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Antioxidant and Antibacterial Activities of Plant and Endophytic Fungi Extracts from *Syzygium myrtifolium* Walp, with LC-HRMS Profiling of Active Extracts

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ABSTRACT. The emergence of antibiotic resistance and oxidative stress-related diseases highlights the urgent need for novel bioactive compounds. This study investigates the potential of *Syzygium myrtifolium* Walp. and its endophytic fungi as sources of antibacterial and antioxidant agents. Sixteen endophytic fungi isolates were obtained from six plant parts and identified morphologically. Thin layer chromatography (TLC)-based chemical profiling demonstrated comparable secondary metabolite patterns between the plant and its endophytic fungi, indicating possible shared biosynthetic pathways. Antibacterial screening using TLC-bioautography and minimum inhibitory concentration (MIC) assays demonstrated that both the plant and endophytic fungi extracts inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*, with *Xylaria* sp. showing notable activity. (SmDh4) exhibiting the strongest activity (MIC = 64 µg/mL). Antioxidant activity determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay indicated high to very high radical inhibition capacity, especially in SmRTd (AAI = 25.91) and SmAk1 (AAI = 24.97). Liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis unique secondary metabolites on endophytic fungi, including L-α-palmitin, α-eleostearic acid, and 8-methylnaphthalene-1,2-diol, which were detected exclusively in the endophytic fungi extracts, highlighting their potential as alternative antibacterial and antioxidant agents.

Keywords: Antibacterial, antioxidant, endophytic fungi, secondary metabolites, *Syzygium myrtifolium* Walp.

INTRODUCTION

The increasing threat of antibiotic-resistant pathogens and the harmful effects of oxidative stress have created an urgent need for natural compounds with antibacterial and antioxidant activities (Ho et al., 2025; Milatovic et al., 2021). This urgency encourages the exploration of new bioactive metabolites, one of which is derived from the plant *Syzygium myrtifolium* Walp., a species belonging to the Myrtaceae family, which is widely distributed in tropical and subtropical regions (Pillai & Sreekala, 2023).

Syzygium myrtifolium Walp. is rich in various bioactive compounds with potential pharmacological properties. Its leaves are known to contain flavonoids, phenolics, alkaloids, saponins, and triterpenoids (Ahmad et al., 2021; Amanah et al., 2023). These compounds exhibit antioxidant, antimicrobial, antiviral, anticancer, and antidiabetic activities (Amanah et al., 2023). Overexploitation of plant and cultivation constraints have driven advances in pharmaceutical sciences and biotechnology, leading to the discovery of environmentally friendly endophytic fungi that can rapidly produce the same or more potent secondary metabolites through biotransformation from their host plant (Zakariyah et al., 2024). This shift began in 1993 with the discovery of paclitaxel from *Taxomyces andreanae*, an endophyte of *Taxus brevifolia*, highlighting the potential of endophytes as alternative sources of plant-derived bioactive compounds, such as taxol (Tiwari & Bae, 2022).

Since then, endophytic fungi have been recognized for forming symbiotic relationships with medicinal plant, significantly influencing their secondary metabolism and metabolite production (Alam et al., 2021). These fungi produce a variety of bioactive compounds, including phenolics, flavonoids, steroids, polyketides, saponins, and alkaloids, which help plant cope with biotic and abiotic stresses and enhance their immune systems (Jha et al., 2023). Several endophytic fungi exhibit

diverse biological activities such as antibacterial, antioxidant, anticancer, antiviral, antidiabetic, anti-inflammatory, and antiparasitic effects (Hashem et al., 2023).

The close mutualistic symbiosis between medicinal plant and their endophytic fungi has drawn significant research interest in exploring their antioxidant and antibacterial activities. This study focuses on *S myrtifolium* Walp. due to its rich profile of bioactive compounds, aiming to assess these biological activities in both the plant and its associated endophytes, as well as to identify the active secondary metabolites using liquid chromatography-high resolution mass spectrometry (LC-HRMS). The findings are expected to contribute to advancements in pharmaceutical sciences and biotechnology.

EXPERIMENTAL SECTION

Material

The plant parts of *S. myrtifolium* Walp. The roots, stems, young petioles, mature petiole, mature leaves (green), and young leaves (red) were used and obtained from Padang, West Sumatra, Indonesia. Chemicals used in this study include dimethyl sulfoxide/DMSO (Merck), Folin-Ciocalteu (Merck), ethanol P.A (Merck), quercetin (Sigma-Aldrich), Dragendroff (Merck), vanillin (Sigma-Aldrich), hexanes, ethyl acetate, methanol, acetone, dichloromethanes, aquadest, chloramphenicol, and Iodonitrotetrazolium/INT (Sigma) and media such as Mueller Hinton Agar/MHA (Criterion), Mueller Hinton broth/MHB (Criterion), Potato Dextrose Broth/PDB (Difco™), Potato Dextrose Agar/PDA (Difco™), Corn Meal Malt Agar/CMMA (Difco™).

Plant Identification and Preparation

Syzygium myrtifolium Walp. was taxonomically identified at the ANDA Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang. The plant parts were then air-dried, ground into a fine powder using a grinder, weighed, and extracted.

Isolation of Endophytic Fungi

Each plant part was stored at low temperature, surface-sterilized using ethanol and sodium hypochlorite, and then dried aseptically. Sterilized samples were cut into 1×1 cm^2 pieces and placed on CMMA supplemented with 0.05 g/L chloramphenicol, then incubated at room temperature for 5 days. Emerging fungi colonies were subcultured on PDA to obtain pure isolates. Each pure isolate was then morphologically identified (Mahmud et al., 2020).

