

Antibacterial and Antioxidant Activities of Endophytic Fungal Extracts from *Pluchea indica* (L.) Less.

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Abstract— This study investigated endophytic fungi associated with *Pluchea indica* (L.) Less, focusing on their chemical profiles and biological potential. A total of five isolates were successfully obtained from leaves and stems. Morphological examination led us to identified the fungal isolates -as *Aspergillus* section *Nigri* 12TBt-3, *Colletotrichum* sp. 12TDn-1, *Diaporthe* sp. (*Phomopsis* sp.) 12TBt-1, *Diaporthe* sp. (*Phomopsis* sp.) 12TBt-2, and *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2. Thin-layer chromatography (TLC) analysis revealed distinct secondary metabolite profiles among the isolates, with the detection of alkaloids and phenolic compounds, particularly in extracts *Aspergillus* section *Nigri* 12TBt-3 and *Diaporthe* sp. (*Phomopsis* sp.) 12TDn2. Antibacterial activity was evaluated qualitatively by TLC bioautography and quantitatively by microdilution assays. Extract *Aspergillus* section *Nigri* 12TBt-3 exhibited moderate inhibitory activity against *Staphylococcus aureus* (MIC 128 µg/mL) and *Escherichia coli* (MIC 256 µg/mL). Antioxidant activity, assessed using the DPPH assay, demonstrated that the extract *Aspergillus* section *Nigri* 12TBt-3 possessed strong activity with an IC₅₀ value of 27.31 µg/mL and an Antioxidant Activity Index (AAI) of 1.14. In addition, total phenolic content analysis revealed that the extract *Aspergillus* section *Nigri* 12TBt-3 contained 257.179 mg GAE/g extract, while *Diaporthe* sp. (*Phomopsis* sp.) 12TDn2 contained 67.249 mg GAE/g extract. Collectively, these findings indicate that endophytic fungi from *Pluchea indica* (L.) Less, particularly isolated *Aspergillus* section *Nigri* 12TBt-3, harbor significant potential as sources of bioactive metabolites with antibacterial and antioxidant properties, supporting their prospective application in biotechnology and pharmaceutical development.

Keywords— Antibacterial; Antioxidant; Endophytic fungi; *Pluchea indica*

1. INTRODUCTION

Pluchea indica (L.) Less, a member of the Asteraceae family, is a tropical plant widely distributed in coastal areas and wetlands of Southeast Asia. Traditionally, this plant has been used to reduce inflammation, promote wound healing, treat digestive disorders, and manage infections. Its biological activities are associated with secondary metabolites, including flavonoids, phenolics, sesquiterpenes, and other bioactive compounds, which have been reported to possess antibacterial, antioxidant, and other pharmacological properties. The chemical diversity of *Pluchea indica* (L.) Less makes it a promising source of bioactive compounds for pharmacological research and natural product development [1-3].

Plant tissues also provide a habitat or endophytic fungi, microorganisms that inhabit internal plant tissues without causing disease symptoms and establish mutualistic relationships with their hosts. Endophytic fungi occupy specific ecological niches within plant tissues and produce a variety of secondary metabolites.

These metabolites have been reported to exhibit antioxidant, anticancer, immunomodulatory, antiviral, antituberculosis, antiparasitic, and insecticidal activities. Endophytic fungi can synthesize compounds similar to those produced by their host plants and perform biotransformations that convert compounds into derivatives with enhanced biological activity [4-5]. Using endophytic fungi as a source of bioactive compounds requires minimal host plant material, reduces environmental impact, shortens cultivation time, lowers costs, and can yield metabolites comparable to those found in the host plant [6].

Secondary metabolites produced by endophytic fungi can exhibit biological activities comparable to or greater than those of the host plant. This highlights their potential as alternative sources of bioactive compounds and provides opportunities to explore metabolites that may not be produced by the host plant itself [7-8].

