

Screening of Endophytic Fungi of *Melaleuca cajuputi* Powell Leaves as Antibacterial Sources

by Hary Widjajanti

Submission date: 21-Sep-2022 12:38PM (UTC+0700)

Submission ID: 1905188285

File name: 38_Proceeding_IMC-SciMath_2022_compressed.pdf (346.86K)

Word count: 4852

Character count: 26674

Screening of Endophytic Fungi of *Melaleuca cajuputi* Powell Leaves as Antibacterial Sources

Hary Widjajanti, Salni, Niken Irfa Nastiti and Elisa Nurnawati

Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Palembang, Indonesia

Keywords: Antibacterial, Endophytic fungi, *Melaleuca cajuputi* Powell.

Abstract: *Melaleuca cajuputi* leaves contain high flavonoids, flavonoids are chemical compounds that are antibacterial. Antibacterial bioactive compounds are found in plants, but the use of plants as an antibacterial source requires a large amount of biomass. Endophytic fungi that live in plant tissue can produce secondary metabolites that potentially as an antibacterial compounds. Isolation of the endophytic fungi for antibacterial sources can reduce the large amount of plant as a source of antibacterial agents. The aims of this research were obtain endophytic fungi of *M. cajuputi* leaves as an antibacterial sources. The research stages were sampling, isolation and purification of endophytic fungi, cultivation and production of secondary metabolites, antibacterial activity and the minimum inhibitory concentration (MIC) tests, thin layer chromatography, and characterization and identification of endophytic fungi that potentially as an antibacterial sources. The results there were 7 isolates of endophytic fungi from *Melaleuca cajuputi* leaves. MC₁, MC₂, MC₃, and MC₄ fungal isolates have a strong antibacterial activities. The MIC of MC₂ and MC₃ extracts on *Escherichia coli* were 4100 µg/mL and 100 µg/mL. The MIC of MC₁ and MC₄ extracts on *Staphylococcus aureus* were 200 µg/mL and 700 µg/mL, respectively. The extract of four endophytic fungi contain phenol and flavonoid that potentially as an antibacterial. Endophytic fungi MC₁ isolate identified as *Scopulariopsis candida*, MC₂ isolate as *Fusarium equiseti*, MC₃ isolate as *Fusarium sporotrichoides*, and MC₄ isolate as *Mucor hiemalis*.

1 INTRODUCTION

A number of plants have been passed down from generation to generation used as medicine by almost every community, including in Indonesia. Plants that are used as medicinal plants, have now begun to be explored scientifically by analyzing the content of active compound that cause medicinal properties. The active compound in the plant are too few or minor, therefore if it is to be developed on a large scale there are constraints from the raw material of the plant that must be in large quantities.

Antibacterial compounds in the form of crude extracts produced from plants when purified, the amount will be even less. These obstacles make it very difficult if the development of bioactive compounds from medicinal plants that are rare and endemic will be carried out. This is because if rare plants are explored for bioactive compounds to be taken, then the sustainability of these plants is threatened. These constraints can be anticipated by isolating microbes, especially endophytic fungi that

are symbiotic with these plants. The technique used to take only a few parts of plants commonly used in traditional medicine, then the isolation of endophytic fungi from the plant parts is done, so that rare medicinal plants will remain sustainable and the development of medicinal compounds from these plants through endophytic fungi can be developed.

The development of medicinal plants based on medicinal plants must be considered aspects of preservation of medicinal plants. One technology can be done was isolate endophytic fungi from parts of medicinal plants that are often used in traditional medicine and selection of bioactive secondary metabolites such as antibacterial from the culture of endophytic fungi, thus the development of medicinal plants based on medicinal plants can be done and the preservation of medicinal plants especially that are already scarce can be maintained.

Endophytic fungi are fungi that live in plant tissues in a certain period and are able to live by forming colonies in plant tissue without endangering the host. Each higher level plant can contain several

20
endophytic microbes that produce secondary metabolites that are suspected as a result of coevolution or genetic recombination from host plants to endophytic microbes (Tan and Zou (2001), Strobel and B. Daisy (2003).

The ability of endophytic fungi to produce secondary metabolites in accordance with their host plants is a very large and reliable opportunity to produce secondary metabolites from endophytic microbes isolated from these host plants. Approximately 300,000 species of plants scattered on this earth, each plant contains one or more endophytic microbes consisting of bacteria and fungi (Strobel and Daisy, 2003). If the endophytes isolated from a medicinal plant can produce the same alkaloids or secondary metabolites as the original plants or even in higher amounts, then we don't need to cut down the original plants to be taken as *simplicia*, which is likely to take decades to be harvested (Radji, 2005). According to Stierlie *et al.* (1995) the use of endophytic microbes in producing active compounds has several advantages, including (1) faster producing of uniform quality, (2) can be produced on a large scale, and (3) the possibility of obtaining new bioactive components by providing conditions that are new different.

M. cajuputi can live on land that has limited nutrients from fertile soil and is rich in nutrients. This plant is also able to live in soil with critical conditions with little nutrient elements. *M. cajuputi* leaves in Indonesia are widely used as raw materials in the production of eucalyptus oil (Widiana *et al.*, 2014). *M. cajuputi* leaves has many benefits, one of which can be used as an antibacterial source (Al-Abd *et al.*, 2015). *Melaleuca cajuputi* has potential as an antibacterial, antioxidant, and even potentially an antifilaria. (Al-Abd² *et al.*, 2016). *M. cajuputi* has been used traditionally for the treatment of diseases such as coughing, stomach cramps, burns, and influenza. This plant also exhibits anti-inflammatory, antidengue, anticancer and anticonvulsant activities. The active compound of *M. cajuputi* leaves were trans caryophyllena, β -selinene, germacrene (C₁₅H₂₆O), neopitadiene, cyclohexakarbonsaldehyde, β -caryophyllena, 3-methoxy benzoic acid trimethylsilan; limonene; 1,4 naphthoquinone-5,8-dihydroxy-2-methoxy; β -carene; α -caryophyllena; cineol; patchulin; ethyl benzene; benzene 1-metil-3 (metiletyl), and 1.3% volatile oil [10]. Essential oils are one of the compounds found in plants that have antibacterial activity (Ajizah, 2014).

