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AKTIVITAS ANTIOKSIDAN DARI METABOLIT SEKUNDER KAPANG ENDOFIT MANGROVE *Aegiceras corniculatum*

(*The Antioxidant of Secondary Metabolites from Aegiceras corniculatum Mangrove Derived Endophytic Fungi*)

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ABSTRAK. *Kapang endofit dari mangrove memiliki potensi dalam menghasilkan metabolit sekunder seperti antioksidan. Antioksidan merupakan senyawa yang banyak diaplikasikan dalam industri sebagai bahan untuk pembuatan produk di bidang pangan maupun kesehatan. Penelitian ini bertujuan untuk mengetahui potensi antioksidan kapang endofit dari mangrove species Aegiceras corniculatum. Sebanyak 8 isolat kapang endofit berhasil diisolasi, yaitu 2 isolat berasal dari daun, 3 isolat berasal dari buah dan 3 isolat berasal dari ranting. Di antara 8 isolat tersebut, hanya 1 isolat yang dihanyakan aktif sebagai antioksidan dengan nilai IC_{50} sebesar 19,28 µL/mL, meskipun nilai ini masih lebih rendah dibandingkan vitamin C yaitu sebesar 6,08 µL/mL. Hasil identifikasi kimia dengan menggunakan GC-MS menunjukkan beberapa senyawa kimia yang memiliki aktivitas aktioksidan di antaranya phenol,3,5-bis(1,1-dimethylethyl), hexadecanoic acid, hexadecanoic acid methyl ester, malic acid, N-aminopyrrolidine, 9-octadecanoic acid, methyl ester (E), benzeneethanol, 4-hydroxy, 1,2-benzenedicarboxylic acid, d-tyrosine, bis(2-methylpropyl) ester 1-nonadecene dan heneicosane. Isolat kapang endofit diidentifikasi dengan penanda Internal Transcribed Spacer (ITS) dan memiliki kemiripan tertinggi dengan Microdochium sp.*

Kata kunci: *Aegiceras corniculatum, antioksidan, kapang endofit, mangrove, Microdochium sp*

ABSTRACT. *Mangrove endophytic fungi potentially produce secondary metabolites such as antioxidant. Antioxidants are compounds that are widely applied in the industry as an ingredient in the manufacture of products in the food and health sector. In this study, we investigated the antioxidant potential of secondary metabolites from fungal endophytic of mangrove species Aegiceras corniculatum. A total of eight endophytic fungi were successfully isolated, two isolates from leaf, three isolates from fruit and three isolates from twig. Between the isolates obtained, only one isolated was active as the antioxidant with IC_{50} value 19.28 µL/mL eventhough still lower than the standard ascorbic acid (6.08 µL/mL). The results of chemical identification using GC-MS showed several chemical compounds that have antioxidant activity including phenol,3,5-bis(1,1-dimethylethyl), hexadecanoic acid, hexadecanoic acid methyl ester, malic acid, N-aminopyrrolidine, 9-octadecanoic acid, methyl ester (E), benzeneethanol, 4-hydroxy, 1,2-benzenedicarboxylic acid, d-tyrosine, bis(2-methylpropyl) ester 1-nonadecene dan heneicosane. The selected fungal endophytic isolated were identified using molecular Internal Transcribed Spacer (ITS) marker and has a high taxonomy similarity with Microdochium sp.*

Keywords: *Aegiceras corniculatum, antioxidant, endophytic fungi, mangrove, Microdochium sp*

1. INTRODUCTION

Indonesia has the largest mangrove forest in the world. It has 202 species of mangrove that scattered along the country (Wardani *et al*., 2016). Mangrove plants have benefit as it contains secondary metabolites. Condition of mangrove's mixed ecosystem causes mangrove fungi to live in extreme environment. This situation potentially produces numerous compounds, including bioactive metabolites (Debbab *et al*., 2013; Hamed *et al*., 2015). Secondary metabolites from mangrove can provide antimicrobial, antimalarial, anticancer and antioxidant materials (Sari & Hasibuan 2017).