Systematic evaluation of the antibacterial and antioxidant activities of endophytic fungi extracts

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associated with *Pluchea indica* (L.) Less is therefore necessary to determine their bioactive potential. Although several studies have reported bioactive metabolites produced by endophytic fungi associated with *Pluchea indica* (L.) Less, research on these microorganisms remains limited. Previous investigations have mainly focused on the biological activities of purified compounds isolated from specific endophytic fungi, such as *Didymella* sp. and *Ascomycota* sp., while studies evaluating the antibacterial activity of crude extracts from taxonomically identified endophytic fungal isolates are still scarce. Moreover, to the best of our knowledge, the antioxidant potential of endophytic fungi associated with *Pluchea indica* has not yet been investigated. Information regarding the phytochemical profiles of bioactive extracts and the localization of active constituents through TLC-bioautography is also lacking. Therefore, further studies are required to comprehensively evaluate the antibacterial and antioxidant activities of endophytic fungi isolated from *Pluchea indica* and to provide preliminary insights into the chemical constituents associated with these biological activities. [9-11]

Such studies can provide insight into the bioactivity of endophytic fungi and support the development of sustainable antibacterial and antioxidant agents with potential applications in pharmaceutical and health-related fields.

2. EXPERIMENTAL SECTION

2.1. Materials

Sample of fresh leaves and stems of *Pluchea indica* (L.) Less were collected from Tawangmangu, Solo, Central Java, Indonesia. The specimens were identified in Herbarium Bogoriense. Ethanol 70%, Na-hypochlorite 5.25%, corn malt meal agar (CMMA, Sigma), potato dextrose agar (PDA, Merck), potato dextrose broth (PDB, Merck), ethyl acetate, dichloromethane, methanol, dimethyl sulfoxide (DMSO, Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), catechin (Sigma), thin layer chromatography plate (silica gel GF₂₅₄, Merck), Folin-Ciocalteu reagent (Merck), gallic acid (Sigma), vanillin (Sigma), chloramphenicol (Merck), iodinitrotetrazolium p-violet (INT, Sigma), H₂SO₄ (Merck), Mueller-Hinton Broth (MHB, Merck), Bacterial isolates, namely *Staphylococcus aureus* InaCC-B4 and *Escherichia coli* InaCC-B5, were used for the antibacterial test.

2.2. Instrumentations

The instruments used in this study included a laminar air flow cabinet (OSK CVB-1300M), autoclave (HIRAYAMA), rotary vacuum evaporator (IKA), UV lamps at 254 and 365 nm (CAMAG), hotplate (IKA C-MAG), analytical balance (Quintix Sartorius), microplate reader (Varioskan LUX, Thermo Fisher Scientific), ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu), and micropipettes (GILSON).

2.3. Procedure

2.3. Isolation of Endophytic Fungi

Parts of *Pluchea indica* (L.) Less (leaves and stems) were washed using running water and followed by surface sterilization using ethanol and Na-hypochlorite. The sterilized samples were cut to approximately 1-2 cm and placed on corn malt meal agar (CMMA) medium supplementing with 0.05 mg/mL chloramphenicol, and incubated at dark and room temperature for 5-7 days. Colonies that appeared were subcultured several times using Potato Dextrose Agar (PDA) to obtain pure isolates. Each pure isolate was then morphologically identified [12].

2.4. Identification of Endophytic Fungi

Fungi isolated identification is carried out based on morphological characteristics according to a previous study [13]. Morphological identification was conducted by observing both macroscopic and microscopic properties. Macroscopic characterizations include observations on color, colony shape, surface, texture, exudate drop, and reverse color. Microscopic characterizations were performed on a light microscope by observing hyphae, hyphae pigmentation, septate, clamp connection, conidia, spores, and other reproductive structures.

2.5. Cultivation and Extraction of Endophytic Fungi

Endophytic fungi were grown on Potato Dextrose Broth (PDB) medium and incubated at room temperature in dark and static conditions for 3 weeks. Following the cultivation phase, the liquid-liquid extraction method was implemented to extract the growth medium and endophytic fungal biomass using ethyl acetate. The ethyl acetate extract was concentrated using a rotary evaporator and then preserved at -4 °C for future utilization [12].

2.6. Chemical Compounds Analysis by Thin Layer Chromatography (TLC)

Extracts were prepared at 10 mg/mL in acetone, then 10 µL of the extract was applied to TLC plates and developed in dichloromethane:methanol (10:1). 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, and Folin-Ciocalteu reagents.

