Deciphering the Bioactive Potential of Four Malaysian *Litsea* Species for Antioxidant, Antifungal, and Antibiofilm Activities

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Summary

This study evaluates the antioxidant, antifungal, and antibiofilm activities of methanolic leaf extracts from four *Litsea* species (*L. glauca, L. fulva, L. rubicunda,* and *L. tomentosa*), highlighting their potential as natural therapeutic agents. The antioxidant activity was assessed using the β-carotene/linoleic acid bleaching assay, DPPH radical scavenging assay, and total phenolic content (TPC) determination. Among the species, *L. glauca* exhibited the highest antioxidant activity, with an 85.2% inhibition of β-carotene bleaching, a DPPH IC_{50} value of 126.2 µg/mL, and the highest TPC of 130.1 mg GA·g^{-1}, demonstrating a strong correlation between phenolic content and antioxidant efficacy. Antifungal activity was evaluated against *Candida albicans*, *Candida lusitanae*, and *Candida auris*, with *L. tomentosa* showing the strongest activity, achieving MIC and MFC values as low as 62.5 µg/mL against *C. albicans*. Antibiofilm activity was also notable, particularly for *L. tomentosa* and *L. rubicunda*, which significantly inhibited biofilm formation in *C. lusitanae* (70.0% and 65.5%, respectively) and *C. auris* (67.2% and 65.8%, respectively). These findings underscore the potential of *Litsea* species as sources of bioactive compounds for medicinal and industrial applications, warranting further research to isolate active constituents, elucidate mechanisms of action, and validate efficacy *in vivo*.

Key words

Lauraceae, *Litsea*, antibiofilm, antifungal, antioxidant

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Introduction

Plant extracts are rich in bioactive compounds, such as polyphenols, flavonoids, alkaloids, and terpenoids, which exhibit antioxidant, antifungal, and antibiofilm activities. These interconnected properties make plant extracts highly valuable for addressing health challenges, including oxidative stress, fungal infections, and biofilm-associated complications (Kowalczewski and Zembrzuska, 2023). The antioxidant activity of plant extracts is primarily attributed to compounds that neutralize reactive oxygen species (ROS), protecting cellular components like lipids, proteins, and DNA from oxidative damage (Kasote et al., 2015). Phenolics and flavonoids, for instance, are effective due to their ability to donate electrons or hydrogen atoms, stabilizing free radicals. This protective effect not only shields host tissues from oxidative stress but also creates an unfavorable environment for fungal pathogens and biofilm formation (Tungmunnithum et al., 2018).

The antifungal activity of plant extracts arises from compounds such as alkaloids, saponins, and essential oils, which disrupt fungal cell membranes, inhibit enzymatic processes, or prevent spore germination. Fungal infections are often exacerbated by oxidative stress, and the antioxidants present in plant extracts can mitigate this stress, reducing fungal growth and enhancing the efficacy of antifungal agents (Zhou et al., 2023). Additionally, many plant extracts demonstrate antibiofilm activity by inhibiting biofilm formation or disrupting established biofilms, which are highly resistant to conventional treatments. Bioactive compounds like terpenoids and flavonoids interfere with microbial quorum-sensing pathways, critical for biofilm development. Antioxidants further complement this activity by reducing ROS levels, which are often involved in signaling pathways that promote biofilm formation and resilience (Silva et al., 2023).

The synergy among these activities is especially significant in plant extracts. Antioxidants not only protect host cells from oxidative damage caused by fungal infections but also weaken the oxidative defenses of fungi, making them more susceptible to antifungal agents (Kaur et al., 2021). Similarly, antioxidants disrupt ROS-mediated signaling pathways, inhibiting biofilm initiation and reducing the structural integrity of established biofilms. Meanwhile, antifungal compounds target fungal cells within biofilms, while antibiofilm compounds weaken the biofilm matrix, enhancing the penetration and effectiveness of antifungal agents (Delattin et al., 2014). Overall, the multifunctional bioactivities of plant extracts neutralizing oxidative stress, inhibiting fungal growth, and disrupting biofilms highlight their potential as natural therapeutic agents for managing oxidative damage, fungal infections, and biofilm-related complications.