M. cajuputi leaves also contain high flavonoids, flavonoids are chemical compounds that are

antibacterial (Al-Abd *et al.*, 2015). The aims of the research were 1). Get endophytic fungi isolates from *Melaleuca cajuputi* Powell that are able to produce secondary metabolites that are antibacterial and antioxidant, 2). Conducting *in vitro* tests to verify the antibacterial and antioxidant properties of secondary metabolites of the plant's endophytic fungi of *Melaleuca cajuputi* Powell, 3). Identify the isolates of endophytic fungi of *Melaleuca cajuputi* Powell which have high potential to produce antibacterial.

13 2 MATERIALS AND METHOD

2.1 Isolation and Purification of Endophytic Fungi

The sterile leaves are cut 2-3 cm aseptically, then placed in a commercial Potato Dextrose Agar powder (PDA) (20 g dextrose, 15 agar, and 4 g potato starch) medium. Incubated at room temperature until the fungi appeared to grow. The different fungal colonies on PDA medium are further purified on new PDA medium.

2.2 Cultivation of Endophytic Fungi and Secondary Metabolite Extraction

50
Twenty agar plug of endophytic fungi isolates inoculated into 500 mL Potato Dextrose Broth (PDB) medium and incubated at room temperature for \pm 30 days. After a change in the color of the medium which indicates the production of secondary metabolites, the medium was extracted using an ethyl acetate solvent (1: 1) and concentrated with a rotary evaporator to obtain a crude extract of secondary metabolites (Jamal *et al.*, 2008).

2.3 Antibacterial Test

7
A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate (BaCl₂.2H₂O), with 9.95 mL of 1% sulfuric acid (H₂SO₄). The 0.5 McFarland solution measured the absorbance using a UV-Vis spectrophotometer (625 nm). The absorbance value obtained is equivalent to 1.5 x 10⁸ bacterial cells/mL (Fatisa, 2013).

Escherichia coli, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Shigella dysenteriae* were inoculated into a physiological saline solution (0.85% NaCl) then homogenized and measured their

absorbance using a UV-Vis spectrophotometer (625 nm). The absorbance of the suspension test bacteria was prepared similar to the McFarland standard solution to reach the density of 1.5×10^8 CFU/mL (Kusla, 2014).

The antibacterial activity test was performed using disc diffusion method (Kirby-Bauer). The bacterial suspension was inoculated as much as 0.1 ml into a Mueller-Hinton Agar (MHA) medium with a spread plate methods. Crude extracts of secondary metabolites made concentrations of 400 µg/disc (4%) and standard antibiotic tetracycline 30 µg/disc (0.3%) as positive control. The disc paper saturated in a solution of secondary metabolite extract and tetracycline antibiotics then inoculated on the surface agar containing bacterial suspension and incubated at room temperature for 24 hours (Islam *et al.*, 2013). The criteria for each concentration of antibacterial compounds tested against standard antibiotics were determined by weak : $A/B \times 100\% < 50\%$; medium : $70\% < A/B \times 100\% < 50\%$; strong : $A/B \times 100\% \geq 70\%$, with A was clear zone diameter of extract and B was clear zone diameter standard antibiotic (Chan, 2007).

2.4 Determination of Minimum Inhibitory Concentration (MIC)

Secondary metabolite extracts that had strong antibacterial activity determined the minimum inhibitory concentrations. Determination of KHM using disc diffusion methods. The concentrations to be used were 4%, 3%, 2%, 1% and 0 (as a control). Paper discs that have been saturated with extracts of each of the existing concentrations and tetracycline as a standard antibiotics are placed on the surface of MHA medium that has been inoculated with the suspension of the test bacteria. Incubated at room temperature for 24 hours. Inhibition zones are measured and a minimum concentration value that can inhibit bacteria was determined.

2.5 Thin Layer Chromatography Analysis

Endophytic fungal extracts which have high antibacterial activity then analyzed by TLC. Thin layer chromatography analysis using silica gel 60 plates with ethyl acetate and n-hexane solvents with the eluent ratio used (ethyl acetate: n-hexane) were 1: 4; 3: 2; 2: 3 and 4: 1. The formed spots were seen by using UV light with a wavelength of 366 nm, and

the Rf value of the compound formed was determined (Jamal *et al.*, 2008).

2.6 Characterization and Identification of Endophytic Fungi

Fungal endophytes were grown on Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Czapek Yeast Extract Agar (CYEA), and Malt Extract Agar (MEA) media at room temperature (28°C) for 7 days. Fungal colonies grown in each medium observed about colony color, colony diameter, medium color around the colony, and colony reverse color (Srikandace, 2007). Microscopic morphology observed were hypha (septate or not), hyaline (colorless) or dark pigmented hyphae (greenish or blackish brown, dark black, grayish black), spores (asexual and sexual) with Henrici's Slide Culture (HSC) methods (Gandjar *et al.*, 1999).

40

3 RESULTS AND DISCUSSION

3.1 Antibacterial Activity of Secondary Metabolite of Endophytic Fungi

60

Isolation and purification of endophytic fungi of *Melaleuca cajuputi* Powell leaves obtained 7 isolates, 5 isolates from the young leaves and 2 isolates from the old leaves. The antibacterial activity test of *Melaleuca cajuputi* endophytic fungi extract on *Escherichia coli* showed that there were two endophytic fungi isolates had the highest antibacterial activity, i.e MC3 and MC5 fungi isolates with 11 mm and 10 mm clear zone diameter. Five isolates did not have antibacterial activity on *E. coli* namely MC1, MC2, MC4, MC6, and MC7 isolates. The antibacterial activity of a secondary metabolite extract varies with bacterial depending on the components of the bioactive compound in the extract.