Mangrove plants probably contain several endophytic microbes on its tissues that can produce secondary metabolites compounds. Endophytic microbes from mangrove are also part of the second biggest marine microbe group (Zhou *et al*., 2018). This kind of endophytic microbes, especially endophytic fungi has an essential role in supporting ecosystem of mangrove on its cycles. It is proven that endophytic fungi are the source of natural compounds that have high biological activity and level structural diversity (Vijaya, 2017). In an ideal way, metabolites compounds from endophytic fungi have a large number of activities in comparison to its host plant (Strobel *et al*., 2004).

The utilization of endophytic fungi from mangrove is potentially to be observed The production of secondary metabolite using endophytic microbes has several advantages, such as short life cycles, so production process is simpler as it does not require big space as a planting area. Moreover, bioactive resources from plant such as fruit, peel, tissue, leaf and stem can destroy the plant itself in a time. Also, the process of collecting the substances that is done repeatedly can cause crisis and extinction for several species of plants (Deshmukh *et al*., 2018).

Endophytic fungi can produce secondary metabolite based on its bioactivity and chemical structure such as antioxidant (Zhou *et al*., 2018; Rahmawati *et al*., 2019), antibacterial (Handayani *et al*., 2017; Mukhlis *et al*., 2018; Prihanto *et al*., 2011), antimicrobial (Basha *et al*., 2012), antivirus (Rajamanikyam *et al*., 2017; Zhang *et al*., 2011), antidiabetic and anti-inflammatory (Rajamanikyam *et al*., 2017). Antioxidants are compounds that mostly used as industrial resources in food and health sector. It can prevent radical oxidation and reduce oxidative stress (Chaudhary *et al*., 2015). Moreover, antioxidant inhibits peroxidation by transfering electron or hyrogen atom of free radicals in order to balance the compounds (Chi *et al*., 2015). Several synthetic antioxidants can be used to inhibit oxidation on food containing oils such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) dan tert-butylhydroquinone (TBHQ). However, the use of synthetic antioxidants is limited as it has health risk such as protein and DNA damage, toxicity, etc. (Zhou *et al*., 2018). As the result, it is a need to find antioxidants sources that are safer and made from sustainable resource. Therefore, the research related to antioxidants from endophytic fungi is essential to be conducted. This research aimed to isolate endophytic fungi of *Aegiceras corniculatum* to analyze its antioxidant activity and identify chemical that is contained in secondary metabolite produced by isolated endophytic fungi. Furthermore, molecular identification was carried out on selected endophytic fungi isolates.

2. METHOD

Isolation of Endophytic Fungi (Modified Handayani *et al.*, 2017)

The fresh leaf, fruit and twig of mangrove species *Aegiceras corniculatum* were collected from Segara Anakan, Central Java, Indonesia. It washed with running water for 10 minutes. The sample is cut into two pieces with a length of the pieces 1 cm. Then, the part of sample is sterilized by dipping in 70% ethanol for 1 min, put into NAOCL 5,3% (w/v) for 5 minutes and dipped again in 70% ethanol (v/v) for 30 seconds. Each pieces were placed on potato dextrose agar (PDA) media and incubated at 27 °C for 7 days. Morphologically different results were transferred to the PDA media and incubated for 7 days at room temperature. (not tilted, PDA with petri dish)

Fermentation and Extraction of Isolated Endophytic Fungi (Rachman *et al.*, 2018)

Fermentation is carried out by taking a loop of mold that grows on a plate containing PDA media and put in an erlenmeyer container containing 100 mL PDB media. It was incubated using shaking incubators at 120 rpm in 14 days, approximately. Extraction was carried out after cultivation for 14 days. The extraction process itself was done using ethyl acetate to separate between the filtrate and the growing mold mycelia. Separation using a vacuum filter using filter paper that has been weighed before. After being tested, the mold mycelia were dried using an oven at 60 ^{o}C for 24 hours. The filtrate was partitioned using a separating funnel with 1:1 ethyl acetate solvent. Extraction was

carried out 3 times. After partitioning, the filtrate is evaporated using a rotary evaporator until the liquid thickens. After that, the drying is done again to evaporate all the ethyl acetate in the mold extract. The final result of the mold extract was weighed and tested for its antioxidant activity.

