Advances in Biological Sciences Research, volume 22
7th International Conference on Biological Science (ICBS 2021)

Histological Observation, Identification, and Secondary Metabolites Content in Endophytic Fungi of Mahogany Tree *(Swietenia mahagoni* Jacq*)*

Utami Sri Hastuti[1,*] Sulisetijono Sulisetijono[1] Chomisatut Thoyibah[1] Siti Hartina Pratiwi[1] Khusnul Khotimah[2]

[1]*Department of Biology, Faculty of Mathematics and Science State University of Malang, Semarang Street, 5th Malang, Indonesia*
[2]*Doctoral Program of Biology Education, Department of Biology, Faculty of Mathematics and Science, State University of Malang, Semarang Street, 5th Malang, Indonesia*
[*]*Corresponding author. Email: utami.sri.fmipa@um.ac.id*

ABSTRACT
The mahogany *(Swietenia mahagoni Jacq.)* tree is a sort of medicinal plant. The phytochemical analysis by previous research has proved that the mahogany bark methanol extract is contained i.e alkaloid, tannin, saponin, phenol hydroquinone, and flavonoid. Some medicinal plant that has mutualism symbiotic interaction with several endophytic fungi species are relatively resistant to infections caused by pathogenic microbial. This research aimed: (1) to determine the endophytic fungi hyphae isolated from leaves and twigs of *S. mahagoni*, (2) to identify endophytic fungi isolated from leaves and twigs *S. mahagoni*, (3) to analyze several secondary metabolite contents that produced from each endophytic fungi species isolated from leaves and twigs of *S. mahagoni*. The leaves and twigs of *S. mahagoni* were prepared to do histological observation. The plant parts were inoculated on a PDA plate medium incubated into 25ºC-27ºC for a week for identification of fungal characteristics. Each endophytic fungi species on PDA medium were cut into 5x1 cm in size inoculated on potato dextrose liquid medium. The liquid culture is centrifugated and the supernatant was taken to detect secondary metabolite contents by using the spectrophotometric method. The data were consisting of macroscopic, microscopic characteristics, and secondary metabolites content of each endophytic fungi species was analyzed descriptively. This research showed that the endophytic fungi hyphae were found, on the twig of *S. mahagoni* at epidermis and parenchyma cell wall, whereas at the leaves found at epidermis, parenchyma, xylem, and stomata guard cell wall. The endophytic fungi isolated from *S. mahagoni* leaves and twigs were: *Colletotrichum gloeosporoides, Colletotrichum theobromicola, Colletotrichum kahawae, Colletotrichum siamense, Rhizoctonia* sp., *Colletotrichum asianum*, *Hansfordia biophila,* and *Mycelia sterilia*. Each endophytic fungi species could produce secondary metabolites, which was: flavonoid, alkaloid, tannin, saponins, and terpenoids with different contents. The flavonoid content ranged from 87,79–178,36 mg/kg; the alkaloid content ranged from 28,00–45,06 mg/kg; the tannin content ranged from 86,99–123,19 mg/kg; the saponin content ranged from 19,98–33,04 mg/kg; the terpenoid content ranged from 10,97–61,84mg/kg.

Keywords*: Endophytic fungi, Identification, Secondary metabolite, Swietenia mahagoni.*

1. INTRODUCTION

Some pathogenic bacteria species have been resistant to synthetic antibiotics, so it needs to examine to find a natural substance that can be used as an eco-friendly antibiotics. The mahogany *(Swietenia mahagoni* Jacq*.)* tree is a sort of medicinal plants. People use the parts of this plant for medicine necessity.

The phytochemical examination by previous research has proved that the mahogany bark methanol extract is contained alkaloid, tannin, saponin, phenol hydroquinone, and flavonoid [1], [2]. The other information has reported that the secondary metabolite from *S. macrophyla* plant is contained triterpenoids, saponins, flavonoid, tannin, catheline, and epicateline [3], [4].

The *S. mahagoni* tree is relatively resistant to some diseases, so it was assumed that there is a mutualism symbiotic interaction between the *S. mahagoni* tree and the endophytic fungi species that live in the plant tissues. Some medicinal plants that have been examined in the previous researches proved that there is a mutualism symbiotic interaction between the plant with several endophytic fungi species and the plant relatively resistant toward pathogenic microbial that is potentially cause infections [5], [6]. *Curcuma longa* has a mutualism symbiotic with endophytic fungi of *Penicillium sp.* which can produce antimicrobial active compounds [7]. Several species of endophytic fungi isolated from the *Hedychium acuminatum* plant can also produce some antimicrobial compounds that can inhibit the *Staphylococcus aureus* and *Bacillus subtilis* bacteria growth, which causes human diseases [8].