Identification of Endophytic Fungi

Fungi identification was carried out based on morphological characteristics according to a previous study (Ilyas et al., 2019). Morphological identification was performed by observing both macroscopic and microscopic phenotypic characteristics. Macroscopic characterization included observations of colony color, shape, surface, texture, exudate droplets, and reverse color. For microscopic observation, fungi mycelia were mounted in a drop of 1% lactophenol blue solution. Microscopic characterization was conducted using a light microscope to observe hyphae, hyphal pigmentation, septation, clamp connections, spores, and other reproductive structures.

Cultivation of Endophytic Fungi

Each isolate was cultivated in 200 mL of potato dextrose broth (PDB; HiMedia™) in 500 mL culture flasks and incubated in the dark for 21 days. Isolation and cultivation of endophytic fungi were carried out aseptically. The isolation and cultivation of endophytic fungi were conducted aseptically (Mahmud et al., 2020).

Extraction of Endophytic Fungi and Plant

Plant extraction was performed by maceration using ethyl acetate with three repetitions. In addition, endophytic fungi isolated from the plant were harvested after incubation, along with their growth in medium and biomass, then blended and macerated using the same solvent. The filtrate was concentrated by rotary evaporation and stored at 20°C. (Mahmud et al., 2020).

Chemical Compounds Analysis by Thin Layer Chromatography (TLC)

Chemical compounds present in plant and endophytic fungi extracts were analyzed using silica gel thin layer chromatography (TLC). Extracts were prepared at 10 mg/mL in ethyl acetate, and then 10 μL of the extract was applied to TLC plates and eluted with dichloromethane:methanol (30:1). Separated compounds were visualized under UV light at λ 254 and λ 366 nm, followed by spraying with vanillin-sulfuric acid (heated at 110°C), Dragendorff's, and Folin-Ciocalteu reagents to detect different classes of metabolites (Kumari et al., 2021).

TLC-Bioautography for Screening Antibacterial and Antioxidant Activities

Screening of antibacterial and antioxidant activities of plant and endophytic fungi extracts (10 mg/mL) was performed using TLC-bioautography. A total of 10 μL of each extract and chloramphenicol as a positive control were applied to TLC plates and dried. Antibacterial screening was performed by dipping the plates in bacterial suspension, incubating at 37°C for 18 hours, then with iodonitrotetrazolium (INT). The white zone indicated bacterial inhibition. Active extracts were developed with CH_2Cl_2:MeOH (30:1), dried, and sprayed again with INT. Antioxidant screening was carried out by spraying plates containing extracts and catechin (positive control) with 0.02% DPPH in methanol. Yellow spots on a purple background indicated antioxidant activity. Active extracts were developed, dried, and sprayed again with DPPH (Wang et al., 2021).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of all extracts was determined by serial microdilution in a 96-well plate (Charria-Giron et al., 2021). Extracts (10240 μg/mL) were serially diluted with MHB to a final concentration of 256 μg/mL. Each well was then inoculated with 100 μL of bacterial suspension (10^6 CFU/mL). Chloramphenicol and broth served as positive and negative controls, respectively. Plates were incubated at 37°C for 24 hours, after which 10 μL of INT (4 mg/mL) was added. MIC was defined as the lowest concentration showing no visible bacterial growth.

Determination of IC_{50} of Active Extract

Antioxidant activity was measured using the DPPH method through serial microdilution in a 96-well plate (Kumari et al., 2021). Extracts (10240 μg/mL) were diluted to a final concentration of 128 μg/mL, and then DPPH solution (61.50 μg/mL) was subsequently added. Catechin and methanol served as positive and negative controls. After 90 minutes of incubation in the dark, absorbance was recorded at l 517 nm. The IC_{50} value was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \text{ X } 100\%$$

AAI (Antioxidant Activity Index) was calculated as the ratio of the DPPH concentration to the IC_{50} value.

RESULTS AND DISCUSSION

Plant and Endophytic Fungi Identification

Identification revealed that this plant belongs to the family Myrtaceae and genus *Syzygium*, identified as *S. myrtifolium* Walp. Six extracts were obtained from the plant, which facilitated the isolation of 16 endophytic fungi strains, as shown in **Table 1** and **Figure 1**. These results align with previous studies showing that a single plant species can be colonized by various endophytic fungi, reflecting the diversity of species capable of forming symbiotic relationships with host plant (Habisukan et al., 2021). The diversity of endophytic fungi in *S. myrtifolium* demonstrates their significant potential as sources of bioactive compounds for therapeutic development, emphasizing the important

role these fungi play in producing such compounds. for example, *Acremonium chrysogenum*, which has been used in the industrial production of cephalosporin antibiotics (Ibrahim et al., 2024).