Extracts of secondary metabolites of endophytic fungi that have the highest antibacterial activity on *Staphylococcus aureus* were extract of MC1 and MC7 fungal isolates, with 10 mm and 11 mm clear zone diameter.

56
5 Table 1: Antibacterial activity of endophytic fungi of *M cajuputi* leaves against *E coli*, *S aureus*, *S typhi*, *S dysenteriae* using the disc-diffusion methods.

Isolates	Zone of inhibition in mm (inhibition %)			
	<i>E coli</i>	<i>S aureus</i>	<i>S typhi</i>	<i>S dysenteriae</i>
MC1	0	10(90.9)+++	0	8,13(42.5)+
MC2	0	0	7(63.4)++	12.04(62.9)++
MC3	11(100)+++	9(81.8)+++	6,12(55.4)++	9.14(47.8)+
MC4	0	0	0	9,02(47.2)+
MC5	10(90.9)+++	9(81.8)+++	0	0
MC6	0	0	0	0
MC7	0	11(100)+++	7,2(65.2)++	8,08(42.3)+
Tetracycline	11	11	11.04	19.12

5
Figures in parentheses are inhibition percentages compared to tetracycline. Antibacterial activity is categorized as strong +++ for inhibition $\geq 70\%$, moderate ++ for inhibition $50 < 70\%$, and weak + for inhibition 50% (Chan *et al.*, 2007).

The extract of MC3 and MC5 endophytic fungal isolates have antibacterial activity against *E. coli* with 11 mm and 10 mm clear zone diameter, also have antibacterial activity against *S. aureus* with both 9 mm clear zone diameter (Table 1). This means that the extract of second metabolites of MC3 and MC5 isolates have a broad spectrum of antibacterial activity, because it has antibacterial activity against Gram-negative and Gram-positive bacteria. Table 2 shows the differences in the content of bioactive compounds found in endophytic fungi isolates that act as antibacterial. Research conducted by Ayepola and Adeniyi (2008), showed that secondary metabolites from leaf extracts of *Eucalyptus camaldulensis* including the plant family Myrtaceae have a broad spectrum of antibacterial activity in inhibiting bacterial growth, both Gram positive and Gram negative bacteria.

3.2 Minimum Inhibitory Concentration (MIC) of Secondary Metabolite Extract of Endophytic Fungi

57
The MIC of secondary metabolite extract of endophytic fungi of *Melaleuca cajuputi* leaves on *Escherichia coli*. MIC of MC5 fungal isolates was 400 $\mu\text{g/mL}$ with inhibition zone diameter 6,16 mm. The MIC of secondary metabolite extract of MC3 endophytic fungal isolates was 100 $\mu\text{g/mL}$ with an inhibition zone diameter 6.02 mm. Based on the MIC value, it showed that MC5 and MC3 endophytic fungi isolates had moderate antibacterial activity, when compared with Batos *et al.* (2016) where the *Eugenia calycina* plant extract has a MIC value of *E. coli* was 500 $\mu\text{g/mL}$, where the range of values of 500 $\mu\text{g/mL}$ - 1000 $\mu\text{g/mL}$ means that it is slightly

active, a value of 100 $\mu\text{g/mL}$ - 500 $\mu\text{g/mL}$ being, and KHM value less than 100 $\mu\text{g/mL}$ means it is very active.

The MIC of the secondary metabolite extract of MC1 isolates on *Staphylococcus aureus* were 200 $\mu\text{g/mL}$ with 6.1 mm inhibitory zone diameter of 6,1 mm. The MIC of MC7 endophytic fungi isolate was 700 $\mu\text{g/mL}$ with a inhibition zone diameter of 6.02 mm. The concentration 700 $\mu\text{g/mL}$ means that it is large enough to obtain a MIC value, compared with the results of a study conducted by Banhos *et al.* (2014) where extracts of the metabolites of the endophytic fungi of the *Myrcia guianensis* plant produced a MIC of *S. aureus* was 25 $\mu\text{g/mL}$. It is suspected that the bioactive compounds in MC7 fungal isolates are not strong enough to inhibit the growth of *S. aureus* at low concentrations, besides it is suspected that *S. aureus* bacteria have experienced resistance to bioactive compounds contained in MC7 fungal isolates. According to Vuong *et al.* (2015), *S. aureus* is one of the bacteria with a wide spread of antibiotic resistance. This bacterium has been resistant to penicillin, methicillin, even vancomycin, one of the antibiotics that are still commonly used.

3.3 Thin Layer Chromatography (TLC) Analysis

51
The results show that the color spot formed on the TLC plates for the three extracts of *Melaleuca cajuputi* endophytic fungal isolates which have relatively strong antibacterial activity, the isolates MC1, MC5, and MC7 were yellow. Based on the results of research conducted by Fadel *et al.* (2018), phenolic compounds when tested using thin layer chromatography plates will be yellow, blue or pink.

The color spot formed for MC3 isolate extract are yellow which shows phenol and brownish yellow compounds, showing flavonoid compounds, according to research conducted by Nugraha *et al.*, (2005) that flavonoid compounds have a brownish-yellow color to a red color. The color spot formed on the plates of each different endophytic fungi isolates showed the components of chemical compounds contained in each different endophytic fungi isolates.