Some species of endophytic fungi have been successfully isolated from *Cananga odorata* leaves, twig, and flower petal, i.e: *Nigrospora sphaerica, Colletotrichum alienum, Mycellia sterilia 1*, *C. kahawae*, *Rhizoctonia* sp., *C. aotearoa, Mycellia sterilia 2*, *C. alatae*, and *C. queenslandicum* (Hastuti *et al.,* 2019). The plants that have mutualism symbiotic interaction with some endophytic fungi species form the endophytic fungi habitat, the plant was protected from some extreme environmental factors, such as air temperature, humidity, and drought. The endophytic fungi can also produce antimicrobial secondary metabolites. The endophytic fungi isolated from the *Hedychium acuminatum* plant can produce secondary metabolites that contain an alkaloids, flavonoids, terpenoids, and tannins [9]. Those substances are antibacterial compounds, so they can protect the host plant where they live from pathogenic bacteria attacks [10], [11].

This research is aimed to isolate and identify endophytic fungi species from *S. mahagoni*, especially on leaves and twigs. The histological observation is also done to determine the endophytic fungi hyphae location on *S. mahagoni* tree tissues. Through this research, it has been proved that endophytic fungi liquid culture isolated from *S. mahagoni* tree contains some antibacterial secondary metabolite compounds, i.e: alkaloids, flavonoids, terpenoids, and tannins, so the liquid culture of endophytic fungi can be used as an eco-friendly natural antibiotic source. This can be another alternative for picking activities on *S. mahagoni* plant parts in order to take a natural medicine, so the *S. mahagoni* tree can be protected from extinction. This research aimed (1) to determine the endophytic fungi hyphae that isolated from leaves and twigs of *S. mahagoni* location, (2) to identify endophytic fungi isolated from *S. mahagoni* leaves and twigs, and (3) to analyze several secondary metabolites compounds content that produced from each species of endophytic fungi which is isolated from leaves and twigs of *S. mahagoni*.

2. METHODOLOGY

2.1. Preparation of Microscopically Observation on Endophytic Fungi Position in S. mahagoni Tree Tissues

The leaves and twigs of the S. mahagoni tree were washed with aquades, then were cut in paradermally and longitudinally, for microscopic slides. Afterwards, the observation was done histologically on the position of endophytic fungi at the tissues of the S. mahagoni tree.

2.2. Isolation and Identification of Endophytic Fungi

The *S. mahagoni* leaves and twigs were washed and dipped in NaOC1 1% for 1 minute, then rinsed in aquades, then were dipped into alcohol 70% for 1 minute and rinsed again with aquades. The leaves part was cut in 1x1 cm^2 in size, the twigs part was cut with the thickness of 0.5 cm. Then, each sample was inoculated on the PDA plate medium which contained chloramphenicol (100 mg/L), and incubated into 25^oC-27^oC for a week. Each endophytic fungi species was inoculated on a PDA slant medium and incubated at 27^oC for 3 days for morphological observation. The culture slide from each endophytic fungi isolate was prepared for microscopical observation and the fungal characteristics were described for identification purposes. The microscope we used for microscopical observation is OLYMPUS microscope CX 21 FS 1.

2.3. Preparation of The Endophytic Fungi Liquid Culture

The secondary metabolite could be obtained from the liquid culture of endophytic fungi. Each colony of endophytic fungi species on PDA plate medium was cut into 5x1 cm^2 in size, and then inoculated on potato dextrose liquid medium and incubated in 27^oC for a week and shook at the speed of 120 rpm. Afterward, the liquid culture is centrifugated at 3000 rpm for 10 minutes. The supernatant was used to detect the contents of secondary metabolite compounds, i.e: alkaloid, flavonoid, saponin, terpenoid, and tannin that are produced by each endophytic fungal isolate.

2.4. Detection of Secondary Metabolite on Liquid Culture of Each Endophytic Fungi

Alkaloid, flavonoid, terpenoids, and tannin contents on the liquid culture of each endophytic fungi species were analyzed by using the spectrophotometric method. The secondary metabolites: alkaloid, flavonoid, saponin, tannin, and terpenoid content were determined with an explanation as follows.

2.4.1 Quantitative Alkaloid Determination [12]

2.4.1.1 Standard Solution Preparation

The atropine solution as a standard solution (100mg/L) was prepared by dissolving 10 mg atropine in chloroform to 100 mL. Afterwards the solutions in some concentrations i.e: 0, 1, 5, 10, 25 and 50 mg/L was prepared.

