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ARTIKEL

ISOLATION AND *IN VITRO* BIOASSAYS OF ENDOPHYTIC FUNGI ASSOCIATED WITH LIDAH BUAYA (*Aloe vera* (L.) Burm.f.)

[*Isolasi dan Uji In Vitro dari Jamur Endofit yang Berasosiasi dengan Tanaman Lidah Buaya* (*Aloe vera* (L.) Burm.f.)]

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ABSTRACT

Endophytic fungi are fungi that live in plant tissues that do not cause disease symptoms, do not harm the host plant, and can produce secondary metabolites such as antibacterial, antifungal, and antiviral compounds. This study was conducted to isolate and evaluate the antibacterial and antioxidant activities of endophytic fungi from *Aloe vera* (L.) Burm. f. against *Staphylococcus aureus* and *Escherichia coli* bacteria. The antibacterial activity assay was carried out qualitatively by thin layer chromatography-direct-bioautography (TLC-DB), and microdilution methods were used for the antibacterial assay against *Staphylococcus aureus* and *Escherichia coli*. Antioxidant activity was determined by free radical scavenging with DPPH reagent using TLC-DB and the microdilution assay. Antibacterial results showed that the extracts LBB-2.4, LBB-2.2, LBAu-1.1, and LBAu-2.1 could inhibit the growth of *S. aureus*. Meanwhile, extracts of endophytic fungi with the isolate codes LBB-2.4, LBB-2.1, LBAp-3, LBAp-1, LBAu-1.1, and LBAu-2.1 could inhibit the growth of *E.coli*. The results of Minimum Inhibitory Concentration (MIC) showed that isolate LBB-2.1 had moderate antibacterial activity with a MIC value of 256 µg/mL against *S.aureus*. The extracts showed weak antioxidant activity against DPPH. It can be concluded that endophytic fungi extracts from *Aloe vera* have potential activity as antibacterial agents.

Keywords: endophytic fungi, *Aloe vera*, antibacterial, antioxidant, TLC-DB, MIC

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ABSTRAK

Jamur endofit merupakan jamur yang hidup pada jaringan tanaman yang tidak menimbulkan gejala penyakit, tidak merugikan tanaman inangnya dan dapat menghasilkan senyawa metabolit sekunder seperti senyawa antibakteri, antijamur, dan antivirus. Penelitian ini dilakukan untuk mengisolasi dan mengevaluasi aktivitas antibakteri dan antioksidan jamur endofit dari Aloe vera (L) Burm.f. terhadap bakteri Staphylococcus aureus dan Escherichia coli. Uji aktivitas antibakteri dilakukan secara kualitatif dengan metode Kromatografi Lapis Tipis-Bioautografi Langsung (KLT-BL) dan metode mikrodilusi digunakan untuk uji antibakteri terhadap S.aureus dan E.coli. Aktivitas antioksidan ditentukan dengan metode peredaman radikal bebas terhadap DPPH menggunakan KLT-BL dan uji mikrodilusi. Hasil antibakteri menunjukkan bahwa ekstrak LBB-2.4, LBB-2.2, LBAu-1.1 dan LBAu-2.1 mampu menghambat pertumbuhan S. aureus. Sedangkan ekstrak jamur endofit dengan kode isolat LBB-2.4, LBB-2.1, LBAp-3, LBAp-1, LBAu-1.1 dan LBAu-2.1 mampu menghambat pertumbuhan E.coli. Hasil Konsentrasi Hambat Minimum (KHM) menunjukkan bahwa isolat LBB-2.1 mempunyai aktivitas antibakteri sedang dengan nilai KHM 256 µg/mL terhadap S.aureus. Semua ekstrak menunjukkan aktivitas antioksidan yang lemah terhadap DPPH. Dapat disimpulkan bahwa ekstrak jamur endofit tanaman lidah buaya mempunyai potensi aktivitas sebagai agen antibakteri.