This is consistent with the statement of Sharma *et al.* (2016), different endophytic fungi in a plant can produce different secondary metabolites. Based on the result of Rf value it is known that there are endophytic fungi isolates that have the same Rf value also have the same color spots. This shows that the possibility of chemical compounds contained in the extract of secondary metabolites of endophytic fungi isolates may be the same. According to Ahamed *et al.* (2017), the Rf was a general characteristic value that can change depending on the polarity of the mobile phase and its fixed phase. The Rf value indicates important information regarding the polarity of the chemical compounds to be identified. In addition, the Rf value can also be used in determining good solvents as a mobile phase in thin layer chromatography tests.

3.4 Characterization and Identification of Endophytic Fungi of *Melaleuca cajuputi*

The macroscopic and microscopic characteristics endophytic fungi isolates of *Melaleuca cajuputi* leaf are presented in Figure 1, 2 and Table 2. Microscopic observations of MC1 endophytic fungi isolates based on Figure 2 shows that hyphal fungi isolates of MC1 endophytes are septate and hyaline-colored, have short conidiophores and there are conidia-forming cells in the cylindrical conidiophore terminal, the conidia are rounded with smooth surfaces.

Based on the microscopic characteristics obtained it is suspected that MC1 endophytic fungi isolates belong to the type of *Scopulariopsis candida*. According to Gandjar *et al.* (1999), *S. candida* has a colony diameter of 3-4 cm within 7 days. Reverse color beige to light brown. Its conidiophores are short, have conidial-forming cells that are cylindrical in shape, conidia in the form of rounded to wide ovals, smooth-walled, and white to beige.

The macroscopic and microscopic characteristics endophytic fungi isolates of *Melaleuca cajuputi* leaf

are presented in Figure 3, 4 and Table 2. Based on microscopic characters of MC3 endophytic fungi isolates obtained that MC3 endophytic fungi isolates was identified as *Fusarium sporotrichiodes*, with characteristic hyphae and insular hyphae, there were two-branched conidia, oval-shaped, three-sided macroconidia, rounded microconidia, and hyaluronic hyphae. According to Samson *et al.* (1995), *F. sporotrichiodes* has a white colony to yellow and finally brown. Microconidia is commonly found in aerial mycelia in the form of ovoid, piriform or fusoid, 3-5 macroconidias.

Based on macroscopic and microscopic characters of MC5 endophytic fungi isolates on Figure 5, 6, and Table 6. Type of hyphae: septate; hyphae color: hyaline; conidia color: hyaline; chlamyospore color: brownish. Based on macroscopic and microscopic characters, MC5 endophytic fungi isolate was identified as *Fusarium equiseti*. According to Samson *et al.*, (1995), *Fusarium equiseti* on PDA medium has a whitish color and becomes creamy to brown in color, has a lot of chlamyospores pale brown when old, smooth-walled or rough, in chains or clumps, in hyphae or conidia. The macroscopic and microscopic characteristics endophytic fungi MC7 isolates of *Melaleuca cajuputi* leaves were presented in Figure 7, 8 and Table 7.

Based on macroscopic and microscopic characters of MC7 endophytic fungi isolates based on Figure 7, 8 and Table 7 obtained results, MC7 endophytic fungi isolates had hyaline-colored hyphae and insulated hyphae, there were rounded and hyaline-colored columns, and had zigospores with rough surfaces. Based on the characteristics obtained, MC7 endophytic fungi isolate is suspected to be a type of *Mucor hiemalis*. The microscopic and macroscopic characteristics are in accordance with Gandjar (1999), *Mucor hiemalis* has a rather creamy yellow colony, a yellowish white reverse color. The columns are round and hyaline, the zigospores are semipurate or round in shape and the surface is rough.

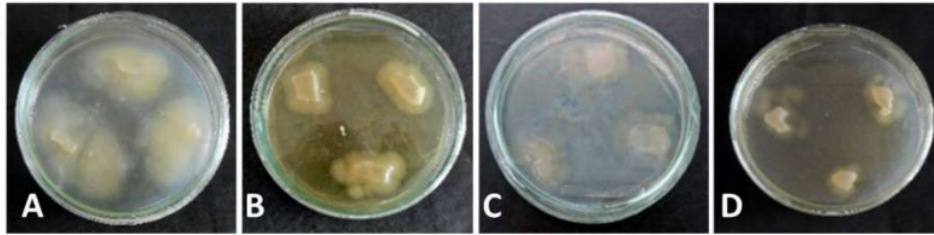


Figure 1: Colony images of MC1 endophytic fungal isolate of *Melaleuca cajuputi* on different medium. (a) PDA, (b) MEA, (c) CDA, (d) CYA.

Table 2: Colony characteristics of MC1 endophytic fungal isolate of *Melaleuca cajuputi* on different medium.

Characteristics	PDA medium	MEA medium	CDA medium	CYA medium
Colony color	White/ yellowish	Cream	Turbid white	Turbid white
Colony reverse color	Turbid yellowish	white/ Cream	Turbid white	Turbid white
Medium color around colony	Turbid white	Turbid white	Turbid white	Turbid white
Colony surface	Smooth	Smooth	Smooth	Smooth
Colony diameter	4.75–5.7cm in 7 days	3.35–3.9cm in 7 days	3.05–3.25cm in 7 days	1.85–2.7cm in 7 days

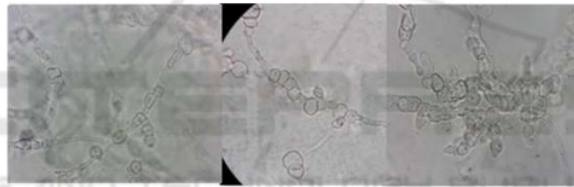


Figure 2: Microscopical characteristics of MC1 isolate.