Phytochemical screening was carried out on the developed TLC plates using specific spray reagents. Dragendorff's reagent was used to detect alkaloids, while Folin-Ciocalteu reagent was applied for the detection of phenolic compounds. The presence of each phytochemical group was determined based on the characteristic color changes observed after reagent application.

2.7. Screening Antibacterial and Antioxidant Activities

Extracts were prepared at 10 mg/mL in acetone. A total of 10 µL of the extract was applied to TLC plates then immersed in the suspension of bacterial culture,

and then incubated the plate in a humid chamber for 18–24 hours at a temperature of 37 °C. After the incubation period was finished, the plates were sprayed with iodinitrotetrazolium p-violet (INT). The inhibition of bacterial growth was viewed by the formation of a clear zone around the active extract. Active extracts were developed with CH₂Cl₂:MeOH (10:1), then the same procedure starting from bacterial dipping was performed to identify the compound spots responsible for the activity.

Antioxidant screening was carried out by spraying TLC plates containing extracts with 0.02% DPPH in methanol. Yellow spots on a purple background indicated antioxidant activity. Active extracts were developed with CH₂Cl₂:MeOH (10:1), dried, and sprayed again with DPPH.

2.8. Determination of Minimum Inhibitory Concentration (MIC) value

The MIC of the extract was determined by the microdilution method in 96-well plates with three replicates [14]. A stock solution of 10,240 µg/mL was serially diluted in MHB, inoculated with bacterial suspension (10⁶ CFU/mL), and incubated at 37 °C for 24 h; afterward, INT was added, and the MIC was defined as the lowest concentration at which no visible growth was observed.

2.9. Determination of IC₅₀ value

The antioxidant activity of the extract was determined using the DPPH assay in 96-well plates with three replicates. A stock solution of 10,240 µg/mL was serially diluted with methanol and mixed with 61.5 µg/mL DPPH solution, then incubated in the dark for 90 min; absorbance was measured at 517 nm, and the IC₅₀ was defined as the concentration required to inhibit 50% of the radical activity and the antioxidant activity index (AAI) was determined based on the ratio of the final DPPH concentration to the IC₅₀ value [6].

2.10. Determination of Total Phenolic Content (TPC) value

The TPC of extracts was determined by the Folin-Ciocalteu method. It was performed in triplicate using gallic acid as the standard solution, and the TPC value was stated as Gallic Acid Equivalent (GAE) per g extract (mg/g) [15].

3. RESULT AND DISCUSSION

3.1. Isolation and Identification of Endophytic Fungi

A total of five endophytic fungal isolates were successfully obtained from the leaves and stems of *Pluchea indica*. Based on morphological characteristics (Fig. 1), the isolates were assigned to three distinct taxa (Table 1). Three isolates, i.e. 12Tbt-1, 12Tbt-2, and 12TDn-2 were identified as *Diaporthe* sp. (*Phomopsis* sp.). *Diaporthe* sp. (*Phomopsis* sp.) is a fungal genus frequently reported as the dominant endophyte in various medicinal plants. This observation is consistent with the findings of Ilyas et al. [13], who reported that

fungal taxa. is commonly the main endophyte in several medicinal plants, and supported by other studies showing that *Phomopsis* sp. is widespread across diverse host plants, particularly in tropical regions [16]. Another isolate, i.e. 12Tbt-3 was identified as *Aspergillus* section *Nigri* (Fig. 2). *Aspergillus* section *Nigri* is a subgroup of *Aspergillus* with black-spored aspergilli. The previous study reported that 20 selected strains of black aspergilli were isolated from rhizospheric *Piper* plants [17]. The remaining isolate, i.e. 12TDn-1 was assigned to *Colletotrichum* sp. Taxa *Colletotrichum* are ubiquitous in higher plant tissues. Genus *Colletotrichum* are distributed worldwide, with diverse host plants, including more than 3000 higher plant species [18]. These three groups indicate that *Pluchea indica* (L.) harbors a heterogeneous community of endophytic fungi with significant ecological and biochemical potential.

