# 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. $MC_1$, $MC_2$, $MC_3$, and $MC_4$ fungal isolates have a strong antibacterial activities. The MIC of $MC_2$ and $MC_3$ extracts on *Escherichia coli* were 400 µg/mL and 100 µg/mL. The MIC of $MC_1$ and $MC_4$ 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 $MC_1$ isolate identified as *Scopulariopsis candida*, $MC_2$ isolate as *Fusarium equiseti*, $MC_3$ isolate as *Fusarium sporotrichoides*, and $MC_4$ 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 that can be done was isolate endophytic fungi from parts of medicinal plants that are often used as traditional medicine and selection of bioactive of 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



Widjajanti, H., Salni, ., Irfa Nastiti, N. and Nurnawati, E.
Screening Endophytic Fungi of Melaleuca cajuputi Powell Leaves as Antibacterial Sources.
DOI: 10.5220/0010139000002775
In *Proceedings of the 1st International MIPAnet Conference on Science and Mathematics (IMC-SciMath 2019)*, pages 196-204
ISBN: 978-989-758-556-2

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 as an antifilaria. (Al-Abd[2] *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, germacrena (C15H26O), neopitadiene, cyclohexakarboksal dehide, β-caryophyllena, 3-methoxy benzoic acid trimethylsilan; limonene; 1,4 naphthoquinone-5,8-dihydroxy-2-methoxy; 3 carena; α-caryophylena; 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.

# 2 MATERIALS AND METHOD

## 2.1 Isolation and Purification of Endophytic Fungi

The sterile leaves are cut 2x1 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

Twenty agar plug of endophytic fungi isolates inoculated into 500 mL Potato Dextrose Broth (PDB) medium and incubated at room temperature for ± 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

A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2.2H_2O$), with 9.95 mL of 1% sulfuric acid ($H_2SO_4$). The 0.5 Mc Farland solution measured the absorbance using a UV-Vis spetrophotometer (625 nm). The absorbance value obtained is equivalent to $1.5x10^8$ bacterial cells/mL (Fatisa, 2013).

*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Shigella dysentriae* 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 $1.5\times10^8$ CFU/mL (Kumala, 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 atroom 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 x 100% <50%; medium : 70% <A/B x 100%>50%; strong : A/Bx100% ≥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 growth on Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Czapek Yeast Extract Agar (CYEA), and Malt Extract Agar (MEA) media at room temperature (28ºC) 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 Cuture (HSC) methods (Gandjar *et al*., 1999).

# 3 RESULTS AND DISCUSSION

## 3.1 Antibacterial Activity of Secondary Metabolite of Endophytic Fungi

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 *Melalleuca 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 bacteria 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.

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 %) | | | |
|---|---|---|---|---|
| | *E coli* | *S aureus* | *S typhi* | *S dysenteriae* |
| 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)$^{+}$ |
| Tetrasicline | 11 | 11 | 11.04 | 19.12 |

Figures in parentheses are inhibition percentages compared to tetracyclin. Antibacterial activity is categorized as strong +++ for inhibition ≥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 secondary 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. This 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

The MIC of secondary metabolite extract of endophytic fungi of *Melaleuca cajuputi* leaves on *Eschrichia coli.* MIC of $MC_5$ fungal isolates was 400 µg/mL with inhibition zone diameter 6,16 mm. The MIC of secondary metabolite extract of $MC_3$ endophytic fungal isolates was 100 µg / mL with an inhibition zone diameter 6.02 mm. Based on the MIC value, it showed that $MC_5$ and $MC_3$ 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 µg / mL, where the range of values of 500 µg / mL - 1000 µg / mL means that it is slightly active, a value of 100 µg / mL - 500 µg / mL being, and KHM value less than 100 µg / mL means it is very active.

The MIC of the secondary metabolite extract of MC1 isolates on *Staphylococcus aureus* were 200 µg/mL with 6.1 mm inhibitory zone diameter of 6,1 mm. The MIC of $MC_7$ endophytic fungi isolate was 700 µg/mL with a inhibition zone diameter of 6.02 mm. The concentration 700 µg/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 µg/mL. It is suspected that the bioactive compounds in $MC_7$ 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

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 Nugrahaningtyas *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 microcondidia, 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; clamydospore 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 chlamydospores 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.