Analysis of Antioxidant (Rachman *et al.*, 2018)

Antioxidant activity of extracted isolated endophytic fungi was analyzed to obtain potential isolate that active as an antioxidant. The antioxidant inhibition test was conducted through 2,2–difenil–1–pikrilhidrazil (DPPH) method at concentration level 100 µL/mL. The stock solution was made in 1000 µL/mL, then pipette as much as 0.3 mL and reacted with DPPH 0.4 mM as much as 0.6 mL and added 2.1 mL methanol so that the total mixture becomes 3 mL. The solution is incubated at 37 °C for 30 minutes. The solution was incubated at 37 °C in 30 minutes. After that, the solution was measured using spectrophotometer with 517 nm wavelength. The inhibition was calculated using equation:

$$Percentage\ of\ DPPH\ scavenger = \frac{A-B}{A}\ x\ 100\%$$

A: control absorbance
B: extract absorbance

Next, IC_{50} of the extract of ethyl acetate which determined as active antioxidant were analyzed. The antioxidant activity was conducted through DPPH method at concentrations level of 5, 20, 25 and 50 µL/mL.

Analysis of Chemical Compounds for Potential Endophytic Fungi (Druzian *et al.*, 2019)

Analysis of chemical compounds was carried out using gas chromatography-mass spectrometry (GS-MS) Shimadzu type GC-100 Plus Series with Rtx-5MS capillary column (diameter 0.25 µm, length 30 m, thickness 0.25 µm). Other conditions in GC-MS include carrier gas using Helium UHP (Ultra High Pure), Split (200 °C), flow control mode: Pressure, column flow: 1.08 mL/min and oven temperature: 60 to 235 °C).

Molecular Identification of Potential Endophytic Fungi

Molecular identification was done by using molecular marker Internal Transcribed Spacer (ITS). DNA isolation of fungal using ZR Fungal Bacteria DNA Kit (Zymo Research, D6005). The stages of DNA amplification are by making a volume of 25 µL PCR master mix containing 9.5 µL alkaline free water, 12.5 µL 2x My Taq HS Red Mix (Bioline, BIO-25047), 1 µL DNA template, 1 µL 20 µmol/µL. Primer uses ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') as the forward primer and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') as the reverse primer. The reactions carried out in the amplification process were 35 cycles with 3 stages, pre-denaturation at 95 °C for 1 minute, denaturation at 95 °C for 15 seconds, annealing at 52 °C for 15 seconds and extension at 72 °C for 45 seconds. PCR product were sent for sequencing at the 1st BASE in Malaysia, Apical Scientific Sdn Bhd. The result of DNA sequencing was analyzed with Base basic local aligment search tool (BLAST) on the National Centre for Biotechnology Information (NCBI).

3. RESULT AND DISCUSSION

Isolation of Endophytic Fungi

Isolation of endophytic fungi from the mangrove *Aegiceras corniculatum* were successfully isolated from leaves, fruit and twig by using PDA. The morphological characters of the endophytic fungi that grow on the media are observed in plain view. Morphological characters observed included surface color and reverse, texture, topography and exudates. Morphologically different fungi were each transferred to PDA media to obtain pure isolates. Eight pure isolates were obtained which 2 isolates were from leaves (Figure 1), 3 isolates from fruit (Figure 2) and 2 isolates from twigs (Figure 3).

Table 1. Morphological characters of endophytic fungi on the leaves, fruits and branches of the mangrove aegiceras corniculatum

No	Isolate Code	Morphological characters
1	DD1	Circular growth pattern, slightly brownish white surface color, brownish yellow reverse color, velvety texture, verrugose topography and no c points
2	DD2	Circular growth pattern, clean white surface color, yellowish-white reverse color, cotton-like texture, topography has button-like protrusions in the center of the colony, forming radial lines and no exudates points
3	DBU1	Circular growth pattern, slightly yellowish white surface color, brownish-white reversal color, hyphae texture almost long like wool cloth, has a protrusion in the center of the colony, does not form radial lines and there are no exudates points
4	DBU2	Circular growth pattern, white surface color, brownish yellow reverse color, smooth hyphal texture and overall more transparent, verrugose topography, does not form radial lines and there are no exudates points
5	DBU3	Circular growth pattern, white surface color, yellowish-white reversal color, long and dense textures of hyphae like cotton, have a prominent area in the center of the colony, do not form radial lines and there are no exudates points
6	DR1	Circular growth pattern, white surface color, yellowish-white inverse color, dense texture like cotton, no radial lines and no exudates points.
7	DR2	Circular growth pattern, slightly brownish white surface color, yellowish-white inverted color, smooth hyphal texture, short like transparent, colonies have irregular grooves and have exudates points
8	DR3	Circular growth pattern, white surface color and brownish part, brownish white reverse color, woolen-like texture, colony has irregular grooves, does not form radial lines and there are no exudates points.