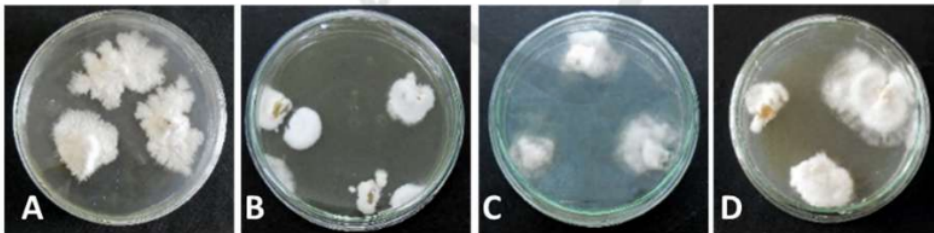


Figure 3: Colony images of MC3 endophytic fungal isolate of *Melaleuca cajuputi* on different medium. (a) PDA, (b) MEA, (c) CDA, (d) CYA.

Table 3: Colony characteristics of MC3 endophytic fungal isolate of *Melaleuca cajuputi* on different medium.

Characteristics	PDA medium	MEA medium	CDA medium	CYA medium
Colony color	White	White	White	White
Colony reverse color	White	Brownish white	White	White
Medium color around colony	Turbid white	Yellowish	Turbid white	Turbid white
Colony surface	Granular and notched	Powdery	Floccose	Floccose
Colony diameter	3.4-4.05 cm in 3 days	1.8-2.2cm in 7 days	3.4-4.05 cm in 3 days	2.35-5cm in 3 days

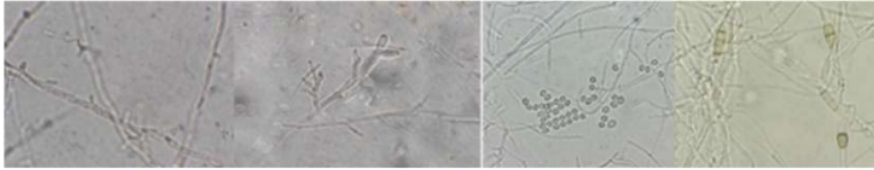
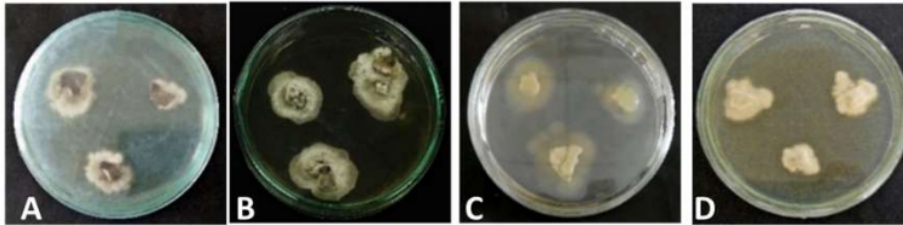


Figure 4: Microscopic characteristics of MC3 isolate.

Figure 5: Colony images of MC5 endophytic fungal isolate of *Melaleuca cajuputi* on different medium. (a) PDA, (b) MEA, (c) CDA, (d) CYA.Table 4: Colony characteristics of MC5 endophytic fungal isolate of *Melaleuca cajuputi* on different medium.

Characteristics	PDA medium	MEA medium	CDA medium	CYA medium
Colony color	Greys white	White	Yellowish turbid white	Cream
Colony reverse color	Brownish white	Brownish white	Yellowish turbid white	Cream
Medium color around colony	Yellowish	Yellowish	Turbid white	Turbid white
Colony surface	Powdery	Powdery	Smooth	Powdery
Colony diameter	1.8-2.5 cm in 7 days	2.95-3.75 cm in 7 days	3.15-3.95 cm in 7 days	1.75-2.35 cm in 7 days

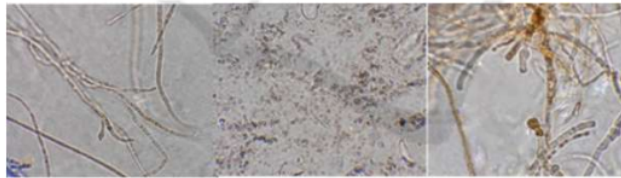


Figure 6: Microscopic characteristics of MC5 isolate.

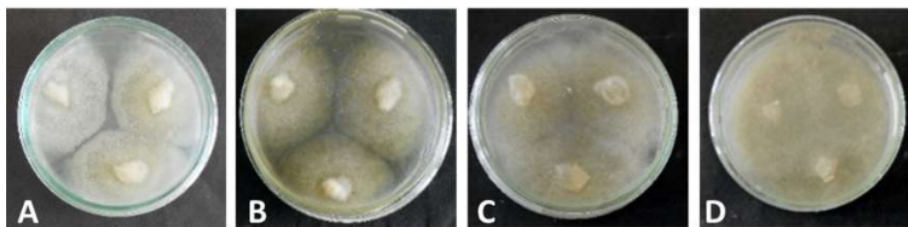
Figure 7: Colony images of MC7 endophytic fungal isolate of *Melaleuca cajuputi* on different medium. (a) PDA, (b) MEA, (c) CDA, (d) CYA.

Table 5: Colony characteristics of MC7 endophytic fungal isolate of *Melaleuca cajuputi* on different medium.

Characteristics	PDA medium	MEA medium	CDA medium	CYA medium
Colony color	Turbid white/yellowish	Turbid white/yellowish	Turbid white	Turbid white
Colony reverse color	White greyish brown	Turbid white/yellowish	Turbid white	Turbid white
Medium color around colony	Yellowish	Turbid white	Turbid white	Turbid white
Colony surface	Cottony	Cottony	Cottony	Cottony
Colony diameter	4.65-5.25 cm in 3 days	4.85-5.55 cm in 3 days	5-5.75 cm in 3 days	5.5-6.05 cm in 3 days

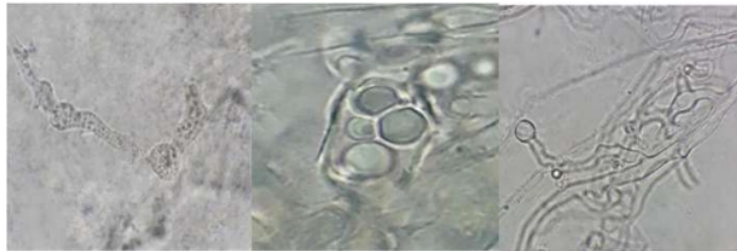


Figure 8: Microscopic characteristics of MC7 isolate.