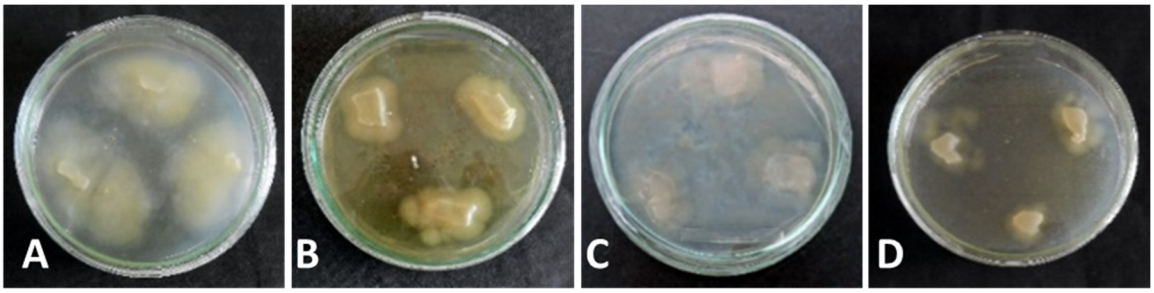


Figure 1: Colony images of MC1 endophytic fungal isolate of *Melaleuca cajuputi* on different medium. (a) PDA, (b) MEA, (c) CDA, (d) CYA.

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 white/ yellowish | 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 |

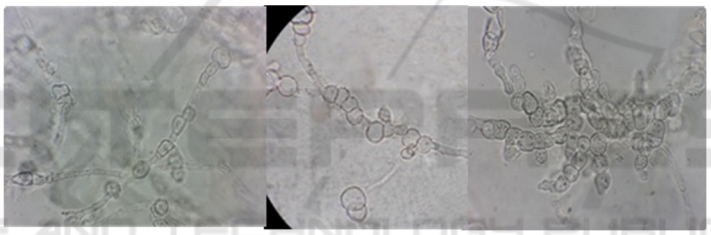



Figure 2: Microscopical characteristics of MC1 isolate.

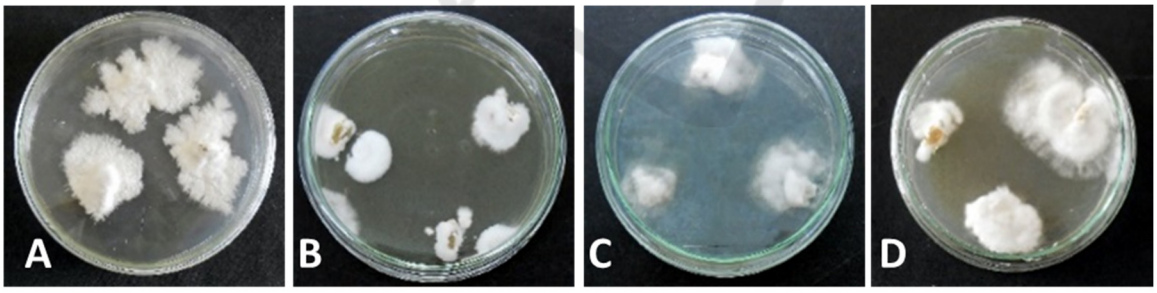


Figure 3: Colony images of MC3 endophytic fungal isolate of *Melaleuca cajuputi* on different medium. (a) PDA, (b) MEA, (c) CDA, (d) CYA.

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 |

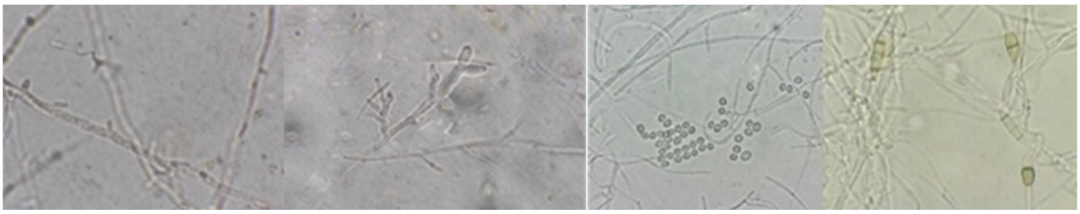

Figure 4: Microscopical characteristics of MC3 isolate.