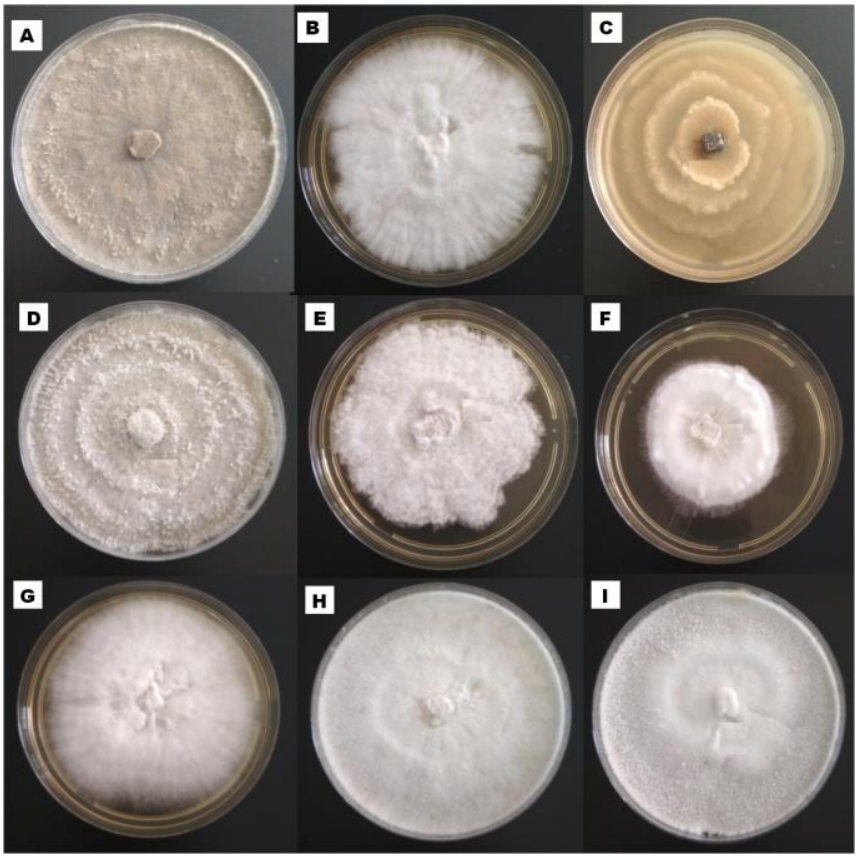

Figure 1. Macroscopic view of representative endophytic fungi inhabiting *Syzygium myrtifolium* Walp., *in vitro* culturing on PDA, 7-10 days incubation at 27°C: (**A**) *Diaporthe* sp. SmAk1 (**B**) *Xylaria* sp. SmBt1(**C**) *Acremonium* sp. SmBt2 (**D**) *Colletotrichum* sp. SmRTd1 (**E**) *Neopestalotiopsis* sp. SmRTd2 (**F**) *Xylaria* sp. SmTd2 (**G**) *Xylaria* sp. SmDh1 (**H**) *Arthrinium* sp. SmDh3, and (I) *Neofusicoccum* sp. SmDm2.

Table 1. List of endophytic fungi isolated from plant parts of *S. myrtifolium* Walp.

No	Plant part	Plant Code	No	Isolate Code	Fungi taxa based on morphology*
1.	Root	SmAk	1.	SmAk1	*Diaporthe* sp.
2.	Stem	SmBt	2.	SmBt1	*Xylaria* sp.
			3.	SmBt2	*Acremonium* sp.
3.	Young petiole	SmRTd	4.	SmRTd1	*Colletotrichum* sp.
			5.	SmRTd2	*Neopestalotiopsis* sp.
			6.	SmRTd3	*Colletotrichum* sp.
			7.	SmRTd4	*Colletotrichum* sp.
4.	Mature petiole	SmTd	8.	SmTd1	*Colletotrichum* sp.
			9.	SmTd2	*Xylaria* sp.
			10.	SmTd3	*Neopestalotiopsis* sp.
5.	Mature Leaf (green)	SmDh	11.	SmDh1	*Xylaria* sp.
			12.	SmDh2	*Arthrinium* sp.
			13.	SmDh3	*Arthrinium* sp.
			14.	SmDh4	*Xylaria* sp.
6.	Young leaf (red)	SmDm	15.	SmDm1	*Arthinium* sp.
			16.	SmDm2	*Neofusicoccum* sp.

(*): Cultures were growth on potato dextrose agar (PDA), 5-7 days incubation at 27°C

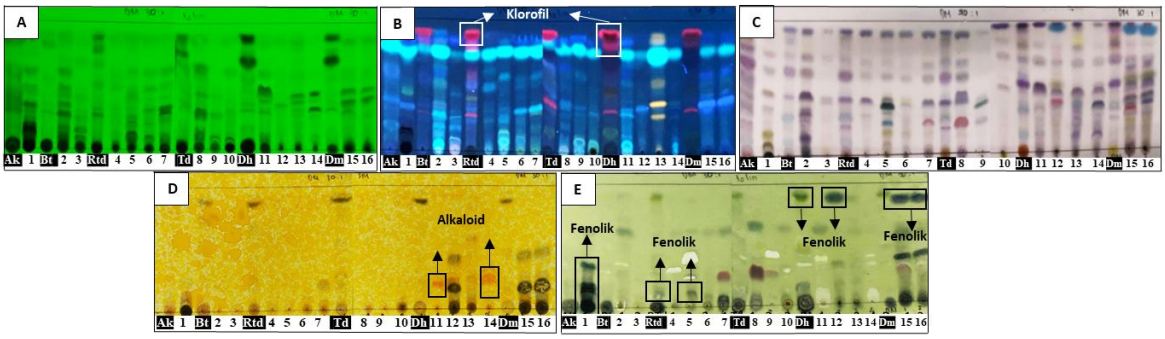

Figure 2. Chromatograms of plant and endophytic fungi extracts with eluted CH_2Cl_2:MeOH (30:1, v/v), visualized under (**A**) UV λ 254 nm, (**B**) UV λ 366 nm, (**C**) vanillin–H_2SO_4, (**D**) Dragendorff, and (**E**) Folin–Ciocalteu. Black boxes indicate plant codes; white boxes indicate endophytic fungi codes.