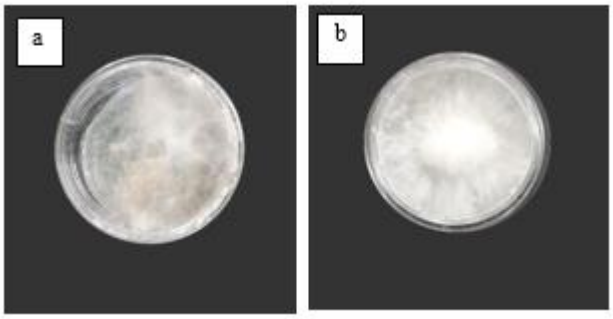

Figure 1. Isolated fungi from the leaf of mangrove *Aegiceras corniculatum;* a) Isolate DD1, b) Isolate DD2

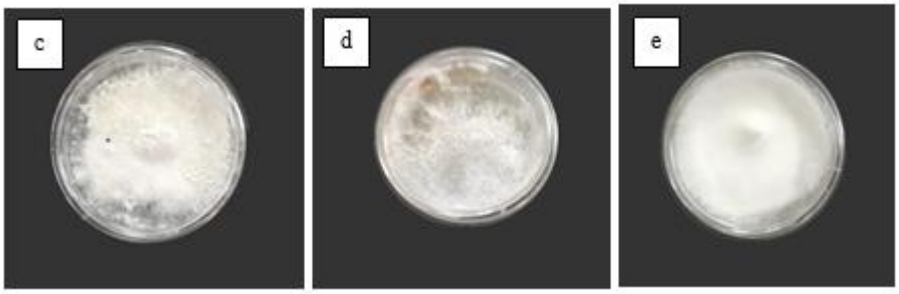

Figure 2. Isolated fungi from the fruit mangrove *Agieceras corniculatum*: c) Isolate DBU1, d) Isolate DBU2, e) Isolate DBU3

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Figure 3. Isolated fungi from the twig of mangrove *Agieceras corniculatum;* f) Isolate DR1, g) Isolate DR2, h) Isolate DR3

In previous study, 24 endophytic taxa, 12 from its leaf and 17 from its twig of mangrove *Aegiceras corniculatum* in South China (Li *et al*., 2016). There were several isolates that can be found from species of mangrove in Indonesia. *Rhizopora apiculata* from Tanjung Api-Api obtained three isolated that has different characateristics, seven isolated from its roots, leaf and stems (Mukhlis *et al*., 2018). There were 12 strains of endophytic fungi from roots, leaf dan stems of *Sonneratia griffithii* (Handayani *et al*., 2017). Moreover, endophytic fungi of *Rhizopora mucrona* that was taken from Sidorajo obtained 12 isolates, three from leaf, four from stem and five from root (Prihanto *et al*., 2011). The difference in the number of isolates obtained in each plant is thought to be due to environmental factors, namely habitat conditions. Environmental conditions in each plant are different and affected to interaction between endophytic fungi and their host plants. In addition, the type of isolate obtained was different from each plant so that it would affect its morphological characteristics. Environmental factors such as season, location and species can influence the diversity or frequency of endophytic fungi in their host plants (De Souza Sebastianes *et al*., 2013). In addition, other factors such as fragment size, plant part and identification difficulties can affect the differences in the number of endophytic species found in each isolated host plant (Costa *et al*., 2012). Host species also contribute to mold formation, such as in mangroves where the acidity index between plant species is relatively low even though they grow in the same area (Costa *et al*., 2012).

Fermentation and Extraction of Isolated Endophytic Fungi

Fermentation is done to grow molds so that they can produce secondary metabolites. The fermentation process in endophytic fungi occurs in the statistical phase which generally occurs until day 14. The statistical phase is the right time for the fungi to produce secondary metabolites optimally (Basha *et al*., 2012) and then extracted to obtain pure secondary metabolites..