Kata kunci: *jamur endofit, Aloe vera, antibakteri, antioksidan, KLT, KHM.*

INTRODUCTION

Medicinal plants have an important position in the pharmacology sector due to their wealth of bioactive compounds. *Aloe vera* is one of the therapeutic plants; it contains vitamins A, B1, B2, B6, B12, C, and E (Thu *et al*., 2013). Some active compounds are also found, such as alkaloids, polyphenols, phytosterols, and indoles, all of which have the ability to act as antioxidants and lessen or prevent the symptoms of diabetes, cancer, heart disease, and neurodegeneration (Nejatzadeh-Barandozi, 2013; Thu *et al*., 2013). This plant has many biological activities, including antimicrobial activity, anti-diabetic effects, antioxidant activity, anti-inflammatory and gastrointestinal protection (Danish *et al*., 2020). Excessive exploitation to obtain secondary metabolite compounds from a natural material can cause extinction, therefore, one solution that can be used is through the development of endophytic microbes as producers of secondary metabolite compounds (Elviasari *et al*., 2016). However, studies related to endophytic fungi isolated from *Aloe vera* have not been widely conducted. More than one million different strains of endophytic fungi have been reported to inhabit about 300,000 plant species (Pál *et al*., 2021).

Endophytic fungi associated with medicinal plants from Indonesia are a promising resource to be developed as active secondary metabolite producers. In general, endophytic fungi and host plants have a mutual-symbiotic relationship, while they can also have parasitic, commensal, saprophytic, or mutualistic connections (Ilyas *et al*., 2019). Many studies have explored the pharmaceutical potential of endophytic fungi associated with Indonesian medicinal plants. Some of them have antibacterial and antioxidant potential such as *Ziziphus spina-christi, Physalis angulata*, as well as several endemic plants from North Maluku (Agusta *et al*., 2022; Palupi *et al*., 2021; Raunsai *et al*., 2023). In this study, endophytic fungi isolated from *Aloe vera* (L.) Burm.f. collected in Duren Sawit, East Jakarta, Indonesia, were evaluated for their antibacterial and antioxidant properties. The results were explored and presented.

MATERIALS AND METHODS

Materials

Aloe vera (L) Burm.f. fresh plant was obtained from Sawah Barat Dalam II, Duren Sawit, East Jakarta, Indonesia. The plant was first determined at the Herbarium Bogoriense, BRIN Cibinong, Indonesia. The plant parts used were the leaves, stems, and roots.

Bacteria used in Antibacterial Assay

Bacterial isolates, namely *S. aureus* InaCC-B4 and *E. coli* InaCC-B5, were used for the antibacterial test. These are microbial collections of the Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN).

Sterilization and Isolation of Endophytic Fungi

Sterilizing and isolating endophytic fungus are referred to Alexopoulos *et al*, (1996) and Ilyas *et al*, (2006). *Aloe vera* was washed using running water and then sterilized on the surface. The surface sterilization process has several stages using two chemical solvents, such as 70% ethanol and 5.25% sodium hypochlorite. The sterilized samples were cut and placed on Corn Meal Malt Agar (CMMA) and incubated at room temperature for 5-7 days. Colonies that appeared were subcultured several times using Potato Dextrose Agar (PDA) to gain pure isolates. The whole process was carried out in laminar airflow.

Morphological Identification of Endophytic Fungi

Initial fungus identification was carried out based on morphological characteristics. Pure endophytic fungi were transferred and grown on PDA plates and then incubated at 27˚C for 5 -7 days. The purified fungal strains were then selected for working and backup cultures. In order to maintain the strain characteristics, the backup cultures were kept based on long-term inactive metabolism (Rohadi *et al*., 2020). Both macroscopic and microscopic characteristics were observed to perform morphological identification. Macroscopic characterizations consist of color, surface, texture, exudate drop, colony shape, and reverse color. Lactophenol was used as a mounting medium to create microscopic slides of each chosen strain. A light microscope was used to undertake microscopic characterizations, which involved observing conidia, spores, septate, pigmentation of the hyphae, clamp connection, and other reproductive features. Microscopic features were studied from colonies on PDA media after 7–15 days of incubation at 27°C.

Cultivation and Extraction of Endophytic Fungi

To cultivate and extract the endophytic fungi, we followed the method used by Praptiwi, *et al.,* (2018). Cultivation was performed by taking pure isolates of each endophytic fungus and transferring them into Potato Dextrose Broth (PDB) media (200 mL). All media were incubated at room temperature for 3 weeks in dark conditions. Following the cultivation phase, the liquid-liquid extraction method was implemented to extract the growth medium and endophytic fungal biomass using an ethyl acetate solvent. The extract was evaporated with a rotary evaporator, and the thick extract was put into vials.

TLC-Based Chemical Compound Analysis

Chemical compound analysis of endophytic fungal extracts was implemented on Thin Layer Chromatography (TLC) plates (silica gel GF_{254}, Merck). Dried extracts were prepared at a concentration of 10 mg/mL. A total of 10 μL of the extract was transferred to the TLC plate and developed in CH_2Cl_2:MeOH (10:1). The KLT plates were visualized under ultraviolet (UV) light at λ 254 nm and 366 nm, followed by spraying with 1% $Ce(SO_4)_2$ and 1% vanillin sulfuric acid reagents.

