

Evaluation of the Potency of Endophytic Fungi Extracts Associated With Potentially Medicinal Plants From Mandalika-Lombok, West Nusa Tenggara

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ABSTRACT

Aim: This study presented to isolate and evaluate the potency of 35 endophytic fungi associated with 12 potential medicinal plants collected from Mandalika-Lombok, West Nusa Tenggara for antibacterial and antioxidant activities.

Methods: Antibacterial screening was carried out by Thin Layer Chromatography (TLC)-bioautography method against *Staphylococcus aureus* and *Escherichia coli*. Antioxidant activity test was carried out by TLC bioautography-Diphenyl picrylhydrazyl (DPPH) method. The active extracts were determined by their Minimum Inhibitory Concentrations (MIC's) and IC₅₀ values by serial dilution method in 96 well microplates.

Results: The results showed that there were 23 active extracts as the antioxidant were categorized as follows: 9 extracts were poor, 7 extracts are moderate, 4 extracts are powerful, 3 extracts are very powerful antioxidant activity. There were 32 extracts inhibited the growth of *S.aureus* were categorized as follows: 19 extracts were weak, 12 extracts were moderate inhibition. There were 18 extracts inhibited the growth of *E.coli* were categorized as follows: 15 extracts were weak and 3 extracts have moderate antibacterial activity against *E.coli*.

Conclusion: Endophytic fungi from potentially medicinal plants collected from Mandalika have the potential as a new antibacterial and antioxidant sources.

Keywords: Mandalika, endophytic fungi, potential medicinal plants, antibacterial, antioxidant

INTRODUCTION

For many generations, in every community medicinal plants have been used for their primary health services, and also used to promote health and longevity in many regions [1]. The used of medicinal plants are related to the bioactive compounds produced by the plants. Plants have been known as the host of endophytic microbes.

Endophytic fungi associated with medicinal plants have gained a lot of interest lately. Endophytic fungi are a group of microorganisms that colonize healthy and living tissues of the plants asymptotically [2], with enormous in their biological diversity [3]. There is a mutualistic relationship between medicinal plants as host and endophytic fungi that colonized it. One species of plant is associated with at least one endophytic microbes [4]. According to

Yu et al. [5] plant provide nutrients for endophytic microbes and the endophytes produce secondary metabolites in returns. Secondary metabolites produced by endophytic fungi could be the same or similar as that produced by the host plant [6]. Endophytic fungi are a source of secondary metabolites that may have a unique structure and have potent activity as antifungal, antibacterial, and cytotoxic [7]. So, there is a possibility to obtain bioactive compounds from endophytes associated with medicinal plants for pharmaceutical uses. Increased bacterial resistance to antibiotics and available drugs encourages the search for new compounds as new antibacterial resource. In the previous paper, we reported potent antibacterial metabolites namely (+)-2,2'-epicytoskyrin A [8] and (+)-bislunatin [9] from the endophytic fungus *Diaporthe* sp. GNB10 which was firstly isolated from *Diaporthe* sp. associated with tea plant *Camellia sinensis* [10]. Screening of antibacterial and antioxidant properties from several medicinal plants has also been carried out. Several endophytic fungi associated with several plants collected from Enggano Island showed good antibacterial activity with the MIC value of 64 µg/ml [11] while some of endophytic fungi from mangosteen (*Garcinia mangostana*) were potential source for antioxidant [12]. On the other hand, there is also growing interest in search for new natural antioxidant compounds. Natural antioxidants have no or negligible side effect [13]. The antioxidant compounds can prevent or delay the oxidative damage by free radicals [14]. In the present study, we isolated endophytic fungi associated with 12 medicinal plants from Mandalika, Lombok and evaluated their potential as antioxidant and antibacterial sources.