4 CONCLUSION

Endophytic fungi obtained from *Melaleuca cajuputi* Powell obtained 7 isolates, 5 isolates obtained from young leaves, and 2 isolates from old leaves. Ethyl acetate extract of secondary metabolites endophytic fungi of *Melaleuca cajuputi* Powell which classified as strong and has a high percentage of antibacterial activity, namely isolates of endophytic fungi MC1, MC3, MC5, and MC7 against *Escherichia coli* and *Staphylococcus aureus*. Minimum inhibitory concentration (MIC) of secondary metabolite extracts of MC5 and MC3 endophytic fungi isolates against *Escherichia coli* were 400 µg / mL and 100 µg / mL respectively, while secondary metabolite extracts of MC1 and MC7 endophytic fungi isolates against *Staphylococcus aureus* bacteria were 200 mg / mL and 700 mg / mL. MC1 fungi isolates were identified as *Scopulariopsis candida*, MC3 fungi isolates were identified as *Fusarium sporotrichioides*, MC5 fungi isolates were identified as *Fusarium equiseti*, and MC7 fungi isolates were identified as *Mucor hiemalis*.

29 REFERENCES

Ahamed, T., Rahman S.K.M., Shohael A.M. 2017. Thin Layer Chromatographic Profiling and Phytochemical Screening of Six Medicinal Plants in Bangladesh. *International Journal of Biosciences*. 11(1) : 131 – 140.

- 17
Ajizah, A. 2004. Sensitivitas *Salmonella typhimurium* terhadap Ekstrak Daun *Psidium guajava* L. *Biosci*. 64(1): 31 – 38.
- Al-Abd¹, N.M., Zurainee Mohammed Nor, Marzida Mansor, Fadzly Azhar, M.S. Hasan dan Mustafa Kassim. 2015. Antioxidant, Antibacterial Activity, and Phytochemical Characterization of *Melaleuca cajuputi* extract. *BMC Complementary and Alternative Med*. 58 : 385 – 397.
- Al-Abd², N.M., Zurainee M.N., Marzida M., MS Hasan dan Mustafa K. 2016. Antifilarial and Antibiotic Activities of Methanolic Extracts of *Melaleuca cajuputi* flowers. *Korean J Parasitol*. 54(3) : 273 – 280.
- 37
Andries, J R., Paulina, N G., dan Aurelia, S. 2014. Uji Aktivitas Antibakteri Ekstrak Bunga Cengkeh terhadap Bakteri *Streptococcus mutans* secara In Vitro. *Jurnal e-Gigi*. 2(2): 1-8.
- 39
Ayepola, O.O. dan Adeniyi B.A. 2008. The Antibacterial Activity of Leaf Extracts of *Eucalyptus camaldulensis* (Myrtaceae). *Journal of Applied Science Research*. 4(11) : 1410 – 1413.
- 22
Banhos, E.F., Souza A.Q.L., Andrade J.C., Souza A.D.L., Koolen H.H.F., dan Albuquerque P.M. 2014. Endophytic Fungi from *Myrcia guianensis* at The Brazilian Amazon : Distribution and Bioactivity. *Brazilian Journal of Microbiology*. 45(1) : 153 – 161.
- Bastos, R.G., Rosa C.P., Oliver J.C., Silva N.C., Dias A.L.T., Da Rocha C Q, Vilegas W., Da Silva G.A., dan Da Silva M.A. 2016. Chemical Characterization and Antimicrobial Activity of Hydroethanolic Crude Extract of *Eugenia florida* DC (Myrtaceae) Leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*. 8(6) : 110 – 115.
- 8
Chan, E W C., Lim, Y Y., dan Mohammed, O. 2007. Antioxidant and Antibacterial Activity of Leaves of