Qualitative Antibacterial Assay

The method used in the antibacterial quantitative assay is TLC-Direct Bioautography (Dewanjee *et al*., 2015). After the samples were transferred to the TLC plate, the plate was sprayed with iodonitrotetrazolium p-violet (INT Sigma). At this stage, the inhibition of bacterial growth was observed to see white zones around the extract. The active extract would be further analyzed using the mobile phase, CH2Cl2:MeOH (10:1), and performed using the same approach. The profile of the bacterial growth inhibition area (white zones) would be seen on the eluted TLC plate.

Quantitative Antibacterial Assay

Serial microdilution in 96-well microplates was used to determine the Minimum Inhibitory Concentration (MIC) of the active extracts (Praptiwi *et al*., 2018; Wulansari *et al*., 2017). Following the dilution procedure, 100 µL of bacterial suspension (10^5 CFU/mL) was added to each well. Cloramphenicol and growth media were used as a positive and negative control. Then, 96-well microplates containing media and samples were covered with parafilm and incubated at 37°C for 18 hours. After that, 10 µL of INT (4 mg/mL) was added to each well. The test was performed in triplicate and there were eight concentrations used in the microwell plate, ranging from 2 - 256 µg/mL. The MIC value was determined based on the formation of a clear color in the well after adding INT solution.

Qualitative Antioxidant Activity

All extracts were tested their for antioxidant activity using the TLC-DB method as previously described by Praptiwi *et al*., (2021). After spraying with 1mM DPPH in methanol, antioxidant activity can be detected by the formation of a white-yellow zone on the purple background of the TLC plate.

Quantitative Antibacterial Activity

The determination of Inhibition Concentration (IC_{50}) of the extracts used the microdilution method as described by Fathoni *et al*., (2022) and Scherer & Godoy, (2009). There were eight concentrations used in the microwell plate, ranging from 4 - 512 µg/mL. The absorbance was calculated using a spectrophotometer (ThermoScientific Varioskan Flash) at λ 517 nm. The absorbance data collected was then analyzed using the Microsoft Excel program to calculate the half-maximal inhibitory concentration (IC_{50}) value. This formula below was used to determine the IC_{50} (Scherer & Godoy, 2009):

$$\text{IC (\%)} = \frac{Abo - Abs}{Abo} \text{ x } 100\ \%$$

IC = Inhibitory Concentration
Abo = the absorbance of the DPPH.
Abs = absorbance of DPPH combined with various extract concentrations.

RESULTS

Characterization of endophytic fungi associated with Aloe vera

A total of nine strains of endophytic fungi were isolated and selected from the stem, root base, and root tip of *Aloe vera*. Furthermore, fungal endophytes were morphologically identified into four fungal taxa; *Chaetomium* sp., *Colletotrichum* sp., dark mycelia sterilia of Dematiaceae, and *Talaromyces* sp. (Table 1). There were nine isolates of endophytic fungi cultivated with PDB media within 21 days (Figure 1).

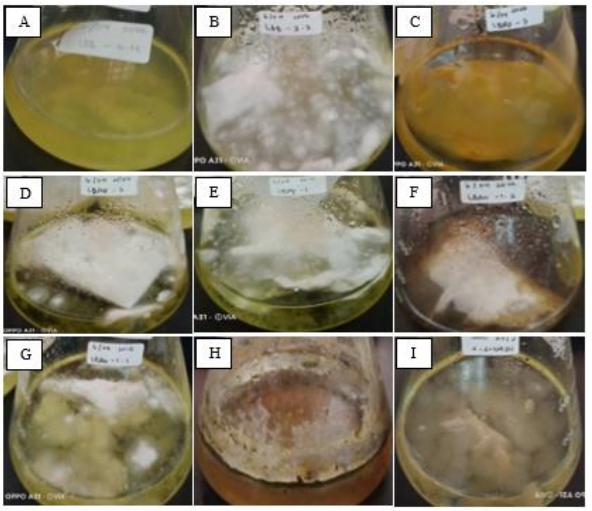

Figure 1. Endophytic fungi cultivated with PDB media within 21 days (*Jamur endofit yang dikultivasi dengan media PDB dalam waktu 21 hari*). A: LBB-2.4, B: LBB-2.1, C: LBAp-3, D: LBAp-2, E: LBAp-1, F: LBAu-1.2, G: LBAu-1.1, H: LBAu-1.3, I: LBAu-2.1.

