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RESEARCH **Open Access**

Antibacterial Activity of Endophytic Fungus Isolates of Mangrove Fruit (*Sonneratia alba*) Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract
Endophytic fungi live and associate in plant tissues and have a mutualistic relationship. Endophytic fungi produce various compounds such as steroids, terpenoids, phenolics, alkaloids which are the same as secondary metabolites from their host plants. The objective of this study is to isolate and identify endophytic fungi from mangrove fruit (*Sonneratia alba*) and to determine the antibacterial activity of endophytic fungi isolates against the growth of *Staphylococcus aureus* and *Escherichia coli*. The type of research is pre-experimental design, one shoot case study. The methods used are isolation, identification and agar diffusion. The pieces of mangrove fruit were disinfected and then cultured on SDA media to grow endophytic fungi isolates. The isolates were cultured repeatedly until pure isolates were obtained. The test of isolate activity against antibacterial was determined by the agar diffusion method with the test material of 2 isolates of endophytic fungi on Nutrient Agar (NA) media. The results showed that the mangrove fruit (*Sonneratia alba*) produced two isolates of endophytic fungi that could inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. It was concluded that the mangrove fruit culture produced 2 isolates, which are isolate 1 *Aspergillus niger* and isolate 2 *Aspergillus flavus*. Isolate 1 and isolate 2 had the potential as antibacterial against the growth of *Staphylococcus aureus* and *Escherichia coli*. Isolate 2 was more effective than isolate 1 in inhibiting the growth of Staphylococcus aureus. It is recommended to test the pharmacological and microbiological activity of the findings of isolates 1 and 2 in vivo.

Keywords: Mangrove, Antibacterial, Endophytic Fungi.

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1. INTRODUCTION

Microbes are very small living things that can live outside the host body (epiphytic microbes) and live inside the host body (endophytic microbes). Currently, endophytic microbes from plants have been widely studied to determine their potential in treatments such as antibacterial. Endophytic microbes in the form of bacteria, molds and yeasts can be isolated from all plant tissues. The screening results showed that each tissue contained endophytic microbes that differed from one plant to another. The number of isolates obtained from one part of the host plant is frequently very large, but only a few microbes are dominant in one host (Kumala, 2014).

Endophytic fungi are fungi that live internally and associate in plant tissues and have a mutualistic relationship with their host plants as protection against herbivores, insects and pathogens. Endophytic fungi can produce various compounds such as steroids, terpenoids, phenolics, alkaloids that have the potential as antioxidants, anti-cancer, antibacterial, antiviral, and anti-fungal (Rollando, 2019). The most commonly found bio actives are alkaloids (Kumala, 2014). Some alkaloids can only be produced by plants infected with fungi (Hidayat, et al, 2016).

The existence of mangroves in Indonesia is very abundant in species as identified by Suryono, (2015) finding 10 species in Segara Anakan. Suryono, et al., (2020) found 6 species in Jepara, Central Java. Kezia, et al., (2019) found 10 species in Barru, South Sulawesi. Mangrove fruit has the potential as a source of antioxidants which are proven to contain alkaloids, flavonoids, phenolic compounds, tannins and steroids (Paputungan, et al., 2017). *Sonneratia alba* extract has antibacterial activity of Streptococcus mutans, Propionibacterium acnes, and Candida albicans. The extract contains secondary metabolites such as alkaloids, phenols, tannins, saponins and flavonoids (Putri, et al., 2016; Kurniawan, et al., 2017). Endophytic fungi isolated from mangrove leaves have the potential to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* (Dwilestari, et al., 2015). Endophytic bacteria from mangroves have the potential as antibacterial against *Bacillus sp* and *Eschericia sp*, *Staphylococcus aureus* and *Escherichia coli* (Lutfia, 2017; Rismawati, 2018). The objective of the study is to isolate and identify endophytic fungi from mangrove fruit and to determine the antibacterial activity of endophytic fungi isolates against *Staphylococcus aureus* and *Escherichia coli* growth. This research is useful for developing the potential of mangroves as medicinal ingredients, especially antibacterial. Finding the types of endophytic fungi isolates on mangrove fruit and proving the activity of the isolates as antibacterial is the novelty of this study.