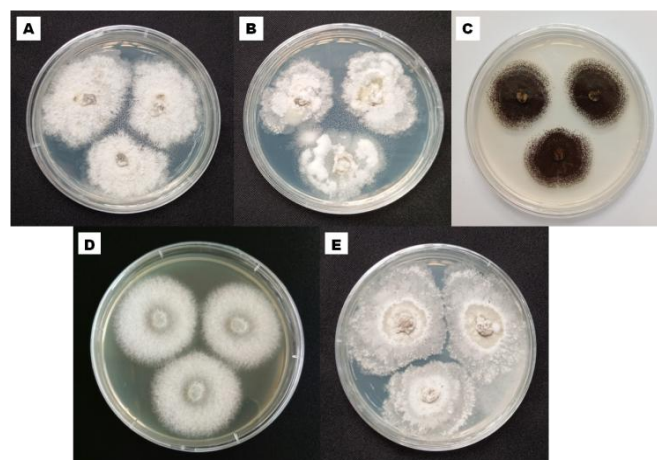
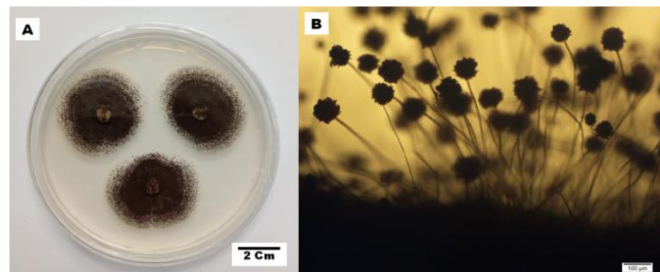


Fig. 1 Macroscopic view of selected endophytic fungi inhabiting *Pluchea indica* (L.) Less., in vitro culturing on PDA, 7 days incubation at T 27°C: *Diaporthe* sp. (*Phomopsis* sp.) 12Tbt-1 (A) *Diaporthe* sp. (*Phomopsis* sp.) 12Tbt-2 (B), *Aspergillus* section *Nigri* 12Tbt-3 (C), *Colletotrichum* sp. 12TDn-1 (D), and *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2 (E)

The diversity and growth of endophytic fungi in *Pluchea indica* are influenced by multiple factors. First, the host plant species play a critical role, as variations in metabolite composition and tissue structure among species allow certain fungal taxa to grow more optimally in specific hosts. Second, the geographic origin of the host plant affects endophytic community composition, as local ecological conditions, including soil microbiota and interactions with other organisms, determine which fungi successfully colonize the plant [16,19]. Third, culture media or laboratory growth conditions influence colony growth rates and the ability of isolates to develop healthy colonies, with variations in nutrient composition leading to significant differences in growth among isolates [20]. This phenomenon suggests that optimal growth of endophytic isolates is often correlated with their capacity to produce bioactive secondary metabolites, which are crucial for enhancing the therapeutic potential of the host plant.

Table 1. List of endophytic fungi isolated from plant parts of *Pluchea indica*

Sample No.	Part of Plant	Isolate Code	Fungal Taxa
1	Stem	12TBt-1	<i>Diaporthe</i> sp. (<i>Phomopsis</i> sp.)
2	Stem	12TBt-2	<i>Diaporthe</i> sp. (<i>Phomopsis</i> sp.)
3	Stem	12TBt-3	<i>Aspergillus</i> section <i>Nigri</i>
4	Leaf	12TDn-1	<i>Colletotrichum</i> sp.
5	Leaf	12TDn-2	<i>Diaporthe</i> sp. (<i>Phomopsis</i> sp.)

**Fig. 2** The macroscopic (A) and microscopic view (B) of fungal endophytes with the most potential antibacterial and antioxidant activity *Aspergillus* section *Nigri* 12TBt-3, cultures were grown in vitro on potato dextrose agar (PDA), 7 days incubation at T 27°C.