MATERIALS AND METHODS

Chemical Reagents

Ethanol, Na-hypochlorite, Corn Meal Agar (Sigma-Aldrich), Malt Extract (Bacto), Yeast Extract (Bacto), Chloramphenicol (Sigma), Potato Dextrose Agar (PDA, Difco), Potato Dextrose Broth (PDB, Difco), vanillin (Sigma-Aldrich) and Cerium (IV) sulfate (Merck), iodinitro tetrazolium p-violet (INT, Sigma), Diphenyl picrylhydrazyl (DPPH, sigma), Mueller Hinton Broth (MHB, Difco), and (+)-catechin (Sigma).

Plant Collection

Twelve potential medicinal plants from Mandalika, Lombok, West Nusa Tenggara-Indonesia namely: *Cassia fistula*, *Ficus septica*, *Geunsia acuminata*, *Ixora cumingiana*, *Kleinhovia hospita*, *Leea aequata*, *Micromelum minutum*, *Moringa oleifera*, *Rauwolfia javanica*, *Schoutenia ovata*, *Tetrastigma lanceolarum*, *Xanthophyllum flavescens* were collected for endophytic fungi isolation. The plant materials were identified and authenticated at the Herbarium Bogoriense, Research Center for Biology.

Isolation of Endophytic Fungi

The plant samples were rinsed under tap water to remove debris and then cut into small fragments. The small fragments were surface sterilized with a sequence of soaking in 70% alcohol for 1 min, 5.3% Na-hypochlorite for 5 minutes, 70% alcohol for 0.5 min and distilled water. After the sterilization process, samples were dried aseptically and cut into small pieces using a sterile blade and then placed on Corn Meal Malt Agar (CMMA) medium and incubated for up to 7 days at room temperature. CMMA medium are consist of corn meal agar (20 g/L), yeast extract (2 g/L) and malt extract (17 g/L) and supplemented with 0.05 g/L

chloramphenicol. Emerging mycelia from the samples were sub-cultured on Potato Dextrose Agar (PDA) many times to obtain pure endophytic fungi isolate.

Identification of Endophytic Fungi

The pure endophytic fungi were transferred and growing out on 80-mm Petri dish and then incubated at 27°C for 5-10 days. Microscopic slides of each selected strains were prepared using 0.01% trypan blue dye in lacto phenol as mounting medium. Fungal identification carried out based on morphological characteristics. The morphological identification was conducted by observing both of the macroscopic and microscopic characterizations. Macroscopic characterization includes observation on color, colony shape, surface, texture, exudates drop, and reverse color. Microscopic characterization was conducted on light microscope by observing hyphae, hyphae pigmentation, septate, clamp connection, conidia, spore and other reproductive structure.

Cultivation and Extraction of Endophytic Fungi

The endophytic fungus was cultured on Potato Dextrose Broth (PDB) medium and incubated at room temperature for 3 weeks under dark condition. After incubation completed, the biomass and media were extracted with ethyl acetate thrice. The extract was concentrated under reduced pressure by a rotary evaporator. The concentrated extract was stored at low temperature for further analysis.

Metabolites Analysis of Endophytic Fungi by Thin Layer Chromatography

The concentrated extract was dissolved in acetone at the concentration of 10 mg/ml. Ten microliters of extract were loaded on thin layer chromatography (TLC) plate (Silica gel GF₂₅₄, Merck) and developed using dichloromethane/methanol (10:1). Separated chemical compounds were examined under UV light (254 nm and 366 nm, Camag). To detect the chemical compounds, the TLC plates were sprayed with spray reagents (1% vanillin-sulphuric acid and 1% cerium (IV) sulfate).

Evaluation of Antibacterial Activity : TLC-Direct Bioautography

Ten microliters of extract (10 mg/ml) were loaded on TLC plate and evaluated for its antibacterial activity against *S. aureus* InaCC B-4 and *E.coli* InaCC B-5 and by Dot Blot test with TLC-Direct Bioautographic method. The active extracts were further analyzed by separating chemical compounds in the extracts with dichloromethane/methanol (10:1) on TLC. The chromatogram on TLC plate was dipped into bacterial suspension and placed on sterile petri dish with wet sterile cotton to keep humidity. The plate was incubated at 37°C for 18 hours. After the incubation period, the plate was sprayed with iodo nitro tetrazolium p-violet (INT, Sigma). Growth inhibition zone was indicated by clear zone formation.