2. RESEARCH METHOD

The type of research used is a pre-experimental design, one shoot case study which was conducted at the Microbiology Laboratory of the University of East Indonesia. The step-by-step procedure was isolation of endophytic fungi, identification of endophytic fungi isolates and in vitro antibacterial activity of endophytic fungi isolates.

Isolation and Purification of Endophytic Fungi were from Mangrove Fruit (*Sonneratia alba*). All glassware used were sterilized in an oven at 180^{o}C for 2 hours. The test material for Mangrove fruit (*Sonneratia alba*) was taken from the village of Bonto Perak, Pangkajene District, Pangkajene Regency and Islands Province of South Sulawesi. Mangrove fruit was washed with running water for 10 minutes. Then, the surface was sterilized by immersing successively into 75% alcohol for 1 minute, 5% sodium hypochlorite (NaOCl) for 5 minutes, and 75% alcohol for 30 seconds. Furthermore, the mangrove fruit was dried in a sterile petri dish which was given sterile

filter paper. Mangrove fruit was cut transversely and longitudinally with a size of ± 1 cm above a sterile glass object. Then, the pieces of Mangrove Fruit were inoculated on SDA medium (Sabouraud Dextrose Agar with 0.005% chloram phenicol added) in a petri dish. Incubated at 25°C for 5-7 days. The results of the isolation of endophytic fungi growing on SDA medium were purified by re-inoculation of single colonies on SDA media and incubated for 3 days at 25°C. The incubation results discovered several types of pure mold based on observations of the shape and color of the colonies on SDA medium. Each colony with a different shape or color was re-cultured repeatedly to obtain a pure colony isolate of endophytic fungi.

The results of endophytic fungi isolates were further identified based on macroscopic and microscopic basis. Macroscopic observations included colony shape and color and microscopic observations (shape and size of hyphae, conidia, spores with a microscope). Fungi were identified by matching microscopic specifications with the literature.

Testing the Antibacterial Activity of Mangrove Endophytic Fungi Against *Staphylococcus aureus* and *Escherichia coli*. The test bacteria *Staphylococcus aureus* and *Escherichia coli* were rejuvenated 1x24 hours and then suspended until a turbidity level was obtained which was equivalent to the Mc Farland standard of 0.5. Mangrove fruit endophytic fungi (*Sonneratia alba*) which had grown on SDA medium were suspended with sterile distilled water. Blank paper discs were immersed in a suspension of endophytic fungi for 30 minutes and then drained. Sterile Nutrient Agar (NA) medium was poured aseptically into sterile petri dishes and allowed to solidify. *Staphylococcus aureus* and *Escherichia coli* suspensions were inoculated on the surface of the NA medium, respectively, using a sterile swab evenly. Paper discs were placed on NA medium that had been inoculated with the test bacteria at approximately the same distance from each other, incubated at 37°C for 1×24 hours. The zone of inhibition formed was measured. The test was administered with 4 times replication. The research has also received a recommendation from the ethics committee of the Health Polytechnic of the Ministry of Health Makassar with the ethics number: 422/KEPK-PTKMKS/V/2019.

3. RESULTS AND DISCUSSION

The results of macroscopic and microscopic observations on isolate 1 and isolate 2 can be seen from the following figure:

Figure 1. Characteristics of fungal isolate 1 macroscopically.

Figure 1 shows the macroscopic characteristics of isolate 1, which is the color of the colonies black with white around, the shape of the colonies is round, the distribution is concentrated, the surface of the colonies is rough.