The genus *Litsea* is distributed widely across tropical and subtropical regions of Asia, Australia, Africa, and the Americas. It is particularly abundant in Southeast Asia, including countries like China, India, and Indonesia. Species within *Litsea* vary in size, ranging from small shrubs to large trees, and are often characterized by their glossy, evergreen leaves and small, often inconspicuous flowers (Li et al., 2024). Many species of *Litsea* produce essential oils, particularly from their leaves, which are rich in terpenoid compounds such as limonene, eucalyptol, and citronellal. These oils are used in various industries, including aromatherapy, perfumery, and food flavoring. *Litsea* species are also of interest for their potential medicinal properties. Several species, such as *L. cubeba*, have been utilized in traditional medicine for their antimicrobial, anti-inflammatory, analgesic, and digestive properties (Kong et al., 2015). Research has suggested that extracts from these plants may exhibit antioxidant, anticancer, and hepatoprotective effects. Furthermore, *Litsea* species have been used for timber production in some regions, with the wood being valued for its durability and use in furniture and construction (Wang et al., 2016). The genus also contributes to the ecosystem as a source of food and shelter for various wildlife species. Overall, *Litsea* is an ecologically and economically important genus with diverse applications in medicine, industry, and environmental conservation.

Litsea glauca Siebold is a shrub or small tree that grows to 8 m high. It is native to tropical and subtropical regions of Asia, commonly found in countries such as India, China, Thailand, Laos, and Vietnam, typically growing in lowland and montane forests. This species is an evergreen tree that can reach heights of 10 to 20 meters, with a straight, slender trunk and a diameter of up to 30 cm. The tree has smooth, glossy, dark green leaves that are lance-shaped, and its bark is often grayish or light brown. The leaves and bark are sometimes used in local remedies to treat ailments such as fever, cough, and digestive disorders. The fruit is used as a substitute for pepper, while powdered leaves are used as flavors (Burkill, 1966). Phytochemical analysis of this species revealed the presence of flavonoids and alkyl acids (Nakabayashi, 1953; Nii et al., 1972).

Litsea fulva Fern.-Vill. is a species of tree, native to tropical and subtropical regions of Southeast Asia. This species is commonly found in countries such as China, Vietnam, and Laos, typically growing in montane and lowland forests. *L. fulva* is an evergreen tree, which can reach heights of 20 to 30 meters and has a straight, robust trunk with a diameter of up to 50 cm. The leaves of *L. fulva* are large, leathery, and glossy, with a distinctive dark green color. The tree produces small, yellowish-green flowers that are arranged in panicles, though the flowers are not particularly showy. The fruit of *L. fulva* is a small, fleshy drupe that is typically purple when ripe (Burkill, 1966). On the basis of the available literature, the composition of the essential oils of this species has been reported (Yen et al., 2013).

Litsea rubicunda Kosterm. is a species of tree typically found in the tropical and subtropical regions of Southeast Asia such as India, Myanmar, Thailand, and Malaysia. It is known for its evergreen nature and can grow up to 30 meters tall, with a straight trunk that can reach diameters of 50 cm or more. The tree has glossy, dark green leaves and produces small, yellowish flowers that are not particularly showy but are important for local pollinators. The decoction of the leaves have been reported to treat stomachache and diarrhoea (Burkill, 1966).

Litsea tomentosa B.Heyne ex Wall. is an evergreen tree that can grow to a height of 8 to 27 meters, with a bole that can reach up to 50 cm in diameter and short buttresses that extend up to 1.5 meters high. It is locally known as *medang gambak* in Peninsular Malaysia and mainly found in Southeast Asia including Thailand, Malaysia, Indonesia, Philippines to New Guinea. The tree is harvested from the wild primarily for its wood, which is used locally and traded as a source of 'medang' timber (Burkill, 1966).