According to the results, DD1 isolate had the highest extract yield (27.45 mg) and DR1 isolate had the lowest yield (12.85 mg) in 100 mL PDB media (Figure 4). Rachman *et al*. (2018) reported the yield the ethyl acetate extract of endophytic fungi isolated from cinnamon twigs. The highest yield (w/v) was 0.045% in Cb.Gm.B9 isolates and the lowest yield was 0.012% in Cb.Gm.B2 and Cb.Gm.B6 isolates (Rachman *et al*., 2018). The yield of endophytic fungi extract derived from the leaves of *Markhamia tomentosa* was reported by Ibrahim *et al*. (2017), where ethyl acetate exract of 554, 180, 437 and 480 mg in 200 mg of solid rice medium were obtained. Secondary metabolite extract obtained from isolates *Jatropha curcas* namely 750, 590, 56, 321 and 501 mg in 1200 mL of media (Kumar *et al*., 2013). The yield produced from extracts of secondary metabolites from mangroves *Avicennia marina* and *Xylocarpus granatum* is 17 mg from leaves, 12-15 mg from stems and 7-14 mg from roots (Rahmawati *et al*., 2019).

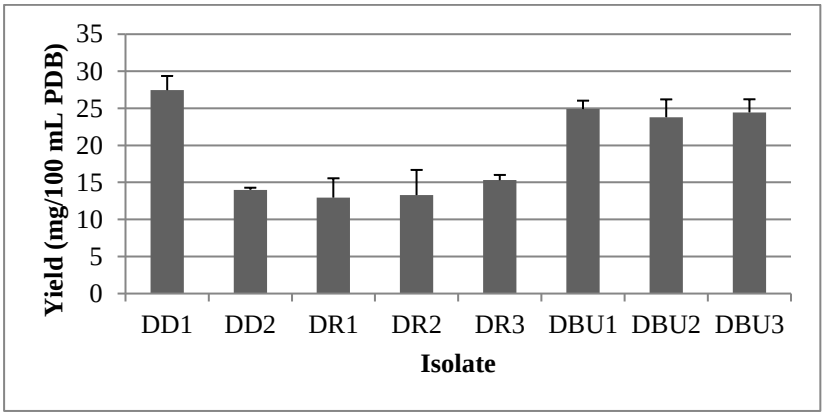

Figure 4. Extract yield of secondary metabolites mangrove *Aegiceras corniculatum* endophytic fungi endophytic fungi by ethyl acetate, whereas DD1, DD2 are fungi from leaves; DR1, DR2, DR3 are fungi from twig; DBU1, DBU2, DBU3 fungi from fruits. Data showed in the mean ± SD (n=3)

Analysis of Antioxidant of the Extract

The antioxidant inhibition test was conducted at 100 µL/mL concentration level to obtain the inhibition percentage. The result showed that all of the endophytic fungi isolated which were successfully isolated had an inhibitory of DPPH scavenger. However, there was only one endophytic fungi (DBU3 isolate showed inhibition over 50% at concentration used (Table 2). The activity of antioxidant is considered active if it is able to inhibit free radical more than 80%, moderate if it inhibits 50-80% of free radical and inactive if the inhibition level less than 50% (Widowati *et al*., 2016).

Table 2. Secondary metabolite antioxidant capacity of secondary metabolites produces by endophytic fungi from *Aegiceras corniculatum*

No	Isolate	Percentage inhibition of DPPH scavenger (%)
1	DD1	22.20
2	DD2	10.09
3	DBU1	41.64
4	DBU2	19.17
5	DBU3	89.80
6	DR1	7.45
7	DR2	4.18
8	DR3	31.58

Abbreviations for percentage of DPPH scavenger are the same as in Figure 4

Ethyl acetate extract which had an inhibition more than 50% were analyzed for IC_{50} The antioxidant activity was further tested using 5, 10, 25 dan 50 µL/mL level of concentration. Table 2 shows the higher level of concentration that is used, there will be an escalation of the percentage of the inhibition level. According to the result, value of the extract of antioxidant from DBU3 isolate on IC_{50} is 19,28 µL/mL.