- Etlingera Species (Zingiberaceae) in Peninsular Malaysia. *Food Chemistry*. 104. 1586-1593.
- 43 Fadel, O., Rodrigues D.G., Girard L., Bauduin P., Castera A.R., L'Hermitte A., Gaillard J.C., dan Diat O. 2018. Separation and Identification of Polar Polyphenols in Oily Formulation Using High Performance Thin Layer Chromatography and Mass Spectroscopy Techniques.
- 11 *Oilseeds & Fats Corps and Lipids*. 25(5) : 1 – 8.
- Fatima, Y. 2013. Daya antibakteri ekstrak kulit dan biji buah pulsa terhadap *Staphylococcus aureus* dan *Escherichia coli* secara in vitro. *Jurnal Peternakan* (10) : 31-38.
- Islam, M R., Shahnaj, P., Rikta, B., Nusrat, J., Nandita, D., dan Ekramul, I. 2013. Antibacterial and Phytochemical Screening of Ethanol Extracts of *Manilkara zapota* Leaves and Bark. *International Journal of Pharma Sciences*. 3(6): 394-397.
- Jamal, Y., Muhammad, I., Ait, K., dan Andria, A. 2008. Diversitas dan Profil Metabolit Sekunder Jamur Endofit yang Diisolasi dari Tumbuhan Gambir (*Uncaria gambler*) Serta Aktivitas Biologisnya Sebagai Antibakteri. *Berita Biologi*. 9 (1). 149-154.
- 21 Kumala, S. 2014. *Mikroba Endofit: Pemanfaatan Mikroba Endofit dalam Bidang Farmasi*. Jakarta: PT. ISFI
- 45 Penerbitan. v + 105 hlm.
- Nugrahaningtyas, K.D., Matsjeh S., dan Wahyuni T.D. 2005. Isolasi dan Identifikasi Senyawa Flavonoid dalam Rimpang Temu Ireng (*Curcuma aeruginosa* Roxb.). *Biofarmasi*. 3(1) : 32 – 38.
- 9 Radji, M. 2005. Peranan Bioteknologi dan Mikroba Endofit dalam Pengembangan Obat Herbal. *Majalah Ilmu Kefarmasian* 2 (3) :113-126.
- Samson, R A., Ellen, S H., Jens, C F., dan Ole, F. 1995. *Introduction to Food-Borne Fungi Fourth Edition*. Netherlands: Centraalbureau voor Schimmelcultures. 1 + 322 hlm.
- 46 Sharma, D., Pramanik A., Agrawal P.K. 2016. Evaluation of Bioactive Secondary Metabolites from Endophytic Fungus *Pestalotiopsis neglecta* BAB-5510 Isolated from Leaves *Cupressus torulosa* D.Don. *Biotech*. 6(1) : 1 – 14.
- 18 Shibuya, H., Agusta, A., Ohashi, K., Maehara, S., dan Simanjuntak, P. 2005. Biooxidation of (+)-Catechin and (-)-Epicatechin into 3,4-Dihydroxy Flavan Derivatives by The Endophytic Fungus *Diaporthe sp.* Isolated from A Tea Plant. *Chem Pharm Bull*. 53(7): 866-867. 9
- Srikandace, Y., Yatri, H., dan Partomuan, S. 2007. Seleksi Mikroba Endofit *Curcuma zedoria* dalam Memproduksi Senyawa Kimia Antimikroba. *Jurnal Ilmu Kefarmasian Indonesia*. 5 (2): 77-84
- 1 Stierle, A., D. Stierle, G. Strobel, G. Bignami, and P. Grothaus. 1995. Bioactive metabolites of the endophytic fungi of pacific yew *Taxus brevifolia*. Elsevier Scientific Publ., Ireland.
- Strobel, GA, and B. Daisy (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiol. and Mol. Biology Rev* 67(4):491-502.
- Tan, RX and WX. Zou. 2001. Endophytes : a rich source of functional metabolites. *Nat Prod. Rep.* 18:448-459.
- 53 Widiana, A., Taufikurrahman, Limin S.H., Hernaman I. dan Manurung R. 2014. The Potential of Gelam Leaves (*Melaleuca cajuputi* Powell) as Cattle Feed. *Pakistan Journal of Nutrition*. 13(6) : 348 – 350.
- 44 Visagie, C M., Houbraken J., Jens, C F., Hong, S B., Klaassen, C H W., Perrone, G., Seifert, K A., Varga J., Yaguchi, T., dan Robert, A S. 2014. Identification and Nomenclature of The Genus *Penicillium*. *Studies In Mycology*. 78: 343-371.
- 10 Vuong, C., Yeh A.J., Cheng G.Y.C., dan Otto M. 2015. Investigational Drugs to Treat Metichilin-resistant *Staphylococcus aureus*. *Expert Opinion Investigational Drug*. 25(1) : 73 – 93.
- 14 Zhao, J., Zhou, L., Wang, J., Shan, T., Zhong, L., Liu, X., dan Gao, X. 2010. Endophytic Fungi for Producing Bioactive Compounds Originally from Their Host Plants. *Formatex*. 567-576

Screening of Endophytic Fungi of Melaleuca cajuputi Powell Leaves as Antibacterial Sources

ORIGINALITY REPORT

23%
SIMILARITY INDEX

18%
INTERNET SOURCES

15%
PUBLICATIONS

8%
STUDENT PAPERS

PRIMARY SOURCES

1 sciencetechindonesia.com **4%**
Internet Source

2 icime.usu.ac.id **1%**
Internet Source

3 Purbowatiningrum Ria Sarjono, Leni Diah Putri, Chlara Eka Budiarti, Nies Suci Mulyani et al. " Antioxidant and antibacterial activities of secondary metabolite endophytic bacteria from papaya leaf () ", IOP Conference Series: Materials Science and Engineering, 2019 **1%**
Publication

4 Submitted to Universitas Sumatera Utara **1%**
Student Paper

5 Chan, E.W.C.. "Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia", Food Chemistry, 2007 **1%**
Publication

6 jpsr.pharmainfo.in **1%**
Internet Source

7	waset.org Internet Source	1 %
8	bennsethicoa.com Internet Source	1 %
9	repository.ub.ac.id Internet Source	1 %
10	Submitted to University of New England Student Paper	<1 %
11	pdfs.semanticscholar.org Internet Source	<1 %
12	irjponline.com Internet Source	<1 %
13	coek.info Internet Source	<1 %
14	digilib.unimed.ac.id Internet Source	<1 %
15	www.ejournal.warmadewa.ac.id Internet Source	<1 %
16	"Microbial Interventions in Agriculture and Environment", Springer Science and Business Media LLC, 2019 Publication	<1 %
17	theses.uin-malang.ac.id Internet Source	<1 %