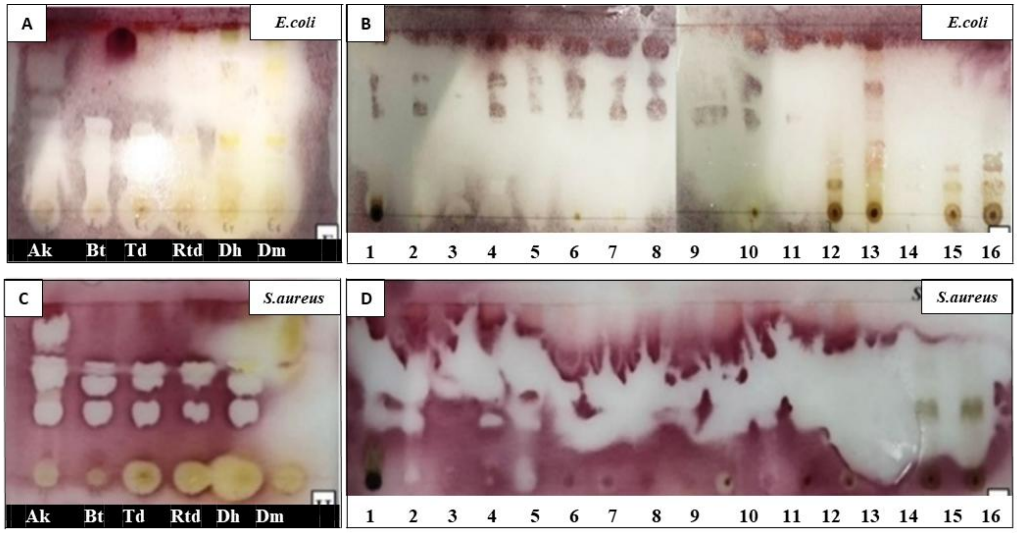

Figure 3. TLC-bioautograms of endophytic plant and fungi with eluted CH_2Cl_2: MeOH (30:1) (**A**). Plant (*E. coli*), (**B**). endophytic fungi (*E. coli*), (**C**). Plant (*S. aureus*), (**D**). endophytic fungi (*S. aureus*). White zones indicate bacterial inhibition and represent antibacterial activity. Black boxes indicate plant codes, and white boxes indicate endophytic fungi codes.

Phytochemical Profile of Extracts by Thin Layer Chromatography (TLC)

The chemical compound profiles of plant extracts and endophytic fungi from *S. myrtifolium* Walp. were analyzed using TLC (**Figure 2**). TLC offers a preliminary overview of the diversity and similarity of compounds present in both plant extracts and endophytic fungi, making it a valuable initial method for mapping secondary metabolites (Wilson & Poole, 2023). Separated chemical compounds were visualized under UV light at λ 254 nm and λ 366 nm. Spots observed at λ 366 nm indicate the presence of compounds containing chromophore groups or conjugated double bond systems (Raunsai et al., 2023).

Figure 2 shows that Folin–Ciocalteu reagent detects phenolic compounds as blue-black spots, Dragendorff's reagent indicates alkaloids with orange spots, and vanillin-H_2SO_4 reveals diverse secondary metabolites through various colored spots (Fathoni et al., 2021). TLC analysis revealed similar retention patterns between the extract of *S. myrtifolium* Walp. and its associated endophytic fungi, suggesting the possibility of shared biosynthetic pathways. This is evident in the SmDh and SmDh2 extracts, where phenolic compounds were detected at Rf = 0.87, indicated by blue-black spots. A similar pattern was observed in the SmRTd extract and its associated endophyte SmRTd2, with phenolic compounds appearing at Rf = 0.12, also marked by blue-black coloration. The metabolites produced by the fungi appeared structurally simpler, likely due to the absence of chlorophyll-derived compounds (Vaishnav & Demain, 2011). Endophytic fungi exhibit significant pharmacological potential, as indicated by the presence of alkaloids in the extracts SmDh1 (Rf = 0.20) and SmDh4 (Rf = 0.27). Additionally, a greater production of phenolic compounds was observed, particularly in the SmAk1 extract (detected at Rf ≤ 0.61), and in extracts SmDh1, SmDm1, and SmDm2 (detected at Rf = 0.87), as illustrated in **Figure 2**. Through horizontal gene transfer, endophytic fungi can produce bioactive metabolites similar to the host plant or new compounds that have potential as alternative sources (Bielecka et al., 2022).

Antibacterial Activity

Screening of antibacterial activity using TLC-dot blot and TLC-bioautography showed that four host plant extracts (SmRTd [3], SmTd [4], SmDh [5], and SmDm [6]) inhibited the growth of both *E. coli* and *S. aureus*. Among the endophytic fungi extracts, three isolates (SmDh4 [14], SmDm1 [15], and SmDm2 [16]) inhibited *E. coli*, while seven extracts (SmBt1 [2], SmDh1 [11], SmDh2 [12], SmDh3 [13], SmDh4 [14], SmDm1 [15], and SmDm2 [16]) showed activity against *S. aureus*.