Chromatographic profiling analysis was also used to characterize the secondary metabolites produced by the isolated endophytic fungus. Analysis with the Thin Layer Chromatography (TLC) method has the aim of knowing the chromatogram pattern of compounds that are present in the extract. There are benefits to this method, including the ability to separate, identify, and quantify various compounds within the extracts (Fathoni *et al*., 2022).

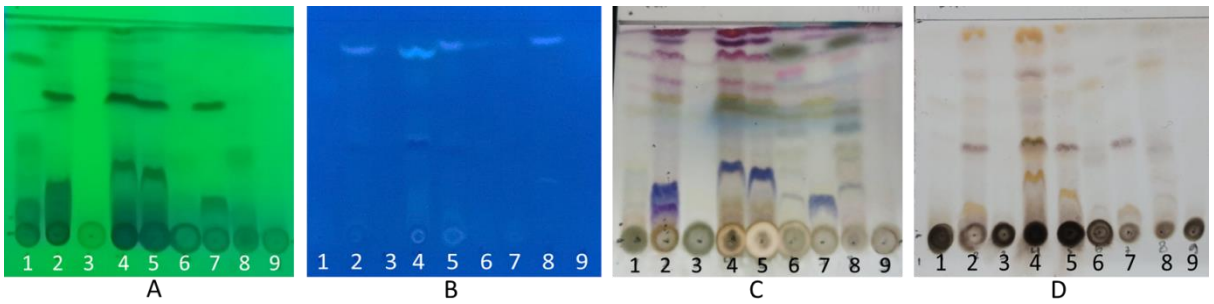

Figure 2. Chromatograms of endophytic fungal extracts developed in dichloromethane-methanol (10:1 v/v), (a) viewed under 254 nm wavelength, (b) viewed under 366 nm wavelength, (c) sprayed with vanillin reagent, (d) sprayed with cerium reagent. Numbers 1 – 9 are representative samples in Table 1. (*Kromatogram ekstrak jamur endofit dikembangkan dalam diklorometan-metanol (10: 1 v / v), (a) dilihat di bawah panjang gelombang 254 nm, (b) dilihat di bawah panjang gelombang 366 nm, (c) disemprot dengan pereaksi vanilin, (d) disemprot dengan pereaksi cerium. Angka 1 – 9 adalah sampel representatif pada Tabel 1*.)

Observation under UV 254 nm revealed the existence of many chemical substances (Figure 2A). Each extract had various chemical components that emit dark colored compounds. Under 366 nm, the observation revealed that the TLC plate emitted bright colored spots and a purple background (Figure 2B). Moreover, after spraying with vanillin and cerium reagents, the distribution of secondary metabolite spots with various retention factors (Rf) and colors can be seen (Figure 2C and 2D).

Qualitative Antibacterial Activity

The TLC dot-blot analysis for antibacterial activity revealed that four samples actively impeded the growth of *S. aureus* and five samples hindered the growth of *E. coli*. The samples were developed on a TLC plate, and some white spots were seen (Figure 3).

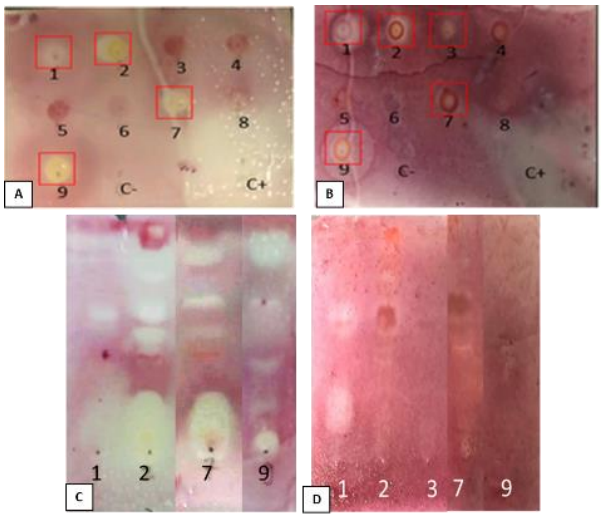

Figure 3. Bioautograms of endophytic fungi associated with *A.vera* plant. TLC dot-blot assay against *S.aureus* (A) and *E. coli* (B) and the active extracts that were developed in dichloromethane : methanol (10:1 v/v) on TLC plates against *S.aureus* (C) and *E. coli* (D). Clear bands indicated antibacterial activity. (*Bioautogram jamur endofit yang terkait dengan tanaman A.vera. Uji dot-blot TLC terhadap S.aureus (A) dan E. (B) dan ekstrak aktif yang dikembangkan dalam diklorometan: metanol (10: 1 v / v) pada pelat TLC terhadap S.aureus (C) dan E. (D). Pita bening menunjukkan aktivitas antibakteri*)