Recently, the chemical composition of the essential oils from *L. glauca*, along with *L. fulva* (Salleh et al., 2024a) and *L. rubicunda* (Salleh et al., 2024b) has been reported, providing new insights into the bioactive compounds present in these species. As part of ongoing research into the pharmacologically active compounds found in Malaysian plants, a study was conducted to evaluate the antioxidant, antifungal, and antibiofilm activities of methanolic leaf extracts from four Malaysian *Litsea* species: *L. glauca*, *L. fulva*, *L. rubicunda*, and *L. tomentosa*. To the best of our knowledge, this study is the first to report these specific activities for these *Litsea* species, contributing to the growing body of evidence supporting their potential medicinal applications. The findings from this study offer promising insights into the possible therapeutic benefits of these plants, particularly in combating oxidative stress, fungal infections, and biofilm formation.

Materials and Methods

Plant Materials

Four *Litsea* species were collected in January 2023 from Behrang, Perak, and Fraser Hill, Pahang, Malaysia. The specimens were identified by Shamsul Khamis, and the voucher specimens have been deposited at the UKMB Herbarium, Universiti Kebangsaan Malaysia (UKM). The details of these species are provided in Table 1.

Plant Extraction

The dried and powdered leaves (100 g) of the aforementioned *Litsea* species were subjected to cold extraction using methanol as the solvent. The resulting extracts were filtered, and the solvent was removed under reduced pressure using a rotary evaporator (Eyela, Japan). The final extracts (w/w) were stored in a freezer until required for the experimental procedures.

Solvents and Chemicals

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

Antioxidant Activity

β-Carotene/Linoleic Acid Bleaching Assay

The β-carotene-linoleic acid bleaching assay as described previously was used with minor modifications (Salleh et al., 2016). A mixture of β-carotene and linoleic acid was prepared by adding together of 0.5 mg β-carotene in 1 mL $CHCl_3$, 25 µL linoleic acid and 200 mg Tween 40. The $CHCl_3$ was then completely evaporated under vacuum and 100 mL of oxygenated distilled water was subsequently added to the residue and mixed gently to form a clear yellowish emulsion. The extracts and BHT were individually dissolved in methanol (2 g/L) and 350 µL volumes of each of them were added to 2.5 mL of the above emulsion in test tubes and mixed thoroughly. The test tubes were incubated in a water bath at 50 °C for 2 h, together with a negative control (blank) contained the same volume of methanol. The absorbance values were measured at 470 nm on UV-vis spectrophotometer. Percentage inhibitions (I%) of the extracts were calculated using the following equation:

$$I\% = [\ A_{\beta\text{-carotene after 2 h}} / A_{\text{initial }\beta\text{-carotene}}\ ] \times 100$$

where $A_{\beta\text{-carotene after 2h}}$ assay is the absorbance values of β-carotene after 2 h assay remaining in the samples and $A_{\text{initial }\beta\text{-carotene}}$ is the absorbance value of β-carotene at the beginning of the experiments. All tests were carried out in triplicate and percentage inhibitions were reported as means ± SD of triplicates.

DPPH Free Radical Scavenging Assay

The free radical scavenging activity was measured by the DPPH method with minor modifications (Salleh and Ahmad, 2016). Each sample of stock solution (1 $mg{\cdot}mL^{-1}$ in MeOH) was diluted in various concentrations (200-25 $\mu g{\cdot}mL^{-1}$). The methanolic solution (1.11 $mg{\cdot}50\ mL^{-1}$) of the stable DPPH radical was prepared and measured immediately at 0 min to obtain A_{blank}. Then, 75 µL (0.1 µM) DPPH methanolic solution was added to 225 µL of each sample solution and allowed to incubate in the dark for 30 min at room temperature. Finally, absorbance was measured at 515 nm using ELX-500 UV plate reader (Bio-Tek, Winooski, VT). The recorded optical densities were used to calculate the percentage of DPPH radical scavenging, which is proportional to the antioxidant power of the sample. Ascorbic acid and BHT were used as reference standards. The percentage inhibitions (I%) of DPPH radicals were calculated as follows:

Table 1. List of *Litsea* species

Name	Locality	Voucher no.	Yield (g)
L. glauca	Behrang, Perak (3° 44' 51.612" N 101° 27' 19.9008" E)	SK35-19	0.92
L. fulva		SK37-19	0.85
L. rubicunda	Fraser Hill, Pahang (3.7119° N 101.7366° E)	SB50-52	0.72
L. tomentosa		SA31-58	0.98

$$I(\%) = [A_{blank} - A_{sample} / A_{blank}] \times 100;$$

where A_{blank} is the absorbance value of the control reaction (containing all reagents except the samples) and A_{sample} is the absorbance value of the test samples. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting the inhibition percentages against concentration of the sample. All tests were carried out in 96-well microplate and IC_{50} values were reported as means ± SD triplicates.

Total Phenolic Content (TPC) Assay

TPC of extracts were determined by gallic acid equivalent with minor modifications (Salleh et al., 2015). A sample of stock solution (1 mg·mL^{-1} in MeOH) was diluted to final concentrations of 1000 µg·mL^{-1}. A 0.1 mL aliquot of sample was pipetted into a test tube containing 0.9 mL of MeOH, then 0.05 mL Folin-Ciocalteu's reagent was added, and the flask was thoroughly shaken. After 3 min, 0.5 mL of 5% Na_2CO_3 solution was added and the mixture was allowed to stand for 2h with intermittent shaking. Then, 2.5 mL of MeOH was added and left to stand in the dark for 1h. The absorbance measurements were recorded at 765 nm. The same procedure was repeated for the standard gallic acid solutions. The concentration of total phenolic contents in the extracts was expressed as mg of gallic acid equivalent per gram of sample. Tests were carried out in triplicate and the gallic acid equivalent value was reported as mean ± SD of triplicate.

Antifungal Activity

Four strains of *Candida* species were used: *C. albicans*, *C. lusitanae*, and *C. auris*. All strains were kept in glycerol (25%) at -80 °C freezer. The pathogens were grown in Sabouroud 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 done to get an absorbance value of 0.008-0.10, which corresponds to 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 were 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 positive control, and wells without antifungal agents served as the negative control. MICs were visually determined and defined as the lowest concentration of the extract that produced no visible growth. Each experiment was performed in triplicate. Then, the minimum fungicidal concentration (MFC) was performed right after the MIC test was completed. 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) (Khan et al., 2020).

Antibiofilm Activity

The antibiofilm of *Syzygium* extracts were analysed at concentration 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 give a final cell density of 106 cells/mL, in a separate sterile 2 mL eppendorf tubes that equivalent to an absorbance of 0.5 at 620 nm wavelength (OD_{620nm}). The suspensions were mixed thoroughly using a vortex mixer for 30 sec. Subsequently, 200 µL of each suspension containing 2×10^5 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. Clorohexidine 0.12% and antifungal agent-free wells were used as positive and negative controls, respectively. Finally, the 96-well plate was incubated for 72 h at 37 °C aerobically, and the medium was replenished aseptically every 24 h. After incubation, the biofilm biomass was quantified using crystal violet (CV) staining according to the method previously reported (Arzmi et al., 2016). Initially, the wells containing biofilms were washed twice with sterile phosphate buffer saline (PBS) to remove the 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_{620nm} using a microtiter plate reader (Tecan NanoQuant Infinite M200, CA). Each assay was performed three times and the mean absorbance values were used to measure the inhibition of biofilm formation as follows:

$I(\%) = [$ (mean $OD_{620\ nm}$ of positive control - mean OD_{620nm} of experimental) / mean OD_{620nm} of positive control)] × 100.

Statistical Analysis

Data obtained from the biological activities are expressed as mean ± SD of triplicate. The statistical analyses were carried out by employing one-way ANOVA, using SPSS version 17. The means were compared with Duncan's multiple comparison test (DMCT) and $P < 0.05$ was considered to indicate statistical significance.