Evaluation of Antioxidant Activity: TLC-Direct Bioautography

The antioxidant activity assay was done by dot blot test and developed chromatogram as in the antibacterial activity test. Ten microliters of extract (10 mg/ml) were loaded on TLC plate and dried followed by spraying the plate with DPPH solution in methanol. The active extract was indicated by yellow spot formation on the purple background. The active extracts were further analyzed by separating chemical compounds in the extracts with

dichloromethane/methanol (10:1) on TLC. The developed chromatogram was sprayed with DPPH solution in methanol. The active chemical compounds were indicated by yellow spots or bands.

Minimum Inhibitory Concentration by Microdilution Broth Method

The concentrated extracts were dissolved in dimethyl sulfoxide at the concentration of 1024 µg/ml as a stock solution, and evaluated for antibacterial activity against *S. aureus* InaCC B-4 and *E. coli* InaCC B-5 and Each well of the first column was added with 100 µl Mueller Hinton Broth (MHB), the rest of the wells were added with 100 µl of MHB as well. Each well of the first column was also added with 100 µl extract and then diluted serially. After completing dilution, each well was added with bacteria suspension (10^6 cfu/ml) and incubated for 24 hours in 37°C. Each extract for antibacterial activity was conducted three times. The range of concentrations in the antibacterial test was 2.0-256 µg/ml. The minimum inhibitory concentration (MIC) was the lowest concentration that inhibits the visible growth of bacteria [15].

Determination of IC₅₀ Values for Free Radical Scavenging Activity

The IC₅₀ values of endophytic extracts were determined using DPPH free radical method. All of the wells were added with 100 µl of methanol. After that, the first column was added with 95 µl of methanol into each well and 5 µl of sample at the concentration of 10,240 µg/ml and homogenized. Serial dilution was carried out by taking 100 µl from the first column and added to the 2nd column. The antioxidant activity test was carried out in triplicate. After the dilution process was completed, each well was added with DPPH 100 µl (61.50 µg/ml). The microplate was incubated at room temperature for 90 minutes at the dark condition. Methanol was used as blank, while catechin was used as positive control. The absorbance of the sample was observed at 517 nm [16].

The percentage of inhibition was calculated as follows:

$$\text{DPPH Inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100$$

A₀ = absorbance of the control

A₁ = absorbance of the sample

The IC₅₀ was calculated from the linear calibration between the inhibition percentage and sample concentration. The antioxidant activity index (AAI) was calculated with the formula below :

$$\text{AAI} = \text{final concentration of DPPH} / \text{IC}_{50}$$

RESULTS AND DISCUSSION

Isolation and Identification of Endophytic Fungi

The healthy plant tissues are good and potential sources for endophytic fungi isolation [17]. However, environmental factors affect the variation of endophytic fungi occurrence and frequency colonization [18]. Moreover, plant age, sampled tissue, and genotype also affected the species composition of endophytic fungi [19].

Totally thirty-five endophytic fungi were obtained from leaves and stem of 12 potential medicinal plants from Mandalika, Lombok. Identification of endophytic fungi were based on their morphological characteristics on potato dextrose agar medium showed that 25 isolates could be identified to the level of genera, and included to 7 genera (*Phomopsis*,

Colletotrichum, *Xylaria*, *Lasiodiplodia*, *Fusarium*, *Schizophyllum* and *Neopestalotiopsis*), 6 isolates were identified to family level (i.e. Dematiaceae) and 4 isolates were identified to class level (i.e. Hyphomycetes (Table 1). The result showed that one species of plant harbors more than one endophytic fungi. Plant tissues that are colonized by more than one endophytic fungi also found in the rhizome of zingiberacious plants [20] and medicinal plants collected from Central Sulawesi [21].