The extracts with antibacterial activity were then determined the minimum inhibitory concentration (MIC) using the microdilution method (Table 1). The results showed that there was only one sample, LBB-2.1, which had moderate antibacterial activity against *S.aureus*. In addition, other extracts had antibacterial activity above 256 ug/mL against both *E.coli* and *S.aureus*. The classification of antibacterial activity refers to Kuete (2010).

Qualitative Antioxidant Activity

The antioxidant activity test with the TLC-DB method showed that there are six extracts that can reduce DPPH activity, as indicated by the white color on the dot blot and eluted plates (Figure 4).

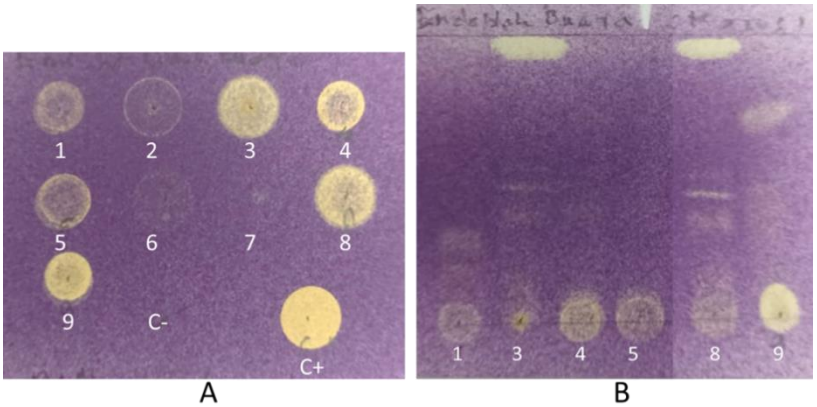

Figure 4. TLC dot-blot (A) and bioautogram (B) on the plate were developed in dichloromethane:methanol (10:1 v/v). Color appearance after 10 minutes of spraying with DPPH (*TLC dot-blot (A) dan bioautogram (B) pada pelat dikembangkan dalam diklorometan: metanol (10: 1 v / v). Penampilan warna setelah 10 menit penyemprotan dengan DPPH*).

The extracts with antioxidant activity were then determined their IC_{50} values, which are shown in Table 1. When compared to the positive control catechin, which had an IC_{50} value of 0.166 ug/mL in the category of very strong antioxidant activity, the other six samples had weak antioxidant activity above 512 ug/mL. The antioxidant activity is categorized based on the study of Scherer & Godoy (2009).

Table 1. Morphological identification and bioassays results of nine fungus endophytes associated with *A.vera* plant

No.	Isolate (*Isolat*)	Plant part (*Bagian Tumbuhan*)	Taxa (*Taksa*)	TLC-DB			MIC (µg/mL)		IC_{50} DPPH (µg/mL)
				E.coli	***S.aureus***	**Antioxidant (*Antioksidan*)**	***E.coli***	***S.aureus***	
1	LBB-2.4	Stem	*Talaromyces* sp.	+	+	+	>256	>256	>512
2	LBB-2.1		*Colletotrichum* sp.	+	+	-	>256	256	-
3	LBAp-3	Root base	*Chaetomium* sp.	+	+	+	>256	>256	>512
4	LBAp-2		*Talaromyces* sp.	-	-	+	-	-	>512
5	LBAp-1		*Talaromyces* sp	-	-	+	-	-	>512
6	LBAu-1.2	Root tip	Dematiaceae	-	-	-	-	-	-
7	LBAu-1.1		*Talaromyces* sp.	-	+	-	-	>256	-
8	LBAu-1.3		Dematiaceae	-	+	+	-	>256	>512
9	LBAu-2.1		*Chaetomium* sp.	+	+	+	>256	>256	>512
10	Catechin			NT	NT	+	NT	NT	0.166
11	Cloramp			+	+	NT	8	8	NT

Notes: Cloramp: Cloramphenicol, NT: Not tested, (+): active, (-): not active. (*Catatan: Cloramp: Cloramphenicol, NT: Tidak diuji, (+): aktif, (-): tidak aktif*)