TLC-bioautography was conducted to separate and identify these bioactive compounds based on their antibacterial activity. The white zone indicates the presence of active antibacterial compounds, resulting from the absence of dehydrogenase enzyme activity in dead bacterial cells (Raunsai et al., 2023), whereas the purple background is produced by the reduction of INT to formazan by bacterial dehydrogenase (Wang et al., 2021). Based on previous findings, *S. zeylanicum* and its endophytic fungus *Penicillium brefeldianum*, isolated from the plant, produce the same compounds, such as p-hydroxybenzaldehyde, and exhibit similar antibacterial activity. This suggests that the similarity in secondary metabolites contributes to their comparable antibacterial effects (Syarifah et al., 2021).

The MIC values presented in **Table 2**, show that the endophytic fungus *Xylaria* sp. isolate SmDh4 exhibited strong antibacterial activity against *Staphylococcus aureus* (MIC = 64 µg/mL) and moderate activity against *Escherichia coli* (MIC = 128 µg/mL), according to the classification by (Dzotam et al., 2018), who defined MIC values <100 µg/mL as strong and 100–500 µg/mL as moderate. This is consistent with the TLC-bioautography result, where SmDh4 displayed a white zone, qualitatively indicating strong antibacterial activity (**Figure 3**), which is further supported by its MIC values. Based on the MIC values, endophytic fungi demonstrate the ability to produce secondary metabolites with notable antibacterial activity. For instance, the SmDh extract showed an MIC of 256 µg/mL, whereas its endophyte SmDh4 exhibited a stronger activity with an MIC of 64 µg/mL, suggesting that the endophyte may produce distinct, more potent antibacterial compounds than its host plant. A similar pattern was observed in SmRTd and its endophyte SmRTd1 against *S. aureus*, with MIC of 256 µg/mL and 128 µg/mL, respectively. These findings are consistent with the TLC-bioautography results, where SmRTd displayed only two active spots, while SmRTd1 showed a broader inhibition zone. However, in some cases, the host plant may exhibit stronger bioactivity, as observed in SmTd (MIC 128 µg/mL) compared to its endophyte SmTd1 (MIC 256 µg/mL). These results sugges t a mutualistic relationship between *S. myrtifolium* Walp. and its endophytic fungi, in which both partners contribute bioactive compounds that inhibit the growth of *S. aureus* and *E. coli*.

Previous studies have shown that, endophytic fungi such as *Xylaria* sp. can produce potent bioactive compounds with significant antibacterial activity. For instance, *Xylaria* sp. isolated from *Ginkgo biloba* leaves was found to produce 7-amino-4-methylcoumarin, exhibiting strong antibacterial effects against *S. aureus* and *E. coli* with MIC values of 16 µg/mL and 10 µg/mL, respectively (Liu et al., 2008).

Table 2. Minimum inhibitory concentration (MIC) of extracts from *Syzygium myrtifolium* Walp. and its endophytic fungi.

No	Extract code	MIC (µg/mL)			
		S.aureus	Activities	*E.coli*	Activities
1	Chloramphenicol**	2	Strong	2	Strong
2	SmAk*	-	NT	-	NT
3	SmAk1	-	NT	-	NT
4	SmBt*	-	NT	-	NT
5	SmBt1	256	Moderat	256	Moderat
6	SmBt2	-	NT	-	NT
7	SmRtd*	256	Moderat	-	NT
8	SmRTd1	128	Moderat	256	Moderat
9	SmRTd2	-	NT	-	NT
10	SmRTd3	-	NT	-	NT
11	SmRTd4	-	NT	-	NT
12	SmTd*	128	Moderat	256	Moderat
13	SmTd1	256	Moderat	-	NT
14	SmTd2	-	NT	-	NT
15	SmTd3	-	NT	-	NT
16	SmDh*	256	Moderat	-	NT
17	SmDh1	256	Moderat	-	NT
18	SmDh2	256	Moderat	-	NT
19	SmDh3	256	Moderat	-	NT
20	**SmDh4**	**64**	**Strong**	**128**	**Moderat**
21	SmDm*	128	Moderat	128	Moderat
22	SmDm1	-	NT	-	NT
23	SmDm2	256	Moderat	-	NT

(*): plant extract, (**): positive control

Antioxidant Activity

The antioxidant activity was evaluated to assess the ability of plant extracts and endophytic fungi extracts from *S. myrtifolium* Walp. to inhibit free radicals, using the DPPH method. This method is based on the donation of hydrogen atoms from antioxidant compounds to stabilize free radicals, converting them into non-radical compounds (1,1-diphenyl-2-picrylhydrazine), as indicated by a color change from purple to yellow (Kusmiati et al., 2018).

According to the qualitative analysis presented in **Figure 4**, using TLC-bioautography assay, plant extracts demonstrated strong antioxidant activity, as evidenced by the numerous yellow spots formed on the TLC. In contrast, six endophytic fungi extracts (SmBt1, SmBt2, SmRTd1, SmTd2, SmDh1, and SmDh4) exhibited weak antioxidant activity. This difference is likely related to variations in secondary metabolite profiles produced by the plants and their associated endophytic fungi. The symbiotic metabolic relationship was further supported by the similar antioxidant activity observed in root extract, where both the plant extract SmAk and the endophytic fungi extract SmAk1 showed activity in polar compounds, indicated by active spots at Rf ≤ 0.39. A comparable pattern was observed in young leaf extracts, with plant extract SmDm and endophytic extracts SmDm1 and SmDm2 exhibiting antioxidant activity across nearly all detected compounds. These results suggest the presence of phenolic compounds, consistent with the data shown in **Figure 2**, which revealed high phenolic content in SmAk, SmDm1, and SmDm2 extracts. Following qualitative TLC analysis, IC_{50} and AAI values of the extracts were determined using the DPPH method.