Table 1: List of endophytic fungi associated with potential medicinal plants from Mandalika-Lombok, West Nusa Tenggara

No	Strain/ Isolate No.	Host Plant	Plant Part	Fungal Taxa
01	1 Bt Lo 2	<i>Moringa oleifera</i> Lam.	Stem	<i>Phomopsis</i> sp.
02	1 Dn Lo 1	<i>Moringa oleifera</i> Lam.	Leaf	Dematiaceae
03	1 Dn Lo 2	<i>Moringa oleifera</i> Lam.	Leaf	Dematiaceae
04	3 Bg Lo 1	<i>Kleinhovia hospita</i> L.	Flower	Dematiaceae
05	3 Bj Lo 1	<i>Kleinhovia hospita</i> L.	Seed	<i>Colletotrichum</i> sp.
06	3 Bt Lo 2	<i>Kleinhovia hospita</i> L.	Stem	<i>Phomopsis</i> sp.
07	3 Bt Lo 3	<i>Kleinhovia hospita</i> L.	Stem	<i>Xylaria</i> sp.
08	3 Dn Lo 1	<i>Kleinhovia hospita</i> L.	Leaf	<i>Colletotrichum</i> sp.
09	4 Bt Lo 3	<i>Cassia fistula</i> L.	Stem	<i>Lasiodiplodia</i> sp.
10	4 Dn Lo 2	<i>Cassia fistula</i> L.	Leaf	Dematiaceae
11	5 Bg Lo 1	<i>Ixora cumingiana</i> Vidal	Flower	Hypomycetes
12	5 Dn Lo 1	<i>Ixora cumingiana</i> Vidal	Leaf	Hypomycetes
13	7 Bh Lo 1	<i>Micromelum minutum</i> (Forst.F.) Wight & Arn..	Fruit	<i>Phomopsis</i> sp.
14	7 Bh Lo 2	<i>Micromelum minutum</i> (Forst.F.) Wight & Arn..	Fruit	<i>Xylaria</i> sp.
15	7 Bh Lo 4	<i>Micromelum minutum</i> (Forst.F.) Wight & Arn..	Fruit	<i>Phomopsis</i> sp.
16	7 Dn Lo 2	<i>Micromelum minutum</i> (Forst.F.) Wight & Arn..	Leaf	<i>Phomopsis</i> sp.
17	8 Bh Lo 3	<i>Leea aequata</i> (Burm.f.) Merr.	Fruit	<i>Fusarium solani</i>
18	8 Bt Lo 2	<i>Leea aequata</i> (Burm.f.) Merr.	Stem	<i>Phomopsis</i> sp.
19	8 Dn Lo 2	<i>Leea aequata</i> (Burm.f.) Merr.	Leaf	<i>Phomopsis</i> sp.
20	9 Bt Lo 1	<i>Xanthophyllum flavescens</i> Roxb.	Stem	<i>Xylaria</i> sp.
21	9 Dn Lo 1	<i>Xanthophyllum flavescens</i> Roxb.	Leaf	<i>Colletotrichum</i> sp.
22	10 Bt Lo 1	<i>Rauvolfia javanica</i> K. & V.	Stem	<i>Phomopsis</i> sp.
23	10 Bt Lo 3	<i>Rauvolfia javanica</i> K. & V.	Stem	<i>Phomopsis</i> sp.
24	10 Dn Lo 1	<i>Rauvolfia javanica</i> K. & V.	Leaf	<i>Phomopsis</i> sp.
25	10 Dn Lo 2	<i>Rauvolfia javanica</i> K. & V.	Leaf	Hypomycetes
26	11 Bt Lo 2	<i>Ficus septica</i> Burm.f.	Stem	Hypomycetes
27	11 Dn Lo 1	<i>Ficus septica</i> Burm.f.	Leaf	<i>Phomopsis</i> sp.
28	12 Bh Lo 1	<i>Geunsia acuminata</i> Juss	Fruit	<i>Schizophyllum</i> sp.
29	12 Dn Lo 1	<i>Geunsia acuminata</i> Juss	Leaf	Dematiaceae