3.2. TLC-Based Chemical Compound Analysis

The mass of crude extracts obtained from each endophytic fungal isolate showed significant variation, despite all cultures being grown under identical conditions and equal medium volumes (**Table 2**). This difference indicates variation in the metabolic production capacity among isolates, which may be influenced by growth rate, differences in secondary metabolite biosynthetic pathways, and the accumulation level of soluble compounds extractable with ethyl acetate.

Table 2. Crude Extract Yield of Endophytic Fungal Isolates Cultivated in PDB medium ist

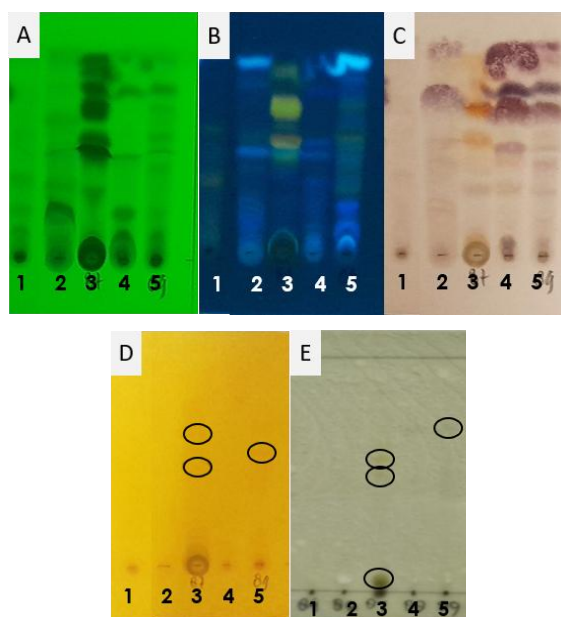
Sample No.	Isolate Name	Isolate Extract (mg/200 mL PDB)
1	<i>Diaporthe</i> sp. (<i>Phomopsis</i> sp.) 12TBt-1	55.1
2	<i>Diaporthe</i> sp. (<i>Phomopsis</i> sp.) 12TBt-2	115.3
3	<i>Aspergillus</i> section <i>Nigri</i> 12TBt-3	279.7
4	<i>Colletotrichum</i> sp. 12TDn-1	158.3
5	<i>Diaporthe</i> sp. (<i>Phomopsis</i> sp.) 12TDn-2	94.3

The chemical profiles of endophytic fungi extracts from *Pluchea indica* (L.) Less were analyzed using thin-layer chromatography (TLC) (**Fig. 3**), with visualization under UV light and by spraying reagents: vanillin-sulfuric acid, Dragendorff, and Folin-Ciocalteu. Vanillin-sulfuric acid was employed for general detection, especially for phenolic, alcoholic, and other easily oxidizable compounds, Dragendorff reagent was applied to detect alkaloids, and Folin-Ciocalteu was sprayed to detect phenolic compounds. Under UV light at 366 nm, compounds appeared as bright fluorescent bands against a dark blue background, indicating the presence of fluorescence-capable molecules, whereas observation under UV light at 254 nm revealed dark bands on a bright background, suggesting UV-absorbing

compounds with conjugated double bonds or chromophoric groups.

Under UV light at 366 nm, the extracts showed bright fluorescent bands, while under UV light at 254 nm, dark bands appeared, indicating the presence of UV-absorbing compounds. Each extract displayed distinct chemical patterns with varying Rf values, reflecting the diversity of secondary metabolites among the endophytic fungal isolates.

Spraying with Dragendorff and Folin-Ciocalteu confirmed the presence of alkaloids and phenolic compounds in extracts *Aspergillus* section *Nigri* 12TBt-3 and *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2. The alkaloid-positive spots were detected at Rf values of approximately 0.43 and 0.22 for *Aspergillus* section *Nigri* 12 TBt-3, whereas *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2 showed a positive spot at an Rf value of approximately 0.32. Following Folin-Ciocalteu treatment, phenolic compounds were detected in both extracts. *Aspergillus* section *Nigri* 12-TBt3 exhibited phenolic-positive spots at Rf values of approximately 0.32, 0.22, and 0.07, while *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2 displayed a phenolic-positive spot at an Rf value of approximately 0.45. The variation in band patterns among extracts indicates that each isolate possesses a unique secondary metabolite profile, which may influence its bioactivity potential, including antioxidant, antimicrobial, and cytotoxic activities [21].