Results and Discussion

The antioxidant activity of methanolic leaf extracts from four *Litsea* species (*L. glauca*, *L. fulva*, *L. rubicunda*, and *L. tomentosa*) was evaluated using the β-carotene/linoleic acid bleaching assay, DPPH free radical scavenging assay, and total phenolic content (TPC) determination. The results are presented in Table 2. β-Carotene/linoleic acid bleaching assay measures the ability of the extracts to inhibit oxidative degradation of β-carotene. Among the four *Litsea* extracts, *L. glauca* exhibited the highest inhibition of β-carotene bleaching, with 85.2 ± 0.2%, indicating strong antioxidant activity.

Table 2. Antioxidant activity of *Litsea* extracts

Extract	β-Carotene/linoleic acid bleaching (I%)	DPPH IC_{50} (μg·mL^{-1})	Total phenolic content (mg GA·g^{-1})
L. glauca	85.2 ± 0.2^{a}	126.2	130.1 ± 0.1^{ab}
L. fulva	70.3 ± 0.1^{ab}	159.4	54.4 ± 0.2^{a}
L. rubicunda	74.2 ± 0.1^{ab}	196.6	79.6 ± 0.2^{ab}
L. tomentosa	72.0 ± 0.3^{a}	178.5	72.0 ± 0.1^{a}
Ascorbic acid	125.5 ± 0.2^{ab}	32.4	ND

Note: Data represent mean ± SD of three independent experiments; ND – not determines. The means were compared with Duncan's multiple comparison test (DMCT) at $P < 0.05$, the means with same small letter within the same column are not significantly different.

The remaining species, *L. fulva* (70.3 ± 0.1%), *L. rubicunda* (74.2 ± 0.1%), and *L. tomentosa* (72.0 ± 0.3%), demonstrated moderate antioxidant activity in this assay. The DPPH assay evaluates the IC_{50} values, representing the concentration required to reduce the DPPH radical by 50%. *L. glauca* showed the strongest radical scavenging activity with an IC_{50} value of 126.2 μg·mL^{-1}, followed by *L. fulva* (159.4 μg·mL^{-1}), *L. rubicunda* (196.6 μg·mL^{-1}), and *L. tomentosa* (178.5 μg·mL^{-1}). However, ascorbic acid, the positive control, exhibited a significantly lower IC_{50} value of 32.4 μg·mL^{-1}, underscoring its superior scavenging activity compared to the extracts. Meawnhile, the TPC of the extracts was determined and expressed as milligrams of gallic acid equivalent per gram (mg GA·g^{-1}). *L. glauca* had the highest TPC (130.1 ± 0.1 mg GA/g), consistent with its superior performance in both antioxidant assays. The other extracts exhibited lower TPC values, with *L. fulva*, *L. rubicunda*, and *L. tomentosa* recording 54.4 ± 0.2, 79.6 ± 0.2, and 72.0 ± 0.1 mg GA·g^{-1}, respectively.

The findings reveal a positive correlation between TPC and antioxidant activity, as demonstrated by the superior performance of *L. glauca* across all antioxidant assays. Phenolic compounds are well-known contributors to antioxidant activity due to their ability to donate hydrogen atoms or electrons, stabilizing free radicals (Kumar et al., 2019). The high TPC of *L. glauca* likely accounts for its strong inhibition of β-carotene bleaching and effective scavenging of DPPH radicals. While *L. fulva*, *L. rubicunda*, and *L. tomentosa* demonstrated moderate antioxidant activity, their lower phenolic content suggests a reduced ability to neutralize oxidative agents. These variations highlight the diverse chemical profiles of *Litsea* species, which may depend on factors such as habitat, extraction method, or specific phytochemical composition (Altemimi et al., 2017). Comparatively, ascorbic acid showed significantly stronger activity in both assays, serving as a benchmark for evaluating the antioxidant potential of the extracts. Despite this, the *Litsea* extracts, particularly *L. glauca*, demonstrate promising antioxidant properties, which may be harnessed for medicinal or industrial applications.