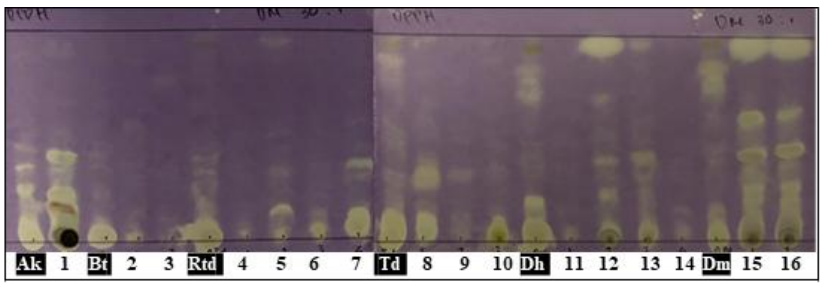

Figure 4. TLC-bioautograms of *S. myrtifolium* Walp. extracts using CH_2Cl_2: MeOH (30:1). Black boxes indicate plant codes, and white boxes indicate endophytic fungi codes.

Table 3. Antioxidant activity of *S. myrtifolium* Walp. plant extracts and endophytic fungi extracts, expressed as IC_{50} (μg/mL) and AAI.

No	Extract code	R^2	IC_{50}	AAI	Category base on AAI Value
1	Catechin**	0.9784	0.833	36.9	Very strong
2	SmAk*	0.9937	10.34	2.97	Strong
3	SmAk1	0.9958	1.23	24.97	Very strong
4	SmBt*	0.9936	47.49	0.65	Weak
5	SmBt1	0.9964	282.73	0.11	Weak
6	SmBt2	0.9995	144.26	0.21	Weak
7	SmRtd*	0.9926	1.19	25.91	Very strong
8	SmRTd1	0.9859	330.63	0.09	Weak
9	SmRTd2	0.9945	121.88	0.25	Weak
10	SmRTd3	0.9979	167.4	0.18	Weak
11	SmRTd4	0.997	177.9	0.17	Weak
12	SmTd*	0.9949	2.71	11.34	Very strong
13	SmTd1	0.9997	287.93	0.11	Weak
14	SmTd2	0.9977	325.82	0.09	Weak
15	SmTd3	0.994	13.34	2.31	Strong
16	SmDh*	0.9955	1.79	17.15	Very strong
17	SmDh1	0.9969	563.03	0.05	Weak
18	SmDh2	0.9927	31.06	1.01	strong
19	SmDh3	0.9913	95.55	0.32	Weak
20	SmDh4	0.9802	303.59	0.1	Weak
21	SmDm*	0.9999	4.69	6.55	Very strong
22	SmDm1	0.9916	3.36	9.15	Very strong
23	SmDm2	0.9926	2.00	15.38	Very strong

(*): plant extract, (**): positive control

Antioxidant activity was evaluated based on IC_{50} and AAI values. IC_{50} indicates the concentration of a compound required to inhibit 50% of DPPH radical activity, reflecting its effectiveness in preventing oxidation (Yusuff et al., 2019). AAI is used to standardize antioxidant activity in DPPH-based assays, with classifications as follows: weak (AAI < 0.5), moderate (0.5 ≤ AAI ≤ 1.0), strong (1.0 < AAI ≤ 2.0), and very strong (AAI > 2.0) (Abarca-vargas et al., 2019).

The results showed that four plant extracts, namely SmRTd, SmTd, SmDh, and SmDm, exhibited very strong antioxidant activity with AAI values of 25.91; 11.34; 17.15; and 6.55, respectively. This strong activity was also reflected in the TLC-bioautography, where antioxidant-active spots appeared at Rf ≤ 0.24, indicating the presence of polar antioxidant compounds. Among the endophytic fungi extracts, three isolates SmAk1 (*Diaporthe* sp.), SmDm1 (*Arthrinium* sp.), and SmDm2 (*Neofusicoccum* sp.) also demonstrated very strong antioxidant activity with AAI values of 24.97; 9.15, and 15.38, respectively. TLC-bioautography revealed that SmAk1 contained active antioxidant compounds at Rf ≤ 0.39, while SmDm1 and SmDm2 showed activity across a broad range of compounds, from polar to non-polar. In addition, SmTd3 (*Neopestalotiopsis* sp) showed strong antioxidant activity with an AAI value of 2.31. The root extract of the plant, SmAk, exhibited strong antioxidant activity with an AAI of 2.97. Supported by TLC results showing dominant polar antioxidant compounds at Rf ≤ 0.39.

Strong antioxidant activity has previously been reported for *Neopestalotiopsis* sp. (IC_{50}: 22.92 μg/mL) and *Diaporthe* sp. (IC_{50}: 37.61 μg/mL) isolated from *Cinnamomum loureiroi*, with eugenol, lauric acid, myristaldehyde, and caprylic acid identified as key antioxidant compounds. Similarly, this study found strong antioxidant activity in *Neopestalotiopsis* sp. (SmDm2) and *Diaporthe* sp. (SmAk1) isolated from *S. myrtifolium*, supporting their potential as natural antioxidant sources (Tanapichatsakul et al., 2019).