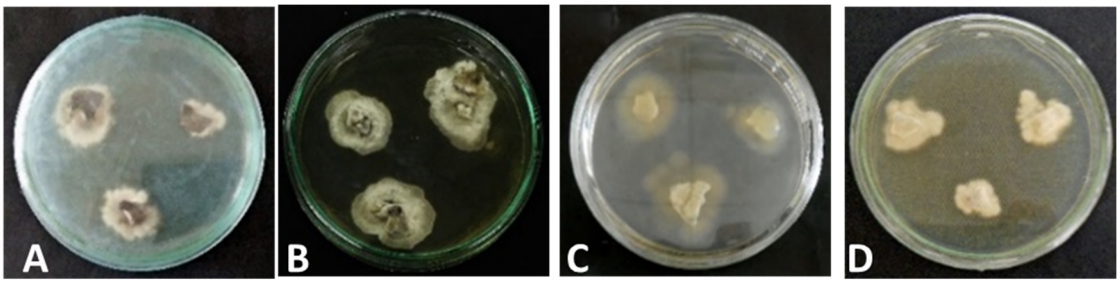


Figure 5: Colony images of MC5 endophytic fungal isolate of *Melaleuca cajuputi* on different medium. (a) PDA, (b) MEA, (c) CDA, (d) CYA.

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 |

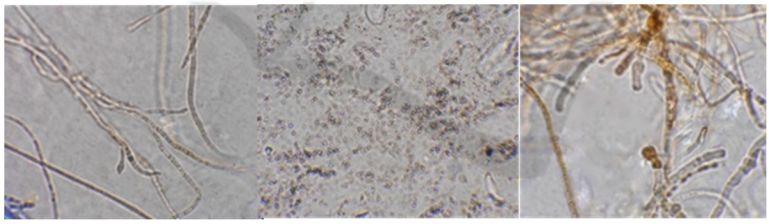

Figure 6: Microscopical characteristics of MC5 isolate.

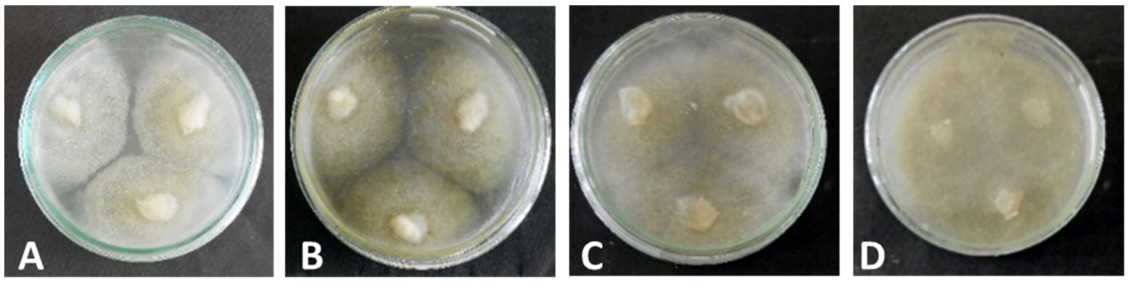


Figure 7: Colony images of MC7 endophytic fungal isolate of *Melaleuca cajuputi* on different medium. (a) PDA, (b) MEA, (c) CDA, (d) CYA.

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 |

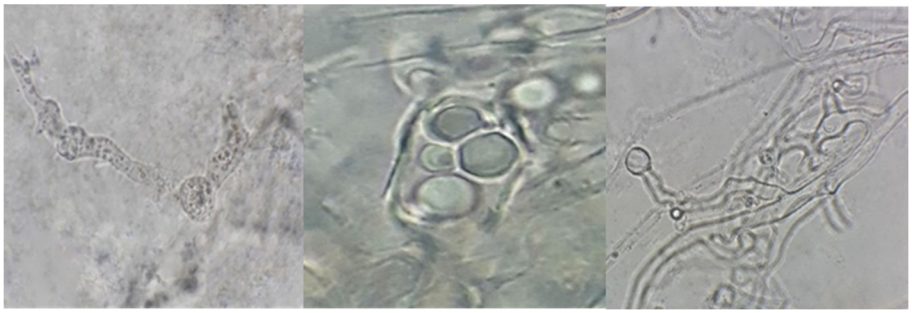

Figure 8: Microscopical characteristics of MC7 isolate.

# 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 sporotrichiodes*, MC5 fungi isolates were identified as *Fusarium equiseti,* , and MC7 fungi isolates were identified as *Mucor hiemalis.*

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