30	12 Dn Lo 3	<i>Geunsia acuminata</i> Juss	Leaf	Dematiaceae
31	13 Bh Lo 2	<i>Tetrastigma lanceolarium</i> Planch	Fruit	<i>Phomopsis</i> sp.
32	13 Bt lo 2	<i>Tetrastigma lanceolarium</i> Planch	Stem	<i>Lasiodiplodia</i> sp.
33	13 Dn Lo 1	<i>Tetrastigma lanceolarium</i> Planch	Leaf	<i>Xylaria</i> sp.
34	14 Bh lo 1	<i>Schoutenia ovata</i> Korth.	Fruit	<i>Neopestalotiopsis</i> sp.
35	14 Bt Lo 2	<i>Schoutenia ovata</i> Korth.	Stem	<i>Xylaria</i> sp.

The majority of isolated endophytic fungi from 12 medicinal plants from Mandalika were *shomopsis*. *Phomopsis* is a rich source of bioactive compounds such as phomoxanthenes as antitubercular and antimalarial [22] and phomoxanthone A as antifungal [23].

Detection of Antibacterial Activity by TLC-Direct Bioautography

Evaluation of antibacterial activity of endophytic fungi associated with medicinal plants from Mandalika against *S. aureus* and *E.coli* was performed by the TLC-direct bioautography method. This method is fast, simple, requires a small amount of sample [24] and rapid detection of antibacterial compounds. The results of TLC-bioautography for antibacterial activity against *S. aureus* were presented in Fig.1 and *E. coli* (Fig.2). There were 32 extracts able to inhibit the growth of *S. aureus* and 18 extracts were able to inhibit the growth of *E. coli*. Clear zone as an indicator of growth inhibition of bacteria on a purple background. A purple background caused by viable microorganisms converse the iodo nitro tetrazolium salt (INT) to color formazan [25].

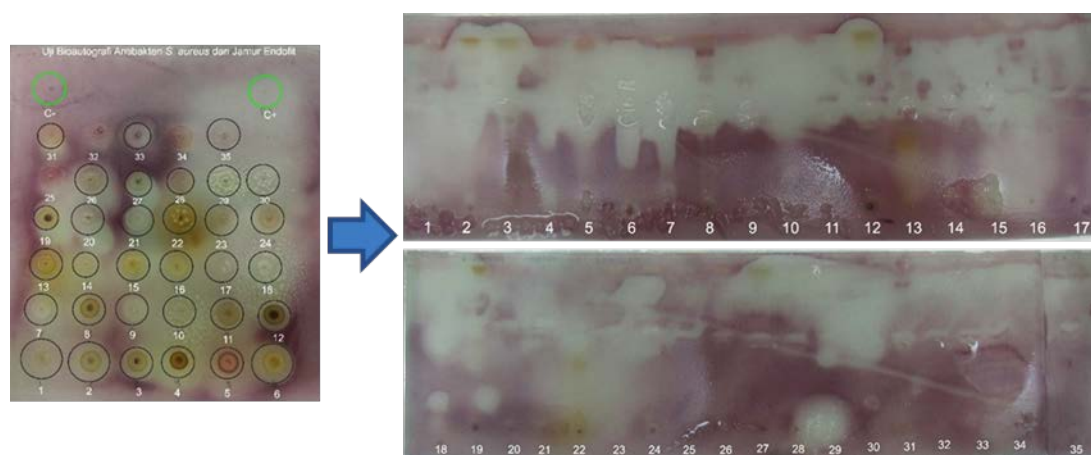


Fig 1: Bioautogram of antibacterial activity of endophytic fungi associated with medicinal plants from Mandalika, Lombok against *S.aureus*. Dot-blot assay (left), develop with mobile phase dichloromethane : methanol (10:1) (right). Clear zone indicated the growth inhibition.