Analysis of Plant and Endophytic Fungi Extracts Using LC-HRMS

Extracts of plant and their associated endophytic fungi that exhibited strong antioxidant and antibacterial activities were selected for LC-HRMS analysis to compare their secondary metabolite profiles. The selected extracts included SmAk, SmAk1, SmDh, SmDh1, SmDh4, SmDm, and SmDm1 (**Figure 5**). SmAk and SmAk1 were selected for their strong antioxidant activity; SmDh for its strong antioxidant and moderate antibacterial activities; SmDh1 and SmDh4 for their moderate to very strong antibacterial activities; SmDm for its moderate antibacterial and very strong antioxidant activity; and SmDm1 for its very strong antioxidant activity. These seven extracts

were selected for LC-HRMS analysis to compare their secondary metabolite profiles. The comparison was based on the five most intense compounds in each extract, selected for their efficiency, biological relevance, and representativeness of the extract's chemical profile. These major constituents are likely to significantly contribute to the extracts biological activity, making them suitable for further pharmacological or biotechnological analysis. The analysis of SmAk and SmAk1 extracts showed four similar compounds and one compound that was only present in SmAk1. Two compounds were known, and three were new compounds with retention times of 14.363, 9.838, 9.916, 9.828, and 7.086 minutes. These compounds are L-α-palmitin ($C_{19}H_{38}O_4$), $C_{14}H_{31}NO$, 2-amino-1,3,4-octadecanetriol ($C_{18}H_{39}NO_3$), $C_{14}H_{31}NO$ found in SmAk and SmAk1 extracts, while $C_{37}H_{62}N_{10}O_2$ is only found in SmAk1. The chromatograms of these compounds are presented in **Figure 5**, and their chemical structures are shown in **Figure 6**.

L-α-palmitin is the major compound found in the SmAk1 extract (*Diaporthe* sp.), which likely contributes to its high antioxidant activity (AAI = 24.97), as also reported by previous researchers who observed strong antioxidant properties of L-α-palmitin isolated from *S. litoralle* (Hidajati et al., 2018). In addition to this major compound, the presence of minor compounds such as 4-methoxycinnamic acid (8.79 min), (7E,7'E)-5,5'-diferulic acid (7.80 min), and ellagic acid (5.37 min) in the SmAk1 extract may also contribute synergistically to its antioxidant activity (Zheng et al., 2024).

Extracts SmDh, SmDh1, and SmDh4 showed five peaks with the highest intensity. One compound is known to be present in SmDh, SmDh1, and SmDh4. The compound is (-)-caryophyllene oxide ($C_{15}H_{24}O$), the retention time is 8.108 min. Previous research demonstrated that β-caryophyllene, an isomer of caryophyllene oxide, exhibits strong antibacterial activity, particularly against *S. aureus*, with MIC values ranging from 3 to 14 μM (Dahham et al., 2015). Three compounds were found in extract SmDh and SmDh4, these compounds are 1,2,3,4-tetramethyl-1,3-cyclopentadiene (C_9H_{14}), IPMP (2-isopropyl-5-methylphenol) ($C_{10}H_{14}O$), and NP-011220 ($C_{11}H_{18}N_2O_2$) respectively at retention times of 9, 131; 9,132; and 5,023; min. IPMP (2-isopropyl-5-methylphenol) likely contributes to the antibacterial activity of SmDh1 and SmDh4 extracts. Previous studies showed that IPMP synergistically inhibits biofilm formation by *S. mutans* through antimicrobial action and suppression of biofilm-related genes, supporting its potential as an effective antibiofilm agent (Korenaga et al., 2024). In addition, α-eleostearic acid ($C_{18}H_{30}O_2$) was detected exclusively in the SmDh1 and SmDh4 extracts, with a retention time of 14.673 minutes (**Figure 5**), and its chemical structure is shown in **Figure 6**.