**Fig. 3** Chromatograms of extracts with eluted CH₂Cl₂:MeOH (10:1), visualized under (A) UV λ 254 nm, (B) UV λ 366 nm, (C) Vanillin-sulfuric acid (heated at 110°C), (D) Dragendorff, and (E) Folin-Ciocalteu reagents

3.3. Antibacterial Activity

The antibacterial activity of the endophytic fungi extracts was qualitatively assessed using the TLC dot-blot method. This approach is regarded as practical, rapid, and efficient for preliminary antimicrobial screening, since it requires only a small amount of extract and allows straightforward observation of the

results. Acetone and methanol were used as negative controls, while chloramphenicol served as the positive control. Among the five extracts tested, *Aspergillus* section *Nigri* 12TBt-3 inhibited the growth of both *Staphylococcus aureus* and *Escherichia coli*, while *Diaporthe* sp. (*Phomopsis* sp.) 12TBt-2 was active only against *Staphylococcus aureus* (Fig. 4). Antibacterial inhibition was visualized as clear or white zones on the TLC plate. The purple background originated from the reduction of iodinitrotetrazolium (INT) into red-purple formazan by dehydrogenase enzymes of metabolically active bacterial cells. In this assay, the absence of color development (clear zones) indicates bacterial growth inhibition, since no reduction of INT occurs in the area where the extract suppresses cell viability [22].

In addition to serving as a qualitative screening, TLC bioautography also enabled the identification of specific spots responsible for antibacterial activity. Active compounds were indicated by the appearance of white inhibition bands along the chromatographic track after development (Fig. 5). This provides preliminary evidence of the location of bioactive metabolites within the crude extract, although synergistic or antagonistic interactions among compounds may still occur.

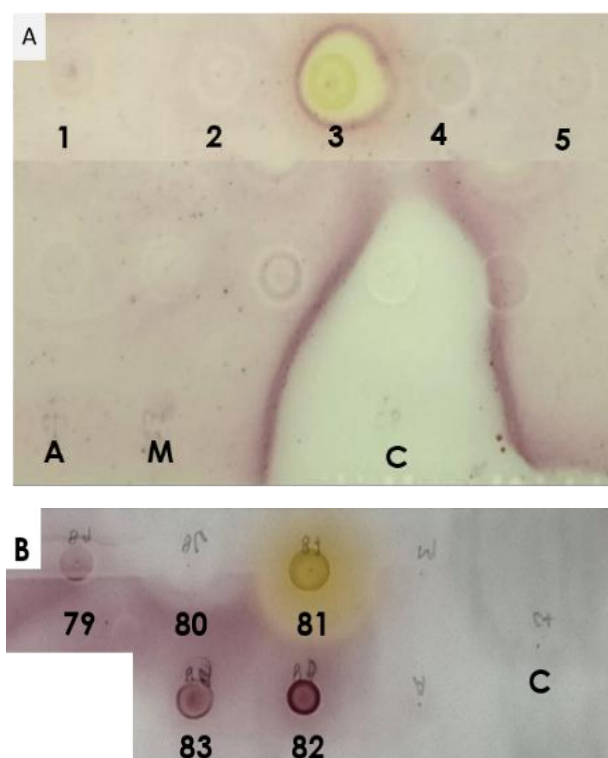


Fig. 4 TLC dot-blot assay against *E. coli* (A) and *S. aureus* (B)

The Quantitative evaluation was further performed using the microdilution method. Quantitative evaluation was further performed using the microdilution method. The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that completely inhibits the visible growth of a microorganism after a defined incubation period. It is commonly determined using broth or agar dilution

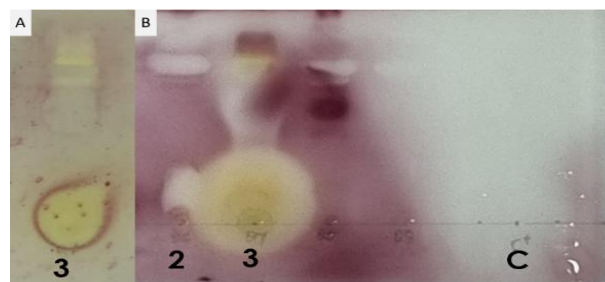


Fig. 5 TLC bioautography as antibacterial of active extract with eluted CH_2Cl_2 :MeOH (10:1) (A) *E. coli*, (B) *S. aureus*

methods, with microdilution assays being the most widely applied for natural product extracts due to their efficiency and reproducibility.