2.4.1.2. Sample and Standard Solution Preparation

The solid sample was crushed and weighed as much as 0,1 g and dissolved in 10 ml DMSO, then added with 1 ml 2N HCL and 5 L brom cresol green solution and 5 L phosphate buffer, homogenized, and then let stand for 60 minutes. The solution was poured into a separatory funnel and mixed with 10 ml chloroform. Afterward, shake the solution and wait until the layer were formed. Take the upper phase of the solution for the next process.

The standard solution was prepared by taking 5 ml atropine solution, adding with 1ml 2N HCL, 5 brom cresol green solution, and 5 ml buffer phosphate, and homogenized. This solution can be used for the next process.

2.4.1.3. The Alkaloid Content Determination

The alkaloid content determination was done. Take 11 ml of the upper phase or the standard solution. If the solution color is yellowish-orange, it was proved that the sample contains an alkaloid. Then the solution was dissolved with chloroform to 5 ml vollume. The absorbance was measured at λ = 470 nm. The alkaloid content concentration is determined by the regression standard equation.

2.4.2. Quantitative Flavonoid Determination [13]

2.4.2.1. Standard Solution Preparation

The quercetin solution as standard solution (100mg/L) were prepared by dissolve 100mg quercetin in distilled water to 100 mL. Afterwards the solution in some concentrations, i.e: 0; 0.5; 1; 10; 25 and 50 mg/L was prepared.

2.4.2.2. Sample and Standard Solution Preparation

The solid sample was crushed and weighed as much as 1 g, then dissolved in methanol to 10 mL, then homogenized and let stand for 30 min. Afterwards filtered with a vacuum filter and centrifugated at 3000 rpm for 10 min. Then the supernatant was taken. The 1 ml liquid sample add with 5 ml methanol then homogenized for 5 min. The solution was filtered with a vacuum filter. Then the filtrate was taken for the next process.

2.4.2.3. The Flavonoid Content Determination

Take 0.1 mL sample solution or standard solution, add with 0.1 mL 2%AL_2CL_3, then homogenized and let stand for 60 min. Afterward, add with distilled water 1 mL in volume. If the solution color becomes red, it was proved that the sample contains flavonoid. The absorbance was measured at λ = 420 nm. The flavonoid content concentration is determined by the regression standard equation.

2.4.3. Quantitative Saponin Determination [14]

2.4.3.1. Standard Solution Preparation

The saponin solution as standard solution (100mg/L) were prepared by dissolve 10 mg saponin in 20% ethanol to 100 mL. Afterward, the solution in some concentrations, i.e: 0; 1; 5; 10; 25; and 50 mg/L was prepared.

2.4.3.2. Sample and Standard Solution Preparation

The solid sample was crushed and weighed as much as 0,1 g, then dissolved in 10mL of 90% ethanol, then homogenized and heated in a water bath at 55^{o}C for 90 min. Afterward, the solution was filtered through filter paper. Then the sludge was reextracted with 10 mL 90% ethanol. Both of the extracts were mixed and heated at 90^{o}C until half of the solution remained. Then the solution was taken in the separating funnel, add with 40 mL diethyl ether, then shook and let stand until the solution was separated. Afterward, take the bottom phase, add 60 mL butanol and 10 mL 5%NaCl, then filtered. The filtrate was dried up in a dry oven at 60^{o}C. The result is dry saponin. The saponin was dilute in 5 mL 20% ethanol.

2.4.3.3. The Saponin Content Determination

Take 5 mL sample or standard solution and add with 0.5ml 0.1 N $FeCl_3$ and 0.5 mL 0.008 M $K_3Fe(CN)_6$, then homogenized and let stand for 30 min. Homogenized again and let stand for a while, if the solution color becomes blue, it proved that the sample contains saponin. Dilute the solution with chloroform to 10 ml in volume. Then measure the absorbance of the

solution by spectrophotometer at λ = 470 nm. The saponin content concentration is determined by regression standard equation.

2.4.4. Quantitative Tannin Determination [12]

2.4.4.1 Standard Solution Preparation

The tannic acid solution as standard solution (50mg/L) were prepared by dissolve 5 mg tannic acid in 20% ethanol to 10 mL Afterwards the solutions in some concentrations i.e: 0; 1; 5; 10; 25 and 50 mg/L was prepared.

2.4.4.2. Sample and Standard Solution Preparation

The solid sample were crushed and weighed as much as 0.1 g, then dissolved in 10 ml methanol, then homogenized and let stand for 30 min. Afterwards filtered with vacuum filter and centrifugated in 3000 rpm for 10 min. Then the supernatant was taken for next process.

2.4.4.3. The Tannin Content Determination

Take 5 ml sample or standard solution and add with 0.5 mL 0.1 M $FeCl_3$ and 0.5 mL 0.008 M $K_3Fe(CN)_6$, then homogenized and let stand for 30 min. The solution dilluted with distilled water to 10 ml in volume. The absorbance were measured at λ = 620 nm. The tannin content concentration determined by regresi standard equation.