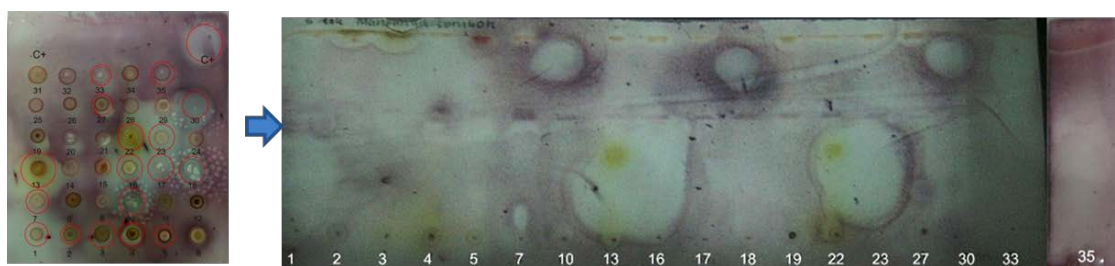


Fig 2: Bioautogram of antibacterial activity of endophytic fungi associated with medicinal plants from Mandalika, Lombok against *E.coli*. Dot-blot assay (left), develop with mobile phase dichloromethane : methanol (10:1) (right). Clear zone indicated the growth inhibition

Table 2: Minimum Inhibitory Concentration of endophytic fungi associated with potential medicinal plants from Mandalika-Lombok.

No	Code	Endophytic fungi	MIC ($\mu\text{g/ml}$)	
			<i>S.aureus</i>	<i>E.coli</i>
01	1 Bt Lo 2	<i>Phomopsis</i> sp.	>256	>256
02	1 Dn Lo 1	Dematiaceae	256	>256
03	1 Dn Lo 2	Dematiaceae	>256	>256
04	3 Bg Lo 1	Dematiaceae	>256	256
05	3 Bj Lo 1	<i>Colletotrichum</i> sp.	256	>256
06	3 Bt Lo 2	<i>Phomopsis</i> sp.	>256	-
07	3 Bt Lo 3	<i>Xylaria</i> sp.	256	256
08	3 Dn Lo 1	<i>Colletotrichum</i> sp.	>256	-
09	4 Bt Lo 3	<i>Lasiodiplodia</i> sp.	>256	-
10	4 Dn Lo 2	Dematiaceae	128	>256
11	5 Bg Lo 1	Hypomycetes	>256	-
12	5 Dn Lo 1	Hypomycetes	256	-
13	7 Bh Lo 1	<i>Phomopsis</i> sp.	>256	>256
14	7 Bh Lo 2	<i>Xylaria</i> sp.	>256	-
15	7 Bh Lo 4	<i>Phomopsis</i> sp.	256	-
16	7 Dn Lo 2	<i>Phomopsis</i> sp.	128	>256
17	8 Bh Lo 3	<i>Fusarium solani</i>	256	>256
18	8 Bt Lo 2	<i>Phomopsis</i> sp.	256	>256
19	8 Dn Lo 2	<i>Phomopsis</i> sp.	>256	>256
20	9 Bt Lo 1	<i>Xylaria</i> sp.	>256	-
21	9 Dn Lo 1	<i>Colletotrichum</i> sp.	>256	-
22	10 Bt Lo 1	<i>Phomopsis</i> sp.	128	128
23	10 Bt Lo 3	<i>Phomopsis</i> sp.	>256	>256
24	10 Dn Lo 2	<i>Phomopsis</i> sp.	-	-
25	10 Dn Lo 1	Hypomycetes	>256	-
26	11 Bt Lo 2	Hypomycetes	>256	-
27	11 Dn Lo 1	<i>Phomopsis</i> sp.	>256	>256
28	12 Bh Lo 1	<i>Schizophyllum</i> sp.	>256	-
29	12 Dn Lo 1	Dematiaceae	128	-
30	12 Dn Lo 3	Dematiaceae	256	>256
31	13 Bh Lo 2	<i>Phomopsis</i> sp.	>256	-
32	13 Bt lo 2	<i>Lasiodiplodia</i> sp.	-	-
33	13 Dn Lo 1	<i>Xylaria</i> sp.	>256	>256
34	14 Bh lo 1	<i>Neopestalotiopsis</i> sp.	-	-
35	14 Bt Lo 2	<i>Xylaria</i> sp.	>256	>256

Table 2. showed that *S.aureus* is more sensitive to endophytic fungi extract than *E.coli*. This may be related to different cell wall structures between *S. aureus* (Gram-positive bacteria) and *E.coli* (Gram-negative bacteria). Gram-negative bacteria have the outer membrane lipopolysaccharide which results in a slow penetration of antibacterial substances [26].