Among the fungal isolates evaluated, only *Aspergillus* section *Nigri* 12TBt-3 demonstrated antibacterial activity against both bacterial strains and was therefore selected for MIC determination. The TLC-bioautography assay further showed several inhibition zones on the chromatographic plates for both test bacteria, suggesting the presence of multiple bioactive constituents with antibacterial properties.

Extract *Aspergillus* section *Nigri* 12TBt-3 exhibited MIC values of 256 $\mu\text{g}/\text{mL}$ against *E. coli* and 128 $\mu\text{g}/\text{mL}$ against *S. aureus*. MIC values <100 $\mu\text{g}/\text{mL}$ are considered strong, those between 100–500 $\mu\text{g}/\text{mL}$ are moderate, and values >1000 $\mu\text{g}/\text{mL}$ are weak. Based on this criterion, extract *Aspergillus* section *Nigri* 12TBt-3 demonstrated moderate antibacterial activity.

3.4. Antioxidant Activity

The antioxidant activity of the endophytic fungal extracts was qualitatively evaluated using the TLC dot-blot assay with DPPH reagent. This method is considered simple, rapid, and efficient for preliminary screening of antioxidant metabolites. The assay is based on the ability of antioxidants to donate a hydrogen atom or an electron to the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH \cdot), converting it into its reduced, non-radical form (DPPH-H). This reduction is accompanied by a color change from purple to yellowish, due to the loss of absorbance at 517 nm [14].

The results revealed that extract *Aspergillus* section *Nigri* 12TBt-3 and *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2 exhibited positive activity, as indicated by the appearance of a yellowish spot against the purple background after spraying with DPPH. Further examination using TLC bioautography demonstrated the presence of bioactive spots responsible for the antioxidant activity. These were visualized as yellowish bands along the chromatographic profile, suggesting the occurrence of secondary metabolites with radical-scavenging potential (Fig. 6). TLC-Bioautography has been regarded as a valuable tool to guide the identification of bioactive constituents within crude extracts.

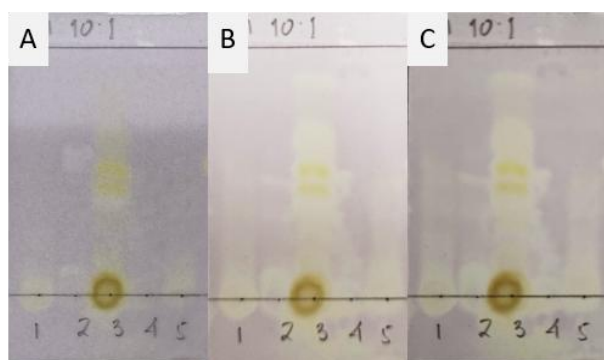


Fig. 6 TLC bioautography as antioxidant with eluted. CH_2Cl_2 :MeOH (10:1) (A) 0 min, (B) 15 min, (C) 30 min

Quantitative assessment using the DPPH radical scavenging assay showed that extract *Aspergillus* section *Nigri* 12TBt-3 had an IC_{50} value of 27.31 $\mu\text{g}/\text{mL}$ and an Antioxidant Activity Index (AAI) of 1.14. Extract *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2 had an IC_{50} value of 271.88 $\mu\text{g}/\text{mL}$ and an Antioxidant Activity Index (AAI) of 0.1131. Catechin was used as a positive control and exhibited a strong antioxidant activity, with an IC_{50} value of 2.0208 $\mu\text{g}/\text{mL}$ and an AAI of 15.217. According to Scherer and Godoy [23], based on the AAI classification, values <0.5 are considered weak, 0.5–1 moderate, 1–2 strong, and >2 very strong. Therefore, extract *Aspergillus* section *Nigri* 12TBt-3 can be categorized as possessing strong antioxidant activity, and extract *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2 can be categorized as possessing weak antioxidant activity, supported by AAI values.