2.4.5. Quantitative Terpenoid Determination [15]

2.4.5.1. Standard Solution Preparation

The linalool solution as standard solution (0.5mg/100mL) were prepared by dissolve 0.5 mg linalool in chloroform to 100 mL. Afterwards the solutions in some concentrations, i.e: 0; 0.01; 0.05; 0.1; 0.25 and 0.5 mg/L was prepared.

2.4.5.2. Sample and Standard Solution Preparation

The solid sample were crushed and weighed as much as 2-10 g, then dissolved in methanol to 25 mL, then homogenized and let stand for 30 min. Afterwards filtered and centrifugated at 3000 rpm for 10 minutes. Then the supernatant was taken and added with chloroform (1 mL supernatant added with 1 mL chloroform each). Afterwards let stand and take the upper phase (non polar) as much as 5 mL. the solution can be used for the next process.

2.4.5.3. The Terpenoid Content Determination

Take 5 mL sample or standard solution and add with 3 mL concentrated H_2SO_4, then homogenized and let stand for a while. If a brownish red colour formed in the solution, it was proved that the sample contains terpenoid. Afterwards dilute the solution with chloroform to 10 ml in volume. Then measured the absorbance at at λ = 538 nm. The terpenoid content concentration determined by regresi standard equation.

2.5. Data Analysis

The data were consisted of macroscopic, microscopic characteristics of each endophytic fungi species, and secondary metabolite content were analyzed descriptively.

3. RESULT AND DISCUSSION

3.1. Histological Observation of Endophytic Fungi on S. mahagoni Data

The observation result of endophytic fungi position was determined based on the hyphae location on twigs and leaves *S. mahagoni* slides microscopic observation. Several histological observations of these endophytic fungi are shown in Figure 1.

Table 1. The Location of Endophytic Fungi on *S. mahagoni* Twigs and Leaves

The Plant Parts	Slice Section	Location
The Twigs	Paradermal	Epidermis cell wall and parenchyma cell wall
The Leaves	Paradermal	Epidermis cell wall, parenchyma cell wall and vascular cell wall, stoma guard cell, hypodermis cell wall, and vascular cell wall tissues

3.2. The Identification of Endophytic Fungi Isolated from Swietenia mahagoni

The endophytic fungi identification from S. mahagoni twigs and leaves showed that there were 8 fungi species, consisting of *Colletotrichum gloeosporoides, Colletotrichum theobromicola, Colletotrichum kahawae, Colletotrichum siamense, Rhizoctonia* sp., *Colletotrichum asianum*, *Hansfordia biophila,* and *Mycelia sterilia* (Figure 2). These fungi

species could be found in another plant. In my previous research, *Colletotrichum kahawae* and *Colletotrichum alienum* were also found as endophytic fungus *Cananga odorata* [16]. *Colletotrichum kahawae* also found in *Cordilyne fruticosa* plant [17]. *Colletotrichum alienum*, *Aspergillus parasiticus,* and *Rhizoctonia* sp. were also found as endophytic fungi in *Hedychium acuminatum* plant as a sort of medicinal plant [8].

The results of the description of the characteristics of endophytic fungal isolate are described in Table 2. The results showed that there were variations in the color of fungal colonies, such as green, white-pink, white-greyish green, and grayish white. The nature of the colony was velvety and the others was cotton-like. The conidia were varied in shape, such as cylindrical, subglobose ellipse, and clavate. *Colletotrichum gloeosporoides* has cylindrical conidia with rounded ends and smooth walls. *Colletotrichum theobromicole* has straight cylindrical conidia and smooth walls. *Colletotrichum kahawae* also has cylindrical conidia with broadly rounded ends and smooth walls (Figure 3).

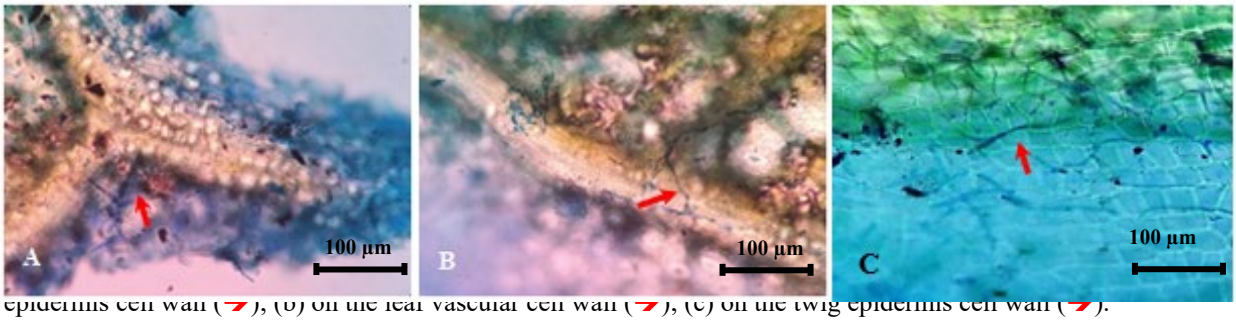
3.3. The Analysis of Secondary Metabolite Content Produced by Each Endophytic Fungi Isolated from S. Mahagoni.

