table 4.

**Table 4.** Antibiofilm activity of *Syzygium* extracts.

Extract (500 µg/mL)	Fungal strains			
	<i>C. albicans</i> 4901	<i>C. albicans</i> ECE1 mutant	<i>C. lusitanae</i>	<i>C. auris</i>
SDL	30.19 ± 1.33	41.41 ± 2.01	66.82 ± 3.61	59.43 ± 2.74
SZL	47.17 ± 3.35	44.44 ± 2.04	59.81 ± 1.90	60.00 ± 2.71
SCL	29.25 ± 2.71	33.33 ± 1.70	66.82 ± 4.00	66.29 ± 2.91
SWL	32.08 ± 2.73	41.41 ± 1.91	69.63 ± 4.11	65.19 ± 3.00

SDL – *S. dyerianum*; SZL – *S. zeylanicum*; SCL – *S. cinereum*; SWL – *S. wrayi*.

The *Syzygium* extracts showed varying degrees of activity in preventing attachment. The extracts effectively prevented biofilm attachment against *C. lusitanae* and *C. auris* (>50%); however, their inhibitory effect was less than 50% against *C. albicans* 4901 and *C. albicans* ECE1 mutants in the biofilm development assay. The highest inhibition of metabolic activity was observed in the *C. auris* biofilm after treatment with *S. cinereum*, resulting in 66.29% inhibition. According to [36], percentage inhibition values between 0 and 100% indicate biofilm inhibition, while growth enhancement is reflected by values below 0%. Above the 50% inhibition mark, the activity is considered good; below 49%, it is poor. This remarkable ability of the plant extracts to interfere with the initial stage of biofilm formation of fungi can be attributed to their interference with forces, such as Brownian sedimentation, Lifshitz-Van der Waals, and electrostatic interactions. These forces play a crucial role in facilitating bacterial deposition and adhesion to surfaces. Moreover, since certain organic and inorganic molecules and other nutrients are important for cell growth and, hence, cell adhesion, it is possible that plant extracts may reduce the availability of these nutrients. The active plant extracts show promise in reducing colonization on various epithelial surfaces of the body, thereby potentially preventing infections [37]. Previously, the glycolid crude extract of *S. cumini* was found to prevent the attachment of *Streptococcus aureus*, a known biofilm-forming bacterium on oral surfaces [38]. In another study, acetone leaf extracts of *S. aromaticum* contained several active compounds, including sesquiterpenes, monoterpenes, hydrocarbons, and phenolic compounds, which resulted in greater reductions in biofilm formation by *Pseudomonas aeruginosa* and *Bacillus cereus* [38]. Moreover, studies reported that the extracts of *S. legatti* and *S. gerrardii* inhibited biofilm formation of *Streptococcus aureus* by 86.00% and 68.00%, respectively [39].

The antioxidant, antifungal, and antibiofilm activities of *Syzygium* species are closely interconnected. The antioxidant capacity of these plants, primarily due to their phenolic compounds, helps neutralize free radicals and reduce oxidative stress. Oxidative stress plays a key role in fungal pathogenesis and biofilm formation, as fungi such as *Candida* spp. utilize reactive oxygen species (ROS) for biofilm development and cell survival [40]. By lowering ROS levels, *Syzygium* extracts may inhibit these processes, reducing both fungal growth and biofilm formation. The antibiofilm results suggest that antioxidant-rich extracts can disrupt biofilms by limiting nutrient availability or interfering with the adhesion mechanisms, which are crucial in the early stages of biofilm development [41]. Ultimately, the synergy among antioxidant, antifungal, and antibiofilm activities highlights the potential of *Syzygium* extracts as promising natural therapeutic agents with applications in both medicine and industry.

## 4. Conclusions

In summary, this paper presents the first systematic study on the antioxidant, antifungal, and antibiofilm activities of Malaysian *Syzygium* extracts, emphasizing their potential as sources of natural bioactive compounds. *S. dyerianum* exhibited the highest antioxidant and antifungal activities, particularly against *Candida albicans*. Meanwhile, *S. cinereum* demonstrated significant antibiofilm activity against *Candida auris*. These findings underscore the potential of *Syzygium* species as valuable candidates for the development of new therapeutic approaches. Therefore, further studies, including bioassay-guided isolation to identify active compounds and comprehensive pharmacological safety evaluations, are warranted to explore their potential as new therapeutic drugs or drug leads.

## Author Contributions

Conceptualization, F.A.M.R. and W.M.N.H.W.S.; methodology, M.H.A.; validation, S.K., F.K., and F.E.; formal analysis, A.S.S.; investigation, F.A.M.R.; writing original draft preparation, F.A.M.R.; writing - review and editing, W.M.N.H.W.S.; supervision, W.M.N.H.W.S.; funding acquisition, M.H.A. All authors have read and agreed to the published version of the manuscript.

## Institutional Review Board Statement

Not applicable.

## Informed Consent Statement

Not applicable.

## Data Availability Statement

The data supporting the findings of this study are available in the article.

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## Conflicts of Interest

The authors declare no conflict of interest.

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