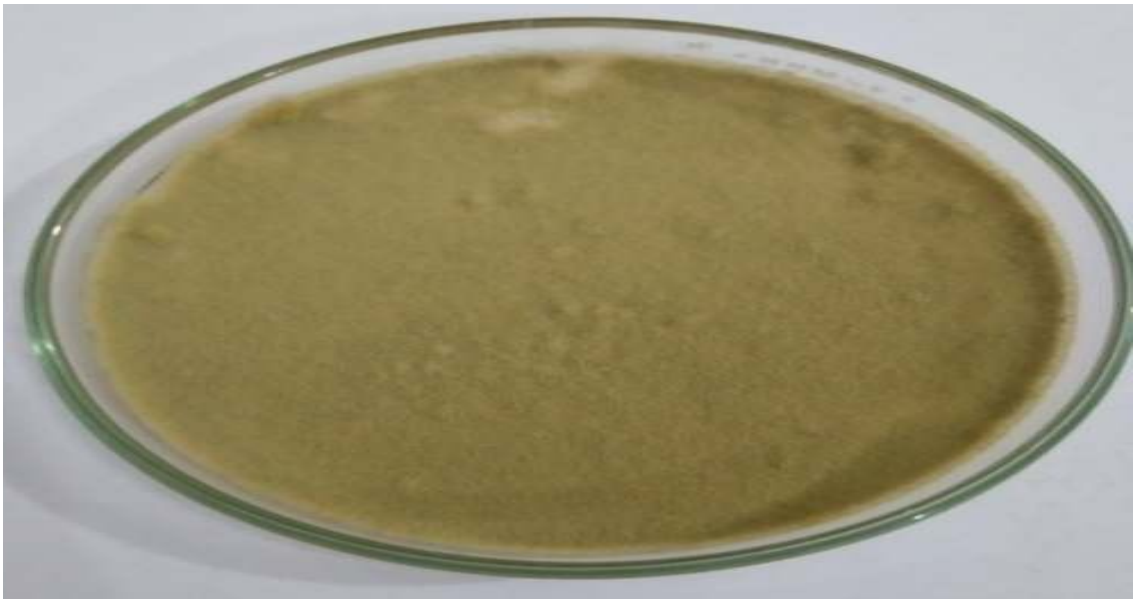

Figure 2. Macroscopic characteristics of fungal isolate 2.

Figure 2 shows the macroscopic characteristics of isolate 2, which is yellowish green colonies, irregular colony shape, spread, smooth colony surface like flour.

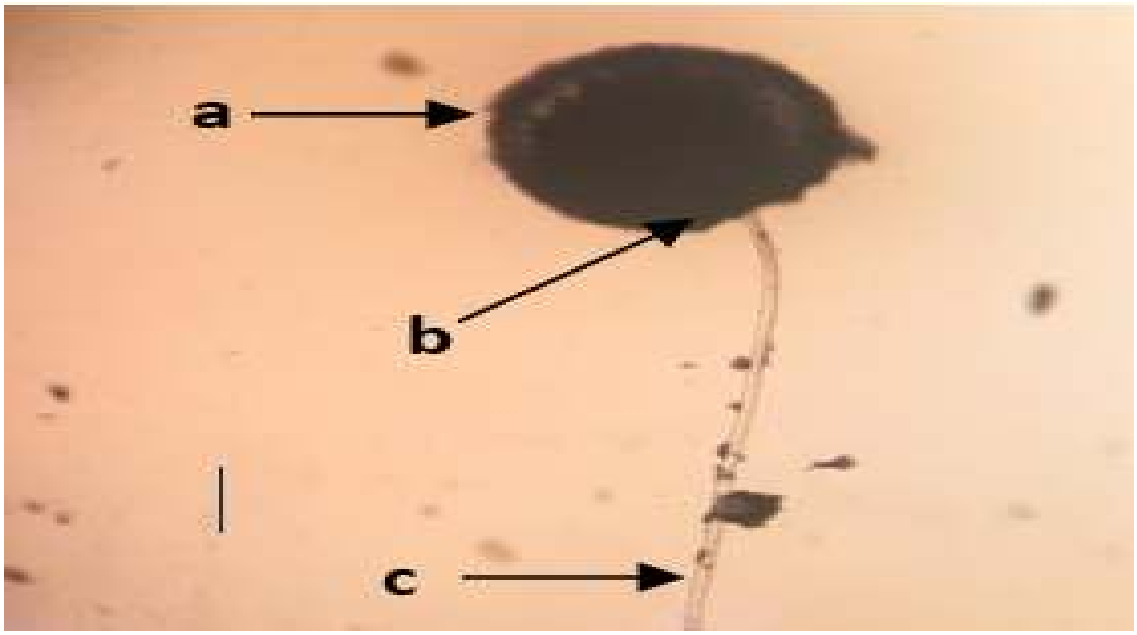

Figure 3. Characteristics of fungal isolate 1 microscopically consisting of: a. Conidia, b. Vesicles and c. Conidiophores.

Figure 3 shows the microscopic characteristics of isolate 1, that is large conidia, round to oval in shape, black in color, conidia in many strands. This fungus has long, thin-walled conidiophores. At the end, it enlarges to form a circle.