Each endophytic fungi species can produce secondary metabolites consisting of flavonoid, alkaloid, tannin and saponin with different contents (Table 3) *Rhizoctonia* sp. has the ability to produce the five kinds of secondary metabolites in the highest number compared with the other endophytic fungi species.

The *S. mahagoni* is one of plant which was rarely attacked by pests and disease. Commonly, the plants that were resistant to pests and disease lived in mutualism symbiotic interaction with some endophytic fungi species which lived in the host plant tissues. The histological observation results showed that the endophytic fungal hyphae were found on epidermis cell wall and parenchyma cell wall twig tissues (Table 1), on epidermal cell wall, xylem vascular cell wall, and stomata guard cell wall of the leaf tissues. The endophytic fungal hyphae did not penetrate into the host plant cell, so it would not cause any damage on the host plant tissue structure. This result was supported the evidence that the endophytic fungi lived in mutualism symbiosis interaction in the host plant. The endophytic fungi also produced some antimicrobial compound such as alkaloid, flavonoid, terpenoid, and tannin [18]. The antimicrobial compound could protect the host plant from microbial attacks. In addition, the endophytic fungi could protect the host plant from the injury environmental factors, such as air temperature, humidity, and drought [19]. The endophytic fungi also take the nutrition in the intercellular spaces of host plant tissues that not used by the host plant cells [20].

The endophytic fungi could obtain another benefit, since it lived inside the host plant which could protect fungi from abiotic factors that could be harm for the fungi, for instance the extreme of air temperature, the humidity, and lack of water [21]. The chemical analysis result on secondary metabolite produced by each endophytic fungi species proved that all endophytic fungi species could produce flavonoid, alkaloid, tannin, saponin, and terpenoid with different contents. The content of each compound was flavonoid ranged from 87,79–178,36 mg/kg; alkaloid ranged from 28,00–45,06 mg/kg; tannin ranged from 86,99–123,19 mg/kg; saponin ranged from 19,98–33,04 mg/kg; terpenoid content ranged from 10,97–61,84mg/kg (Table 3). Based on our results indicated that the endophytic fungi liquid cultures could be used as an eco-friendly antibiotic source.

This research results were (1) the endophytic fungi hyphae isolated from *S. mahagoni* plant, on the twig were found at epidermal cell wall and parenchymal cell wall, whereas at the leaves were found at epidermal cell wall, parenchymal cell wall, xylem cell wall, and stomata guard cell wall; (2) the endophytic fungi isolated from *S. mahagoni* leaves and twigs were *Colletotrichum gloeosporoides, Colletotrichum* theobromicola, Colletotrichum kahawae, Colletotrichum siamense, Rhizoctonia sp., Colletotrichum asianum, Hansfordia biophila and Mycelia sterilia, (3) each species of endophytic fungi could produce secondary metabolite, which were flavonoid, alkaloid, tannin, saponins, and terpenoids with different concentrations.



epidermis cell wall (➔), (b) on the leaf vascular cell wall (➔), (c) on the twig epidermis cell wall (➔).