The antibiofilm activity was evaluated against three *Candida* species (*C. albicans*, *C. lusitanae*, and *C. auris*) at a concentration of 500 μg/mL. The percentage inhibition of biofilm formation is presented in Table 3. Among the tested extracts, *L. fulva* demonstrated the highest biofilm inhibition against *C. albicans* at 45.2 ± 0.2%, followed by *L. tomentosa* (35.4 ± 0.3%), *L. glauca* (32.5 ± 0.5%), and *L. rubicunda* (30.8 ± 0.7%). Although all extracts showed some level of inhibitory activity, the inhibition was below 50%, suggesting limited efficacy against *C. albicans* biofilm formation at this concentration. Besides, all extracts demonstrated good antibiofilm activity against *C. lusitanae*, with inhibition percentages exceeding 60%. *L. tomentosa* exhibited the highest inhibition at 70.0 ± 0.2%, followed closely by *L. glauca* (69.2 ± 0.6%), *L. rubicunda* (65.5 ± 0.4%), and *L. fulva* (60.2 ± 0.9%). These results highlight the potential of *Litsea* extracts to effectively target *C. lusitanae* biofilms. In addition, the extracts also displayed strong antibiofilm activity against *C. auris*. *L. tomentosa* achieved the highest inhibition (67.2 ± 0.5%), followed by *L. rubicunda* (65.8 ± 0.8%), *L. fulva* (60.0 ± 0.5%), and *L. glauca* (55.2 ± 0.7%). These findings suggest that *L. tomentosa* and *L. rubicunda* are particularly effective in reducing *C. auris* biofilm formation.

Table 3. Antibiofilm activity (% inhibition) of *Litsea* extracts

Extracts	*Candida albicans* (I%)	*Candida lusitanae* (I%)	*Candida auris* (I%)
L. glauca	32.5 ± 0.5^{a}	69.2 ± 0.6^{ab}	55.2 ± 0.7^{ab}
L. fulva	45.2 ± 0.2^{a}	60.2 ± 0.9^{a}	60.0 ± 0.5^{ab}
L. rubicunda	30.8 ± 0.7^{a}	65.5 ± 0.4^{a}	65.8 ± 0.8^{ab}
L. tomentosa	35.4 ± 0.3^{ab}	70.0 ± 0.2^{ab}	67.2 ± 0.5^{a}

Note: Data represent mean ± SD of three independent experiments. The means were compared with Duncan's multiple comparison test at $P < 0.05$, the means with same small letter are not significantly different.

Biofilm formation is a critical virulence factor for *Candida* species, contributing to their resistance against antifungal drugs and immune responses. The tested *Litsea* extracts demonstrated varying degrees of antibiofilm activity, with higher efficacy observed against *C. lusitanae* and *C. auris*. The moderate inhibition observed in *C. albicans* suggests that higher concentrations or alternative extraction methods may be required to enhance efficacy (Slobodníkova et al., 2016). The antibiofilm activity of the *Litsea* extracts can be attributed to their bioactive compounds, including phenolic compounds, flavonoids, and tannins. These compounds likely interfere with adhesion mechanisms, nutrient availability, or microbial communication pathways essential for biofilm development (Villanueva et al., 2023). Notably, *L. tomentosa* consistently showed strong antibiofilm activity, highlighting its potential as a natural antifungal agent. These findings underscore the importance of *Litsea* extracts as promising candidates for controlling biofilm-related infections.