DISCUSSION

Characterization of endophytic fungi associated with *Aloe vera*

Table 1 shows that of the total nine selected strains, based on their morphological characteristics, three genera and one group of sterile strains were identified as Dematiaceae. The dynamic and complex habitats of higher vascular plants are influenced by a multitude of factors that impact the composition and structure of endophytes that occupy the plant niches found in their roots, stems, branches, and leaves (Rubini *et alet al*., 2005). Several species of the common fungus *Chaetomium* are found as endophytes, coexisting with healthy plants, and have a worldwide range. *Chaetomium* fungi have been reported to produce structurally distinct and complex natural compounds with a range of important biological functions, including antioxidant, antimalarial, cytotoxic, anticancer, and enzyme inhibitory properties. (Zhang *et al*., 2012). Anthracnose in a variety of plant hosts is caused by the significant pathogenic genus *Colletotrichum*. Nonetheless, they represent one of the most often isolated endophytic fungal groupings, with a broad spectrum of host species. Some of the benefits of endophytic *Colletotrichum* species to their hosts include disease resistance, drought tolerance, and growth enhancement (Dini-Andreote, 2020). Genus *Talaromyces* has been more and more widely reported for endophytic occurrence. *Talaromyces* was found to be a common phyllosphere, phylloplane, and endophyte fungus in different medicinal plants, which has potential for antimicrobial activity and novel secondary metabolites (Zhao *et al*., 2021).

Based on Figure 1, it can be seen that the growth of endophytic fungi varies in each part of the tissue. After 21 days, some fungi had good growth, while others did not show significant mass gain. According to Sun *et al*. (2012), the population of endophytic fungi was greatly impacted by the species and types of host plants, where the growth of endophytic fungal colonies was greater in certain parts of the plant.

Regarding the chemical compound analysis, aromatic groups are indicated by substances with a λ_{max} of 200–400 nm (Praptiwi *et al*., 2018). In Figure 2C, it can be seen that many purple to blue colored spots appeared when treated with vanillin reagent. These colors indicate the presence of terpenoids (Ambarwati *et al*., 2015). Moreover, a prominently brown area appeared after the plate was sprayed with cerium (Figure 2D). These colors signify the presence of phenolic compounds (Azizah & Afghani Jayuska, 2015).

Biological Assays of Endophytic Fungi Cultures

Endophytic microorganisms have been used in numerous studies to explore their pharmaceutical properties. Endophytic fungi are a viable source of novel natural products and can produce bioactive chemicals that have the ability to suppress infections (Jalil *et al*., 2022). In this study, six types of endophytic fungi from *Aloe vera* (L) Burm.f. performed antimicrobial activity, but for the DPPH assay, all extracts exhibited weak antioxidant activity. The antibacterial activity was found in several plant parts, such as the stem, root base and root tip. A study by Palupi *et al*., (2023) also displayed that endophytic fungi from *Uncaria gambier* isolated from various plant parts could inhibit the growth of *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Micrococcus luteus.* Some studies have isolated endophytic fungi associated with *Aloe vera* from several countries. Five endophytic fungi were isolated from *Aloe vera* in India, and one of them showed activity in antioxidant, wound healing, and anticancer (Ameen *et al*., 2021). A total of nine endophytic fungi have also been isolated from the leaves and roots of *Aloe vera* from India, and some of them can be used as bio-inoculums to increase agricultural productivity (Yadav *et al*., 2016). Several studies from Bara *et al*. (2013) and El-Amrani *et al*. (2016) have successfully isolated eleven metabolites from *Talaromyces wortmanii* such as atropisomers and wortmannin derivatives, and that three of them were active as antibiotics, as well as pestalotiopamide E and pestalotiopin B that were isolated from *Aureobasidium pullulans* could exhibit antiproliferative activity. Both fungal endophytes were isolated from *Aloe vera* in Morocco and Egypt.

In this study, eight types of endophytic fungi from *Aloe vera* (L.) Burm.f. exhibited antibacterial and antioxidant activities. In order to investigate the antibacterial and antioxidant potencies of the active endophytic fungus, future research is required to isolate and identify the active chemicals causing the activities of these fungal endophytes and also to see other pharmaceutical potential.

CONCLUSION

A variety of endophytic fungi were found in *Aloe vera* (L.) Burm.f. gathered in Duren Sawit, East Jakarta, and many of the endophytic fungi that were isolated produced compounds with antibacterial activity. These suggest that endophytic fungi from *Aloe vera* (L.) Burm.f. should be further explored and studied as possible sources of active metabolites.