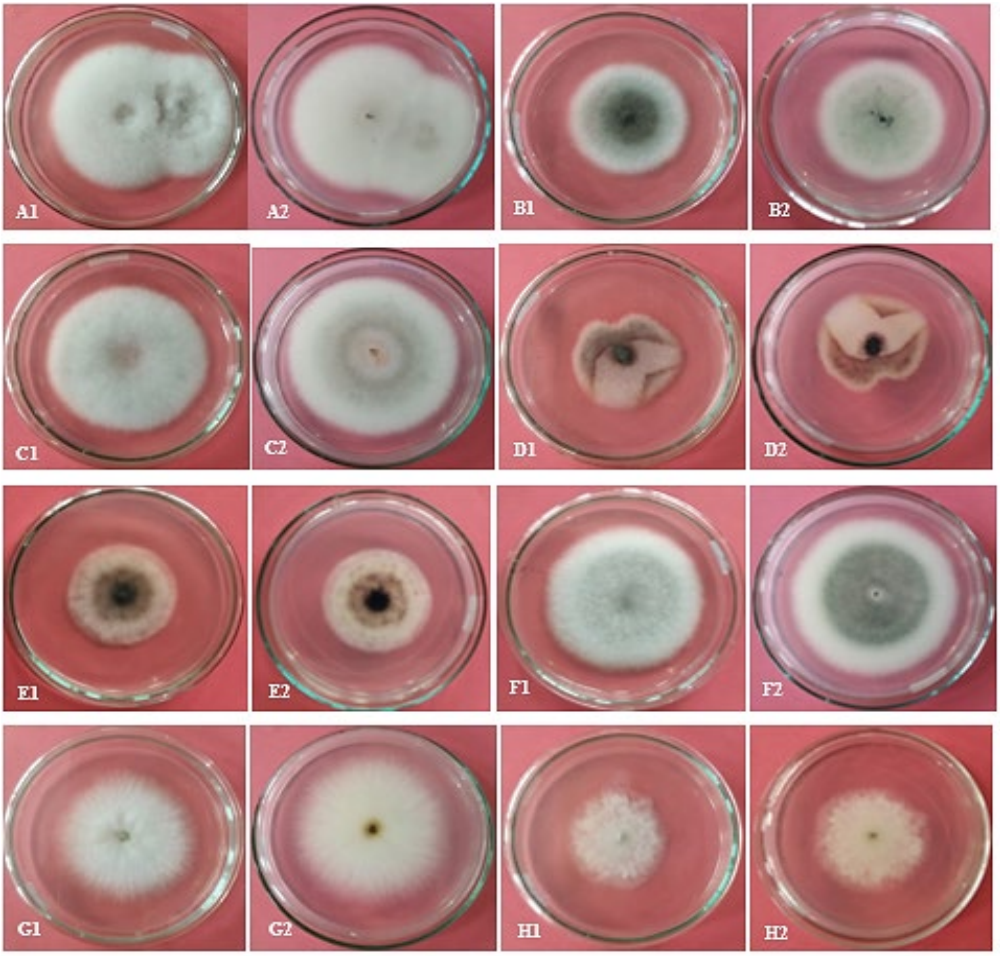


Figure 2 Each endophytic fungi colonies that inoculated on PDA medium after incubation for a week**.** Description: 1. Upper view 2. Reverse view; A. *Colletotrichum gloeosporoides*, B. *Colletotrichum theobromicola*, C. *Colletotrichum kahawae*, D. *Colletotrichum siamense*, E. *Rhizoctonia* sp., F. *Colletotrichum asianum*, G. *Hansfordia biophila*, H. Mycellia sterilia.

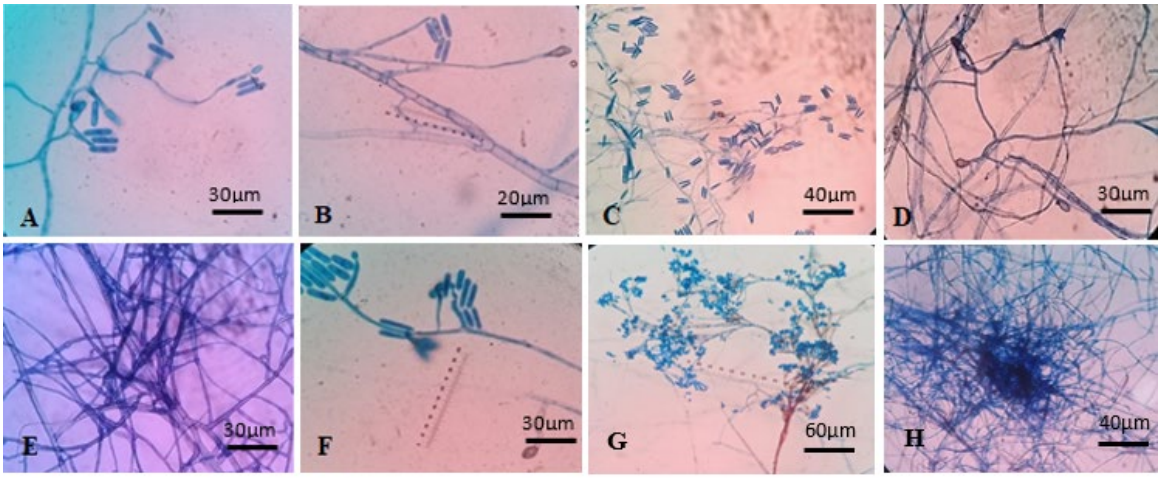


Figure 3 Microscopic Photo of Each Endophytic Fungi Species Isolated from *S. mahagoni* Plant**.** A) *Colletotrichum gloeosporoides*, B) *Colletotrichum theobromicola*, C) *Colletotrichum kahawae*, D) *Colletotrichum siamense*, E) *Rhizoctonia* sp., F) *Colletotrichum asianum*, G) *Hansfordia biophila*, H) Mycellia sterilia.