3.5. Total Phenolic Content

The total phenolic content (TPC) of endophytic fungal extracts was determined using the Folin–Ciocalteu method. The results showed that extract *Aspergillus* section *Nigri* 12TBt-3 contained 257.179 mg GAE/g extract, while extract *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2 contained 67.249 mg GAE/g extract, indicating variation in phenolic metabolite levels between isolates.

Phenolic compounds are well known for their biological significance, particularly as antioxidants, due to their ability to donate hydrogen atoms or electrons to neutralize free radicals. The present findings are consistent with the antioxidant assay, in which extract *Aspergillus* section *Nigri* 12TBt-3, with a higher phenolic content, exhibited strong antioxidant activity (IC_{50} 27.31 $\mu\text{g}/\text{mL}$; AAI 1.1373), while extract *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2, with a lower phenolic content, showed weak activity (IC_{50} 271.88 $\mu\text{g}/\text{mL}$; AAI 0.1131). These results support previous studies that reported a positive correlation between phenolic content and antioxidant potential [15].

4. CONCLUSION

Five endophytic fungi from the leaves and stems of *Pluchea indica* (L.) Less were successfully isolated and identified into three main taxa namely *Aspergillus* section *Nigri* 12TBt-3, *Colletotrichum* sp. 12TDn-1, and *Diaporthe* sp. (*Phomopsis* sp.) 12TBt-1, 12TBt-2, and

12TDn-2. Chemical profile analysis using thin-layer chromatography (TLC) revealed the diversity of secondary metabolites in each isolate, with alkaloids and phenolic compounds detected in extracts *Aspergillus* section *Nigri* 12TBt-3 and *Diaporthe* sp. (*Phomopsis* sp.) 12TDn-2.

Antibacterial assays demonstrated that extract *Aspergillus* section *Nigri* 12TBt-3 exhibited moderate activity, with MIC values of 128 $\mu\text{g}/\text{mL}$ against *Staphylococcus aureus* and >256 $\mu\text{g}/\text{mL}$ against *Escherichia coli*. Furthermore, extract *Aspergillus* section *Nigri* 12TBt-3 showed antioxidant activity in the DPPH assay with an IC_{50} value of 27.31 $\mu\text{g}/\text{mL}$ and an AAI of 1.14, categorized as strong. The total phenolic content was 257.179 mg GAE/g extract for *Aspergillus* section *Nigri* 12TBt-3.

This study was limited to the evaluation of crude extracts obtained from each endophytic fungal isolate. The specific metabolites responsible for the observed antibacterial and antioxidant activities were not identified. Consequently, further investigation is needed to isolate and characterize the active compounds underlying these biological activities.

Overall, these findings indicate that endophytic fungi from *Pluchea indica* (L.) Less represent a promising source of bioactive metabolites with antibacterial and antioxidant activities, as well as significant phenolic content, making them valuable candidates for further development in biotechnology and pharmaceutical applications.

5. AUTHOR'S DECLARATION

5.1. Supporting Information

There is no supporting information in this paper.

5.2. Acknowledgements

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5.3. Conflict of Interest

The authors declared that no conflicts of interest regarding the publication of this article.

5.4. Author Contributions

All authors are the main contributors to this paper. The authorship contributions such as AFI, data collection, analysis of data, and drafting of the manuscript. S, helped with the conceptualization of the study and supervision. AF, sampling, data collection, and helped with the conceptualization of the study. MI, isolation and identification of microbial specimens. AA, conceptualization of the study, supervision, and manuscript preparation.

5.5. AI Statement

ChatGPT was utilize to enhance the clarity, grammar, and overall readability of this manuscript. All technical

content, data interpretation, and conclusion were solely developed and verified by the authors. The final version of the manuscript was thoroughly reviewed to ensure accuracy, coherence, and alignment with the study's findings.

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