The results in the table 2also show that endophytic fungi which have MIC values of 128 µg/ml are two 2 isolates of *Phomopsis* sp. which is associated with the *Cassia fistula* and *Raufolvia javanica* plants, classified as moderate antibacterial activity [27]. The previous study showed that endophytic fungi associated with *C.fistula* were able to inhibit the growth of *E.coli*, *S.aureus*, *P.aeruginosa* [28]. However, antibacterial activity of endophytic fungi from *R. javanica*, *M.minutum* and *G.acuminate* have not been found so far.

Determination of Antioxidant Activity of Endophytic Extracts

Screening of antioxidant compounds in the extracts was done by TLC-bioautography method based on the DPPH radical scavenging assay. The DPPH assay for antioxidant assay is fast, simple, low cost for detecting extract or compounds with antioxidant activity [29].

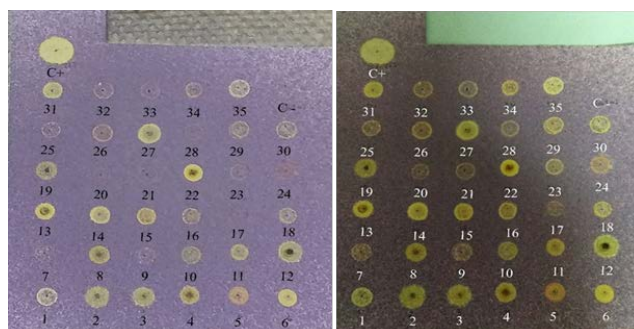


Fig. 3: TLC- dot blot for antioxidant activity of endophytic fungi associated with medicinal plants collected from Mandalika, Lombok by DPPH assay. Observed at 0 min (left), 30 min. (right)

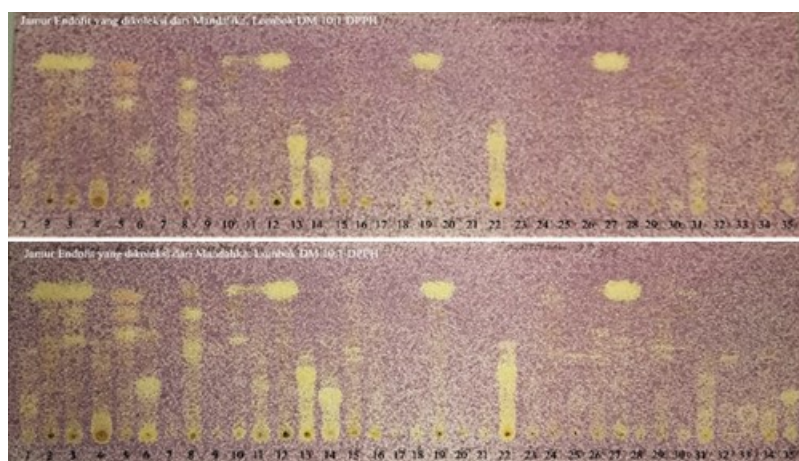


Fig. 4: Bioautogram for antioxidant activity of endophytic fungi associated with medicinal plants collected from Mandalika, Lombok by DPPH assay. Compounds with antioxidant activity indicated by white yellowish bands.