Table 2. The Description of Macroscopic and Microscopic Characteristics of Each Endophytic Fungi Species Isolated from *S. mahagoni*

Species	Isolate Code	The Plant Part	Colony Morphology	Appresorium (shape, size)	Conidia (colour, shape, size, wall, character)	Sclerotium (shape,size)
Colletotrichum gloeosporoides	A	Leaf	Colour: grayish white, reverse: grayish white, orange and there are black spot, cottony	Irregular, size: 22,5 x 12,5 µm	Colour: hyaline, shape: cylindrical with rounded edge, smooth wall, size: 25x7 µm	-
Colletotrichum theobromicola	B	Leaf	Colour: grayish white, reverse: blackish gray, velvety	Ellipsoidal, irregular, size: 22,5 x 10 µm	Colour: hyaline, fusiform, cylindrical, smooth wall, size: 45 x 5 µm	-
Colletotrichum kahawae	C	Leaf	Colour: grayish white, orange, reverse: gray, orange on the middle part, cottony	Irregular with lobes in the middle, size: 15x12,5 µm	Colour: hyaline, cylindrical with rounded edge, smooth wall, size: 25 x 7,5 µm	-
Colletotrichum siamense	D	Leaf	Colour: pinkish gray, reverse: pinkish gray with black spot, cottony	Solitary, ellipsoidal, irregular, size: 25 x 15 µm	Colour: hyaline, subglobose or clavate, size: 25 x 10 µm	-
Rhizoctonia sp.	E	Leaf	Colour: grayish white, reverse: black, velvety	-	-	Colour: Black
Colletotrichum asianum	F	Leaf	Colour: blackish gray, reverse: pinkish gray with black spot in the medium base, velvety	Irregular, ellipsoidal with lobes in the middle, size: 37,5 x 15 µm	Colour: hyaline, ellipsoidal, size: 27,5x12,5 µm	-
Hansfordia biophila	G	Twig	Colour: white, reverse: grayish white, cottony	-	Colour: hyaline, ellipsoidal, size: 10x3 µm	-
Mycellia sterilia	H	Twig	Colour: white, reverse: yellowish white, cottony	-	-	-

Table 3. The Result of Secondary Metabolite Compound Content Produced by Each Endophytic Fungi Species

Isolate Code	Isolates	Secondary Metabolites Content (mg/kg)				
		Flavonoid	Alkaloid	Tannin	Saponin	Terpenoid
A	*Colletotrichum gloeosporoides*	87.73	28.00	86.99	19.98	10.97
B	*Colletotrichum theobromicola*	96.72	29.69	90.57	21.27	16.01
C	*Colletotrichum kahawae*	115.86	33.29	98.22	24.03	26.75
D	*Colletotrichum siamense*	142.42	38.29	108.83	27.86	41.67
E	*Rhizoctonia* sp.	178.36	45.06	123.19	33.04	61.84
F	*Colletotrichum asianum*	145.55	38.88	110.08	28.31	43.42
G	*Hansfordia biophila*	148.28	39.40	111.17	28.71	44.96
H	Mycellia sterilia	101.41	30.57	92.45	21.95	18.64

ACKNOWLEDGMENT

I would like to say thanks to FMIPA for the financial support through PNPB faculty program so the research can be done.

REFERENCES

[1] U.L. Qodri, Masruri, E.P. Utomo, Skrining fitokimia metabolit sekunder ekstrak metanol dari kulit batang mahoni (*Swietenia mahagoni* Jacq.), Kimia Student Journal, vol 2, 2014, pp. 480-484.

[2] D.S. Prasetyono, A-Z Daftar Tanaman Obat Ampuh di Sekitar Kita, Yogyakarta: FlashBooks, 2012.

[3] T.S. Suhesti, W.K. Dhadhang, Nuryanti, Penjaringan senyawa antikanker pada kulit batang kayu mahoni (*Swietenia mahogani* Jacq.) dan uji aktivitasnya terhadap larva udang *Arthemia salina* Leach, Jurnal Ilmiah Keperawatan, vol. 3, 2007, pp. 155-162.

[4] S. Falah, M. Safithri, T. Katayama, T. Suzuki, Hypoglycemic effect of mahogany (*Swietenia macrophylla* King) bark extracts in alloxan-induced diabetic rats, Wood Research Journal, vol. 1, 2010, pp. 89-94.

[5] K. Clay, C. Schardl, Evolutionary origins and ecological consequences of endophyte symbiosis with grasses, Am Nat, vol. 160, 2002, pp. 99–127.