18	link.springer.com Internet Source	<1 %
19	www.science.gov Internet Source	<1 %
20	Sogandi, P Nilasari. "Isolation and molecular identification of Endophytic bacteria from Noni fruits (<i>Morinda citrifolia</i> L.) and their antibacterial activity", IOP Conference Series: Earth and Environmental Science, 2019 Publication	<1 %
21	simakip.uhamka.ac.id Internet Source	<1 %
22	tede.ufam.edu.br Internet Source	<1 %
23	Kapila K. Liyanage, Sehroon Khan, Siraprapa Brooks, Peter E. Mortimer, Samantha C. Karunarathna, Jianchu Xu, Kevin D. Hyde. "Morpho-Molecular Characterization of Two <i>Ampelomyces</i> spp. (Pleosporales) Strains Mycoparasites of Powdery Mildew of <i>Hevea brasiliensis</i> ", <i>Frontiers in Microbiology</i> , 2018 Publication	<1 %
24	www.mdpi.com Internet Source	<1 %
25	doaj.org Internet Source	<1 %

26

"Advances in Endophytic Fungal Research",
Springer Nature, 2019

Publication

<1 %

27

Generoso da Silva GONÇALVES Fernando,
Conceição de ARAÚJO Jonatas, da Silva BRASIL
Dionízio, Asfury RODRIGUES Rodrigo et al.
"Chromatography and bioautography of
endophytic fungi extracts of *Uncaria
tomentosa* (Willd.) DC with antibacterial
activity", Journal of Medicinal Plants Research,
2019

Publication

<1 %

28

ugspace.ug.edu.gh

Internet Source

<1 %

29

univendspace.univen.ac.za

Internet Source

<1 %

30

Hairil Fiqri, Adzani Gaisani Arda, Khodijah
Adrebi, Wahyu Diah Proborini, Rahma Micho
Widyanto. "Evaluation of Total Phenolic, Total
Flavonoid, and In Vitro Cytotoxic Activity of
Syzygium cumini Extract in Cervical Cancer
Cell", Journal of Physics: Conference Series,
2020

Publication

<1 %

31

cdn.intechopen.com

Internet Source

<1 %

32

Internet Source

<1 %

33

pubs.rsc.org

Internet Source

<1 %

34

www.hilarispublisher.com

Internet Source

<1 %

35

www.researchgate.net

Internet Source

<1 %

36

V Sutanti, D Fuadiyah, LH Hidayat, T Agnizarridlo, K S Anggiarta. " Analysis of the effect of extracted yellow kepok banana peels () on the size and morphology of ", Journal of Physics: Conference Series, 2020

Publication

<1 %

37

ejournal.stikes-bth.ac.id

Internet Source

<1 %

38

scitepress.org

Internet Source

<1 %

39

Submitted to Biotechnology High School

Student Paper

<1 %

40

Pradeepa Vasudeva Samaga, Vittal Ravishankar Rai, Kuriya Madavu Lokanatha Rai. " Production of an antimicrobial cytochalasan by an endophytic HYML55 from and its RNA secondary structure analysis ", Chemistry and Ecology, 2014

<1 %

41 Warley de Borges, Keyller Borges, Pierina Bonato, Suraia Said, Monica Pupo.
"Endophytic Fungi: Natural Products, Enzymes and Biotransformation Reactions", Current Organic Chemistry, 2009
Publication

42 academicjournals.org
Internet Source

43 biomedpharmajournal.org
Internet Source

44 dergipark.org.tr
Internet Source

45 e-journal.uajy.ac.id
Internet Source

46 ujcontent.uj.ac.za
Internet Source

47 www.journalmrji.com
Internet Source

48 www.smujo.id
Internet Source

49 Advances in Endophytic Research, 2014.
Publication

50 Submitted to B.S. Abdur Rahman University
Student Paper

51 Kangle Niu, Zhengyao Liu, Yuhui Feng, Tianlong Gao, Zhenzhen Wang, Piaopiao Zhang, Zhiqiang Du, Daming Gao, Xu Fang. "Efficient Synthesis of Rare Disaccharides by Engineered β -Glucosidase Based on Hydropathy Index", American Chemical Society (ACS), 2020
Publication

52 repository.uinjkt.ac.id
Internet Source

53 sith.itb.ac.id
Internet Source

54 www.hindawi.com
Internet Source

55 Akhmad Endang Zainal Hasan, Dimas Andrianto, Husnawati, Faisal Rahman, Nurliani Bermawie, Heddy Julistiono, Eny Ida Riyanti. "ISOLATION AND ANTI-YEAST ACTIVITY OF SECONDARY METABOLITES OF SOURSOP LEAF ENDOPHYTIC FUNGI", Indonesian Journal of Applied Research (IJAR), 2022
Publication

56 Mora Obare Ruth, A. Indieka Stephen, Matasyoh Josphat. "Antibacterial activity of endophytic fungi isolated from leaves of medicinal Plant *Leucas martinicensis* L.

growing in a Kenyan tropical forest", African
Journal of Biochemistry Research, 2020

Publication

57

Ramesha Alurappa, Srinivas Chowdappa.
"Antimicrobial Activity and Phytochemical
Analysis of Endophytic Fungal Extracts
Isolated from Ethno-Pharmaceutical Plant
Rauwolfia tetraphylla L.", Journal of Pure and
Applied Microbiology, 2018

Publication

<1 %

58

eprints.um.edu.my

Internet Source

<1 %

59

www.ncbi.nlm.nih.gov

Internet Source

<1 %

60

"Fungi and their Role in Sustainable
Development: Current Perspectives", Springer
Science and Business Media LLC, 2018

Publication

<1 %

61

A. Shah, M.A. Rather, Q.P. Hassan, M.A. Aga,
S. Mushtaq, A.M. Shah, A. Hussain, S.A. Baba,
Z. Ahmad. " Discovery of anti-microbial and
anti-tubercular molecules from : an
endophyte of ", Journal of Applied
Microbiology, 2017

Publication

<1 %

62

Microbial Diversity and Biotechnology in Food
Security, 2014.

Publication

<1 %

63

ejournal.unsrat.ac.id

Internet Source

<1 %

64

www.isca.in

Internet Source

<1 %

Exclude quotes On

Exclude matches Off

Exclude bibliography Off