Table 3. IC_{50} value of DBU3 isolate and control (ascorbic acid)

Sample	Concentration (µL/mL)	Percentage inhibition of DPPH scavenger (%)	IC_{50} (µL/mL)
DBU3	5	39.51	19.28
	10	44.47	
	25	53.48	
	50	71.45	
Ascorbic Acid	3	39.28	6.08
	6	48.44	
	9	61.86	
	12	71.03	

DBU3 is selected isolate, from fruit mangrove *Aegiceras corniculatum*

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According to the result, it can be concluded that DBU3 endophytic fungi isolated has active antioxidant activity. The IC_{50} on the antioxidant activity test were divided into several groups very active if it has IC_{50}<10 mg/L, active if it reach the value of IC_{50}<100 mg/L and inactive if the value of IC_{50}>100 mg/L (Çam and Durmaz 2009). Therefore, research conducted by Zhou *et al.* (2018), there were 2 isolates, HHL55 *(Cytospora rhuzophorae)* and HHL38 *(Seridium ceratosporum)* from mangrove *Rhizophora stylosa* which had the most potential as antioxidants with IC_{50} values 0,33 ± 0,02 and 0,37 ± 0,02 mg/mL. Antioxidant activity produced from the mangrove endophytic fungi *Aspergillus sp.* Y16 has an IC value of 1.45 mg / mL (Chen *et al*., 2011). Endophytic fungi from the mangrove fruit of *Acanthus ilicifolius* L produced IC_{50} values ranging from 10,2 to 15,3 μg/mL in 4 components resulting from fermentation (Yan *et al*., 2020). Bioactivity that can be produced by endophytic fungi is influenced by environmental conditions. Secondary metabolites produced by endophytic fungi are a response to environmental stress, so that different environments may affect the conditions of endophytic fungi in producing secondary metabolite compounds. Different conditions such as salinity, pressure and temperature in marine organisms can produce different and unique natural product potentials in each species (Hamed *et al*., 2015). In addition, factors such as season, age, environment and location can affect the biological compounds produced by endophytic fungi (Strobel *et al*., 2004).

Identification of chemical compounds

Analysis of Gas Chromatography Mass Spectroscopy (GC-MS) indicated 50 compounds that were contained in ethyl acetate extract. There were only 22 compounds that have *similarity* value more than 80% according to library bank NISTI4.lib (Table 4).

Table 4. The chemical compound secondary metabolites of DBU3 isolate from GC-MS

Retention Time (minutes)	Compound according library bank
8.5	Malic Acid
11.0	Hexane-1,3,4-triol, 3,5- dimethyl-
10.0	N-Aminopyrrolidine
11.7	dl-Mevalonic acid lactone
14.1	Benzeneethanol, 4- hydroxy-
14.4	2,6,10- Trimethyltridecane
15.3	Eicosane
17.3	Phenol, 3,5-bis(1,1-dimethylethyl)-
18.2	d-Tyrosine
18.6	9-Tricosene, (Z)-
18.7	Heneicosane
18.9	Pentadecanoic acid
19.1	i-Propyl 12-methyltetradecanoate
19.6	1,2- Benzenedicarboxylic acid, bis(2- methylpropyl) ester
20.1	Hexadecanoic acid, methyl ester
20.2	Pyrrolo [1,2- a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-
20.3	Eicosane, 2,4- dimethyl-
20.4	n-Hexadecanoic acid
20.6	Phthalic acid, isobutyl non-5-yn-3-yl ester
20.8	1-Nonadecene
20.9	9-Octadecanoic acid, methyl ester, (E)-
31.9	Bis(2-ethylhexyl) phthalate

Based on the result of chemical profiling, it was suspected that active antioxidant compound on extract ethyl acetate produce by DBU3 isolate is phenol,3,5-bis(1,1-dimethylethyl) and