[6] K.H. Kogel, P. Franken, R. Hückelhoven, Endophyte or parasite-what decides?, Current Opinion in Plant Biology, vol. 9, 2006, pp. 358–363.

[7] D. Singh, V. Rathod, S. Niganagouda, J. Herimath, P. Kulkarni, Biosynthesis of silver nanoparticle by endophytic fungi *Penicillum* sp. isolated from *Curcuma longa* (turmeric) and its antibacterial activity against pathogenic gram negative bacteria, J. Pharm Res, vol. 7, 2013, pp. 448–453.

[8] U.S. Hastuti, D. Rahmawati, R.Y. Sari, R.D. Fitri, P.M. Al Asna, Antimicrobial activity of endophytic fungi isolated from a medicinal plant, *Hedychium acuminatum* Roscoe, in: AIP Conference Proceedings, 2019. http://doi.org/10/1063/1.5061895.

[9] U.S. Hastuti, P.M. Al-Asna, D. Rahmawati, Histologic observation, identification, and secondary metabolites analysis of endophytic fungi isolated from a medicinal plant, *Hedychium acuminatum* Roscoe, in: AIP Proceeding, 2018. DOI: 10.1063/1.5050166

[10] V. Verma, S. Singh, R. Kharwar, Histological investigation of fungal endophytes in healthy tissues of *Azadirachta indica* A, Juss, Kasetsart Natural Science Journal, vol. 46, pp. 229–237.

[11] K.A. Selim, A.A. El-Beih, T.M. AbdEl-Rahman, Al. El-Diwany, Biology of endophytic fungi, Current Research in Environtment & Applied, Mycology, vol. 2, pp. 31-82.

[12] D. Vijay, S. Rajendra, Estimation of total phenol, tannin, alkaloid and flavonoid in hibiscus tiliaceus

Linn, Wood Extracts. Research and Review: Jornal of Pharmacognosy and Phytochemistry, vol. 2, 2014.

[13] O.R. Alara, N.H. Abdurahman, C.I. Ukaegbu, Soxhlet extraction of phenolic compounds from *Vernonia cinerea* leaves and its antioxidant activity, Journal of Applied Research on Medicinal and Aromatic Plants, vol. 11, 2018, pp. 12-17. DOI: https://doi.org/10.1016/j.jarmap.2018.07.003

[14] A. Chinelo, C.U. Okeke, O. Bibian, V. Cinyere, A. Emeka, Determination of saponin content of various parts of six citrus species, International Research Journal of Pure and Applied Chemistry, vol. 4, 2014.

[15] C. Indumathi, G. Durgadevi, S. Nithyavani, P.K. Gayathri, Estimation of terpenoid content and its antimicrobial property in *Enicostemma litorrale*, International Journal of ChemTech Research, vol. 6, 2014, pp. 4264-4267.

[16] U.S. Hastuti, D. Rahmawati, R.Y. Sari, Histologic observation, identification and secondary metabolites analysis of endophytic fungi isolated from *Cananga odorata* (Lam.) Hook. F. & Thomson, in: IOP Conf. Series: Materials Science and Engineering 546, 2019, 022005. DOI: 10.1088/1757-899X/546/2/022005

[17] P.K. Mukti, U.S. Hastuti, Sulisetijono, Characterization, identification, and histological observation of isolated endophytic fungi from *Cordilyne fruticosa* (L.) A. Chev, Proceeding Biology Education Conference, vol. 15, 2018, pp. 862-869. p-ISSN:2528-5742.

[18] V.M. Siqueira, R. Conti, J.M. Araujo, C.M. de Souza-Motta, Endophytic fungi from the medicinal plant *Lippia sidoides* Cham and their antimicrobial activity, Symbiosis, vol. 53, 2011, pp. 89–95. DOI: 10.1007/s13199-011-0113-7

[19] D.C. Fontana, S. de Paula, A.G. Torres, V.H.M. de Souza, S.F. Pascholati, D. Schmidt, D.D. Neto, Endophytic fungi: biological control and induced resistance to phytopathogens and abiotic stresses, Pathogens, vol. 10, 2021, pp. 1-28. DOI: https://doi.org/10.3390/ pathogens10050570

[20] E.R. Worcher, H.E. Giaugue, S.N. Kivlin, Fungal symbionts alter plant drought response, Microb Ecol, vol. 65, 2013, pp. 671-678.

[21] M.A.C. Latz, B. Jensen, D.B. Collinge, H.J.L. Jørgensen, Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression, Plant Ecology & Diversity, vol. 11, 2018, pp. 555-567. DOI: https://doi.org/10.1080/17550874.2018.1534146