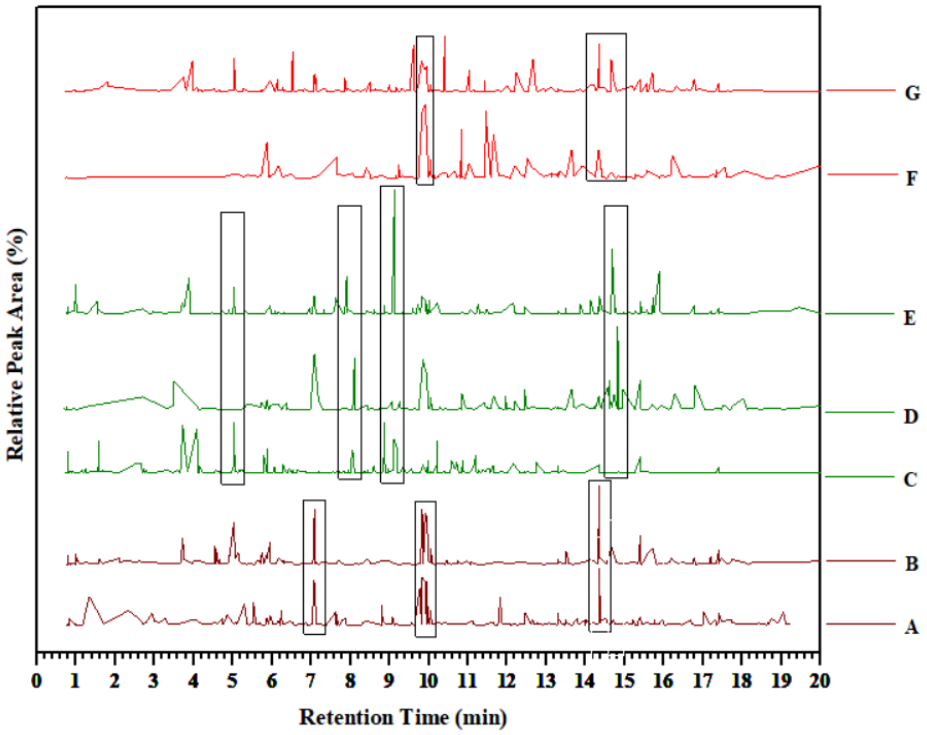

Figure 5. Chromatogram of plant extracts and endophytic fungi from *Syzygium myrtifolium* Walp obtained by LC-HRMS analysis. (**A**) SmAk, (**B**) SmAk1, (**C**) SmDH, (**D**) SmDH1, (**E**) SmDH4, (**F**) SmDm, (**G**) SmDm1. The boxes indicate shared secondary metabolites between the plant and its endophytic fungi.

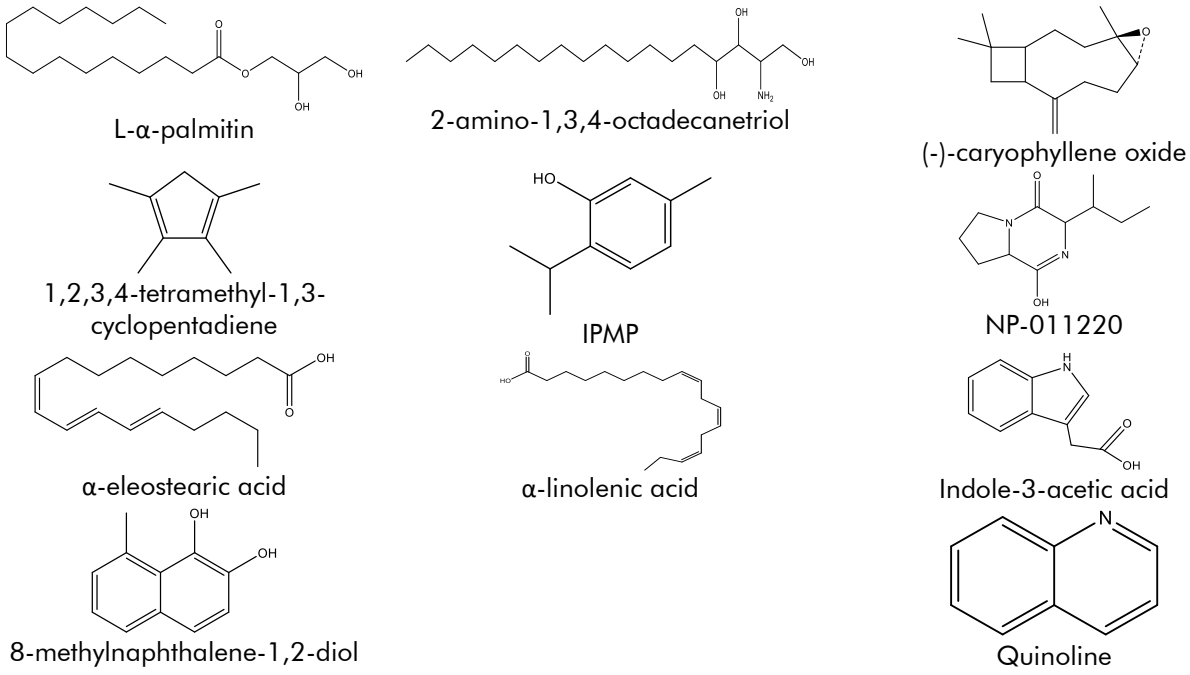

Figure 6. Structure of major secondary metabolite compounds from SmAk, SmAk1, SmDh, SmDh1, SmDh4, SmDm, and SmDm1 extracts.

LC-HRMS analysis of SmDm and SmDm1 extracts showed five peaks with the highest intensity. two compounds were detected in both extracts: α-Linolenic acid ($C_{18}H_{30}O_2$) at 14.66 minutes, and 2-amino-1,3,4-octadecanetriol ($C_{18}H_{39}NO_3$) at 9.912 minutes. In addition, Three other compounds were found exclusively in the SmDm1 extract: 8-methylnaphthalene-1,2-diol ($C_{11}H_{10}O_2$), indole-3-acetic acid ($C_{10}H_9NO_2$), and quinoline (C_9H_7N) with retention times of 10.411, 6.528, and 6,529 minutes, respectively **(Figures 5 and 6)**. α-linolenic acid present in the young leaf extract exhibits strong antioxidant activity, which is consistent with the findings of studies conducted by (Yammine et al., 2024).

CONCLUSIONS

In this study, plant extracts and endophytic fungi isolates were successfully obtained from *S. myrtifolium* Walp. The SmTd and SmDm extracts exhibited moderate antibacterial activity, while the endophytic fungi isolate SmDh4 (*Xylaria* sp.) showed strong antibacterial effects. The highest antioxidant activity was observed in the SmRTd extract, followed by SmAk1 (*Diaporthe* sp.), which also demonstrated very strong antioxidant potential. Based on LC-HRMS analysis, the compounds (−)-caryophyllene oxide and IPMP were identified as contributors to the antibacterial activity, whereas L-α-palmitin and α-linolenic acid were associated with antioxidant effects. These findings suggest that *S. myrtifolium* and its endophytic fungi are promising sources of bioactive compounds for further pharmacological development.

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