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AUTHORSHIP CONTRIBUTION

All authors have contributed equally to this paper. The following are each author's specific roles. MMR: conceptualization of the study, data analysis, and manuscript drafting. OST: Collection of the samples in the field and conducting biological activity assays. ASK, P, and KDP: Sample preparation, collecting and analyzing data. MI: isolation and identification of microbial specimens and manuscript writing. RR: conceptualization of the study and analysis of data. E: data collection and manuscript writing. AA: conceptualization of the study, supervision, and manuscript preparation.

REFERENCES

Agusta, A., Lestari, H. P., Ridwan, R., Ilyas, M., Evana, E., Praptiwi, P. 2022. Antioxidant activity of endophytic fungi culture extracts of Christ's thorn jujube (*Ziziphus spina-christi*). *Journal of Research in Pharmacy*, 26(6), pp.1758–1770. https://doi.org/10.29228/jrp.266

Alexopoulos, C. J., Mims, C. W., & Blackwell, M. 1996. *Introductory mycology.* (Issue Ed. 4). John Wiley and Sons.

Ambarwati, N., Rakhmawati, R., Wahyuni, D. S. C. 2015. Uji toksisitas fraksi daun ambre (*Geranium radula*) terhadap *Artemia salina* dan profil kandungan kimia fraksi teraktif. *Biofarmasi*, 13(1), pp.15–24. https://doi.org/10.13057/biofar/f130103

Ameen, F., Stephenson, S. L., AlNadhari, S., Yassin, M. A. 2021. Isolation, identification and bioactivity analysis of an endophytic fungus isolated from *Aloe vera* collected from Asir desert, Saudi Arabia. *Bioprocess and Biosystems Engineering*, *44*, pp. 1063–1070. https://doi.org/10.1007/s00449-020-02507-1

Azizah, N., Afghani Jayuska, H. 2015. Aktivitas anti rayap ekstrak daun jeruk bali (*Citrus maxima* (Burm.) Merr.) terhadap rayap tanah *Coptotermes* sp. *Jurnal Kimia Khatulistiwa, 4*(3). pp.33-39

Bara, R., Aly, A. H., Pretsch, A., Wray, V., Wang, B., Proksch, P., Debbab, A. 2013. Antibiotically active metabolites from *Talaromyces wortmannii*, an endophyte of *Aloe vera. The Journal of Antibiotics*, 66(8), pp. 491–493. https://doi.org/10.1038/ja.2013.28

Danish, P., Ali, Q., Hafeez, M. M., Malik, A. 2020. Antifungal and antibacterial activity of *Aloe vera* plant extract. *Biological and Clinical Sciences Research Journal*, *2*020(1). https://doi.org/10.54112/bcsrj.v2020i1.4

Dewanjee, S., Gangopadhyay, M., Bhattacharya, N., Khanra, R., & Dua, T. K. 2015. Bioautography and its scope in the field of natural product chemistry. *Journal of Pharmaceutical Analysis*, 5(2), pp. 75–84.

El-Amrani, M., Ebada, S. S., Gad, H. A., Proksch, P. 2016. Pestalotiopamide E and pestalotiopin B from an endophytic fungus *Aureobasidium pullulans* isolated from *Aloe vera* leaves. *Phytochemistry Letters*, 18, pp. 95–98. https://doi.org/10.1016/j.phytol.2016.09.006

Elviasari, J., Rusli, R., Ramadhan, A. M. 2016. Identifikasi Metabolit Sekunder Dan Uji Aktivitas Antibakteri Isolat Jamur Endofit Daun Beluntas (*Pluchea Indica* (L.) LESS.). *Jurnal Sains Dan Kesehatan*, 1(5), pp. 214–220. https://doi.org/10.25026/jsk.v1i5.42

Fathoni, A., Ilyas, M., Wulansari, D., Agusta, A. 2022. Antibacterial and Antioxidant Activities of Fungal Endophytes Isolated from Medicinal Plants in Simeulue Island, Aceh. *HAYATI Journal of Biosciences*, 29(6), pp. 720–732. https://doi.org/10.4308/hjb.29.6.720-732

Ilyas, M., Rahmansyah, M., Kanti, A. 2006. *Seri panduan: Teknik isolasi fungi*. LIPI-Press, Jakarta.