Table 3: IC₅₀ for antioxidant activity and antioxidant activity index (AAI) of endophytic fungi associated with potentially medicinal plants from Mandalika-Lombok

No	Code	Endophytic fungi	IC ₅₀ (ug/ml)	AAI	Category of AAI
01	1 Bt Lo 2	<i>Phomopsis</i> sp.	> 128	< 0.24	Weak-moderate
02	1 Dn Lo 1	Dematiaceae	54.21	0.57	Moderate
03	1 Dn Lo 2	Dematiaceae	20.7	1.49	Strong
04	3 Bg Lo 1	Dematiaceae	61.88	0.50	Moderate
05	3 Bj Lo 1	<i>Colletotrichum</i> sp.	> 128	< 0.24	Weak-moderate
06	3 Bt Lo 2	<i>Phomopsis</i> sp.	74.21	0.41	Moderate
07	3 Dn Lo 1	<i>Colletotrichum</i> sp.	60.07	0.51	Moderate
08	4 Dn Lo 2	Dematiaceae	> 128	< 0.24	Weak-moderate
09	5 Bg Lo 1	Hypomycetes	97.6	0.32	Moderate
10	5 Dn Lo 1	Hypomycetes	13.58	2.26	Very strong
11	7 Bh Lo 1	<i>Phomopsis</i> sp.	23.04	1.33	Strong
12	7 Bh Lo 2	<i>Xylaria</i> sp.	23.88	1.29	Strong
13	7 Bh Lo 4	<i>Phomopsis</i> sp.	123.87	0.26	Moderate
14	7 Dn Lo 2	<i>Phomopsis</i> sp.	> 128	< 0.24	Weak-moderate
15	8 Bt Lo 2	<i>Phomopsis</i> sp.	> 128	< 0.24	Weak-moderate
16	8 Dn Lo 2	<i>Phomopsis</i> sp.	28.57	1.08	Strong
17	10 Bt Lo 1	<i>Phomopsis</i> sp.	12.16	2.53	Very strong
18	11 Dn Lo 1	<i>Phomopsis</i> sp.	12.43	2.47	Very strong
19	12 Dn Lo 1	Dematiaceae	> 128	< 0.24	Weak-moderate
20	12 Dn Lo 3	Dematiaceae	> 128	< 0.24	Weak-moderate
21	13 Bh Lo 2	<i>Phomopsis</i> sp.	45.74	0.67	Moderate
22	14 Bh Lo 1	<i>Neopestalotiopsis</i> sp.	> 128	< 0.24	Weak-moderate
23	14 Bt Lo 2	<i>Xylaria</i> sp.	> 128	< 0.24	Weak-moderate

Table 3 showed that several endophytic fungi extracts have strong and very strong antioxidant activity *in-vitro* based on the IC₅₀ and AAI value. The endophytic fungi that have strong antioxidant activity are 1DnLo-2 (Dematiaceae) associated with *M. oleifera*, 7BhLo-1 (*Phomopsis* sp.) and 7BhLo-2 (*Xylaria* sp.) associated with *M. minutum*, and 8DnLo-2 (*Phomopsis* sp.) associated with *L. indica*. Extracts with very strong antioxidant activity are 5 Dn Lo 1 from *I. cumingiana*, 10 BtLo1 (*Phomopsis* sp.) from *R. Javanica* and 11DnLo1 (*Phomopsis* sp.) from *F. septica*.

The antioxidant activity of endophytic fungi might be related to the bioactive compounds their produced. These bioactive compounds might be the same or similar to those produced by the host plant. Methanol extract of *M. oleifera* mature leaves has potent antioxidant activity [20, 31] with the IC₅₀ value of $18.15 \pm 0.92 \mu\text{g/ml}$ in DPPH assay. Endophytic fungi associated with *L. indica* (8DnLo 2) also has strong antioxidant activity that might be related to the phenolic content produced by endophytic fungi. The previous study on *L. indica* extract showed strong correlation between total phenolic content and antioxidant activity [32].

CONCLUSION

The present study reveals that endophytic fungi associated with the potential medicinal plants from Mandalika Lombok could be used as the sources to the search of new compounds for antibacterial and antioxidant. Further studies that aimed to isolate, purified and characterized biologically active compounds responsible for antibacterial and antioxidant activities.

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