The antifungal activity was evaluated against *Candida albicans*, *Candida lusitanae*, and *Candida auris*. The MIC and MFC values are summarized in Table 4. Among the extracts, *L. tomentosa* exhibited the strongest antifungal activity against *C. albicans*, with both MIC and MFC values of 62.5 $\mu g \cdot mL^{-1}$, indicating its potent fungicidal effect. In contrast, *L. glauca*, *L. fulva*, and *L. rubicunda* showed moderate activity with MIC and MFC values of 125 $\mu g \cdot mL^{-1}$ and 250 $\mu g \cdot mL^{-1}$, respectively. Against *C. lusitanae*, *L. tomentosa* again demonstrated the highest efficacy, with MIC and MFC values of 125 $\mu g \cdot mL^{-1}$. The other extracts (*L. glauca*, *L. fulva*, and *L. rubicunda*) displayed consistent activity, with MIC and MFC values of 250 $\mu g \cdot mL^{-1}$. For *C. auris*, *L. tomentosa* and *L. fulva* showed the best antifungal activity, with MIC and MFC values of 125 $\mu g \cdot mL^{-1}$. *L. glauca* and *L. rubicunda* displayed slightly weaker activity, with both MIC and MFC values at 250 $\mu g \cdot mL^{-1}$. The results indicate that *L. tomentosa* consistently exhibited the strongest antifungal activity across all tested *Candida* species, with low MIC and MFC values. This highlights its potential as a natural antifungal agent. The activity of *L. fulva* against *C. auris* was also notable, suggesting species-specific efficacy among the *Litsea* extracts. The antifungal properties of these extracts are likely attributed to the presence of phenolic compounds, flavonoids, tannins, and other bioactive metabolites. These compounds may disrupt fungal cell membranes, interfere with cell wall synthesis, or inhibit DNA replication, leading to the inhibition of fungal growth and survival (Aboody and Mickymaray, 2020).

In a nutshell, the antioxidant, antifungal, and antibiofilm activities of *Syzygium* species are closely linked. The antioxidant capacity of these plants, largely due to their phenolic compounds, plays a crucial role in neutralizing free radicals and mitigating oxidative stress. Oxidative stress is a significant factor in fungal pathogenesis and biofilm formation, particularly for fungi like *Candida* that depend on reactive oxygen species (ROS) for biofilm development and cellular survival. By reducing ROS levels, the antioxidant properties of *Syzygium* extracts may effectively inhibit fungal proliferation and biofilm establishment. The combined antioxidant, antifungal, and antibiofilm activities underscore the potential of *Syzygium* extracts as powerful natural therapeutic agents, offering valuable applications in both medical and industrial settings.

Conclusions

This study demonstrates the significant antioxidant, antifungal, and antibiofilm potential of methanolic leaf extracts from four *Litsea* species (*L. glauca*, *L. fulva*, *L. rubicunda*, and *L. tomentosa*). Among these, *L. glauca* exhibited the highest antioxidant activity, with superior β-carotene bleaching inhibition, DPPH radical scavenging ability, and total phenolic content, while *L. tomentosa* showed the strongest antifungal and antibiofilm activities, particularly against *C. lusitanae* and *C. auris*. The bioactivities of these extracts are likely due to the presence of phenolic compounds, flavonoids, and tannins, which contribute to their ability to neutralize free radicals, inhibit fungal growth, and prevent biofilm formation. These findings highlight the potential of *Litsea* species as natural therapeutic agents for medical and industrial applications. Future research should focus on isolating and identifying the active compounds, understanding their mechanisms of action, exploring synergistic effects with existing antifungal agents, evaluating their efficacy *in vivo*, and developing sustainable methods for their extraction and use.

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Table 4. Antifungal activity (MIC and MFC in $\mu g \cdot mL^{-1}$) of *Litsea* extracts

Extracts	*Candida albicans*		*Candida lusitanae*		*Candida auris*	
	MIC	MFC	MIC	MFC	MIC	MFC
L. glauca	125	125	250	250	250	250
L. fulva	250	250	250	250	125	125
L. rubicunda	250	250	250	250	250	250
L. tomentosa	62.5	62.5	125	125	125	125

CRediT Authorship Contribution Statement

Audrey Ashleeynus Allhin: Conceptualization, investigation, performed the experiments and original draft preparation. **Wan Mohd Nuzul Hakimi Wan Salleh and Nurunajah Ab Ghani:** Funding, supervision and manuscript editing. **Abubakar Siddiq Salihu:** Field collection and data analysis of antioxidant. **Mohd Hafiz Arzmi:** Data analysis of antifungal and antibiofilm.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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