Ilyas, M., Wulansari, D., Fathoni, A., Agusta, A. 2019. An assemblages of fungal endophytes isolated from medicinal plants collected from Toba and Samosir, North Sumatra. *IOP Conference Series: Earth and Environmental Science*, 308(1), p.12070. https://doi.org/10.1088/1755-1315/308/1/012070

Jalil, M. T. M., Zakaria, N. A., Suhaimi, N. S. M., Ibrahim, D. 2022. Crude extracts of an endophytic fungus attenuate the growth of pathogenic bacteria in aquaculture. *Iranian Journal of Microbiology*, 14(3), pp. 383–394. https://doi.org/10.18502/ijm.v14i3.9780

Kuete, V. 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. *Planta Medica*, 76(14), pp. 1479–1491.

Nejatzadeh-Barandozi, F. 2013. Antibacterial activities and antioxidant capacity of *Aloe vera*. *Organic and Medicinal Chemistry Letters*, 3(5), pp. 1–8. https://doi.org/10.1186/2191-2858-3-5

Pál, S. E., Tóth, R., Nosanchuk, J. D., Vágvölgyi, C., Németh, T., Gácser, A. 2021. A *Candida parapsilosis* overexpression collection reveals genes required for pathogenesis. *Journal of Fungi*, 7(2), p.97. https://doi.org/10.3390/jof7020097

Palupi, K. D., Ilyas, M., Agusta, A. 2021. Endophytic fungi inhabiting *Physalis angulata* L. plant: diversity, antioxidant, and antibacterial activities of their ethyl acetate extracts. *Journal of Basic and Clinical Physiology and Pharmacology*, 32(4), pp. 823–829. https://doi.org/10.1515/jbcpp-2020-0479

Palupi, K. D., Kamal, A. S., Marlina, L., Hafid, L., Tarigan, E. B., Raunsai, M. M., Ilyas, M., Kanti, A., Agusta, A. 2023. Biodiversity and antibacterial activities of endophytic fungi associated with *Uncaria gambier* (hunter) roxb. var. udang from Jasinga, Bogor, Indonesia. *Berita Biologi*, 22(2), pp. 205–213. https://doi.org/10.55981/beritabiologi.2023.713

Praptiwi, Ilyas, M., Palupi, K. D., Fathoni, A., Evana, Raunsai, M. M., Agusta, A. 2021. Rapid screening of antibacterial and antioxidant metabolites from endophytic fungi isolated from

Papuacedrus papuana by TLC-bioautography. *Jurnal Kimia Dan Kemasan*, 43(2), pp. 110–116. https://doi.org/10.24817/jkk.v43i2.6980

Praptiwi, P., Raunsai, M., Wulansari, D., Fathoni, A., Agusta, A. 2018. Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plants from Central Sulawesi. *Journal of Applied Pharmaceutical Science*, 8(8), pp. 69–74.

Raunsai, M. M., Palupi, K. D., Praptiwi, P., Ilyas, M., Agusta, A. 2023. Bioprospection of endophytic fungi associated with plants from Central Halmahera, North Maluku as antibacterial and antioxidant agents. *AIP Conference Proceedings*, 2972(1), p. 050018. https://doi.org/10.1063/5.0183362

Scherer, R., Godoy, H. T. (2009). Antioxidant activity index (AAI) by the 2, 2-diphenyl-1-picrylhydrazyl method. *Food Chemistry*, 112(3), pp. 654–658. https://doi.org/10.1016/j.foodchem.2008.06.026

Sun, X., Ding, Q., Hyde, K. D., Guo, L. D. 2012. Community structure and preference of endophytic fungi of three woody plants in a mixed forest. *Fungal Ecology*, 5(5), pp. 624–632. https://doi.org/10.1016/j.funeco.2012.04.001

Thu, K., Mon, Y. Y., Khaing, T. A., & Tun, O. M. 2013. Study on Phytochemical Properties, Antibacterial Activity and Cytotoxicity of *Aloe vera* L. *International Journal of Biotechnology and Bioengineering*, 7(5), pp. 285–289.

Wulansari, D., Putra, A. P., Ilyas, M., Praptiwi, P., Fathoni, A., Palupi, K. D., Agusta, A. 2017. Skrining beberapa jamur endofit tumbuhan dari Pulau Enggano, Bengkulu sebagai antibakteri dan antioksidan. *BERITA BIOLOGI*, 15(3), pp. 227–235.

Yadav, R., Singh, A. V., Kumar, M., Yadav, S. 2016. Phytochemical analysis and plant growth promoting properties of endophytic fungi isolated from Tulsi and *Aloe vera*. *Int J Agric Stat Sci*, 12(1), pp. 239–248.