benzeneethanol, 4-hydroxy- which was a part of phenolic compounds. According to the research that is conducted by Wei *et al*. (2011) it is reported that mangrove *Aegiceras Corniculatum* extract using ethyl acetate perform as a good antioxidant, especially on phenolic component. Other components that are considered to be involved an antioxidant activity from DBU3 isolate are hexadecanoic acid and octadecanoic acid as it has strong antioxidant activity (Patra *et al*., 2015). Apart from acting as an anticancer, anti-inflammatory and antimicrobial role, 1,2-benzenedicarboxylic acid compounds, bis (2-methylpropyl) ester have bioactivity as antimicrobials and antioxidants (Asghar *et al*., 2011; Ezhilan & Neelamegam, 2011). The bis (2-ethylhexyl) phthalate compound is one of the compounds that has antioxidant activity (Asghar *et al*., 2011). Eicosane, 1-Nonadecene, Hexadecanoic acid and Heneicosane are included in compounds that have antioxidant activity (Premathilaka & Silva, 2016). The octadecanoid acid methyl ester compound or stearic acid ester is also included in compounds that have antioxidant activity (Rahman *et al*., 2014). Another compound, tyrosol, found in endophytic fungi of GFV1 and GFM12 isolates has strong antioxidant activity (Radhakrishnan, 2017). Compounds of [1, 2-A] pyrazine-1, 4-dione, hexahydro-3- (2-methylpropyl)- found in mangroves *Streptomyces sp.* VITMK isolates are nontoxic compounds that have potential as antioxidants that can ward off free radicals (Manimaran & Krishnan, 2017). The N-aminopyrrolidine compound which is included in the amine group has activity as an antioxidant (Tavadyan *et al*., 2017). Malic acid compounds show high antioxidant activity and can inhibit the lipase enzyme (Alakolanga *et al*., 2015). Bioactivity that can be produced by endophytic fungi is influenced by environmental conditions. Secondary metabolites produced by endophytic fungi are a response to environmental stress, so that different environments may affect the conditions of endophytic fungi in producing secondary metabolite compounds.

Molecular Identification of Endophytic Fungi

Molecular Identification for DBU3 sample was conducted using ITS. The result of electrophoresis of PCR DNA product of endophytic fungi showed the formation of a single tape that proves that the process of DNA isolation was successful. Marker that was used to read the result during the electrophoresis uses DNA marker 1 kb. It also showed the location of the DBU3 sample tape which around 600 base pair (Figure 5).

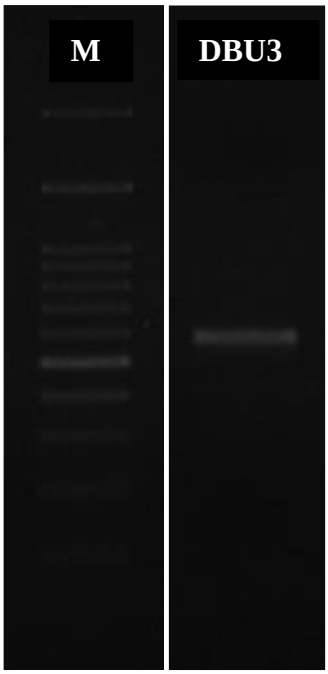

Figure 5. PCR Product from endophytic fungi DBU3 isolate with ITS 1 and ITS 4. DNA Marker 1 Kb.

Based on the BLAST results from the sequencing obtained, the endophytic fungi of DBU3 isolates was identified as *Microdochium sp*. *Microdochium* is a species known as snow mold. Many species of *Microdochium* were identified in aquatic or marine environments (Liu *et al*., 2016). Several species of *Microdochium* are pathogens in plants. One group of Microdochium that causes diseases in plants such as *Microdochium bolleyi* which causes root necrosis and damage to grass (Hong *et al*., 2016), *Microdochium paspali* species are known to produce seashore paspalum in *Paspalum vaginatum* (Zhang *et al*., 2015). Some species from the *Microdochium* group are non-pathogenic and some are endophytic, such as *Microdochium lycopodinum* and *Microdochium phragmitis* which are isolated from plants that do not cause negative effects (Ernst *et al*., 2011). Some of the endophytes found include *Microdochium* sp in *Rhoe spathacea* roots from nurseries in Banten Indonesia (Alvin *et al*., 2016) and wheat in China (Gagkaeva *et al*., 2020). *Microdochium lycopodinum* species found in plants as endophytes include the leaves of *Lycopodium annotinum* in Austria (Hernándes-Restrepo *et al*., 2016). *Microdochium* sp found in roots extracted with ethyl acetate has antibacterial activity that can

inhibit *Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* (Alvin *et al*., 2016).

4. CONCLUSION

Endophytic fungi that is successfully isolated from mangrove *Aegiceras corniculatum* obtained eight isolates from its leaf, fruit and twig. The selected isolated has the highest inhibiton of DPPH scavenger with the IC_{50} value 19.28 µL/mL. This research discovers sustainable use of species especially endophytic fungi from mangrove as material for bioproduction of antioxidant.

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