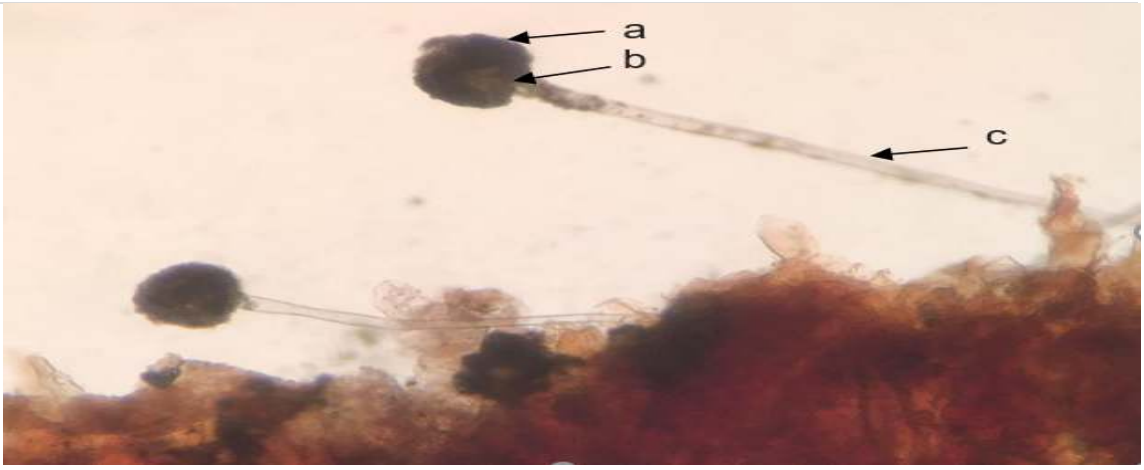

Figure 4. Characteristics of fungal isolate 2 microscopically consisting of: a. Conidia, b. Vesicles and c. conidiophores.

Figure 4 shows the microscopic characteristics of isolate 2, which is conidia in many-shaped series. This fungus has thin-walled conidiophores.

Table 1. Results of Measurement of the Growth Inhibition Zone of *Staphylococcus aureus* and *Escherichia coli.*

Sample	Treatment	Diameter of Bacterial Growth Inhibition Zone (mm)				Average
		1	2	3	4	
Staphylococcus aureus	Isolate 1	9,5	9,5	10	10	9,75
	Isolate 2	14,5	13,25	12	12,25	13
Escherichia coli	Isolate 1	10	9,75	10,5	10	10,06
	Isolate 2	11,5	11	10,75	10,5	10,93

The distribution of mangrove plants in Indonesia is very large, for intanstance, in Barru Regency, South Sulawesi, 7 species were found (Saru, et al., 2019) and in Rote Ndao, East Nusa Tenggara, 11 species were found (Ngoma, et al., 2020). It provides an opportunity to utilize mangrove species for treatment, for example as an antibacterial. The use of mangroves has been examined in the form of extracts, but isolating endophytic fungi from mangrove fruit is a more efficient way of utilization. The use of endophytic microbes as antibacterial has been performed on other plant species such as katokkon chili (Rolando, 2019), cucumber (Yuanwar and Ainy, 2019), gotu kola (Hidayat, et al., 2018), secang wood (Amirullah, et al., 2019); areca nut (Nuryanti and Astuti, 2019), and beluntas leaves (Setiawan and Musdalipah, 2018).

The results of the study found two pure isolates of endophytic fungi from mangrove fruit (*Sonneratia alba*). The colony was identified as Aspergillus niger which is in accordance with Hidayat's, et al., (2018) statement that black colonies are a marker of the presence of Aspergillus niger. On microscopic observation of isolate 1, conidia were found to be large, round to oval in shape, black in color, conidia in series to be numerous. This fungus has long, thin-walled conidiophores. At the end, it enlarges to form a circle. Based on these characteristics, the isolate was suspected to be the fungus Aspergillus niger. It is in accordance with the statement of Gandjar (2000) that the characteristic of Aspergillus niger is that the conidia heads are black, round in shape, and tend to split into columns in old colonies. The stipe of conidiophores is smooth-walled, hyaline in color, but may also be brownish. The vesicles are spherical to

semicircular, and 50–100m in diameter. Conidia are spherical to semi-spherical measuring 3.5-5.0 m, brown in color and have irregular ornamentation in the form of protrusions and spines. The findings of *Aspergillus sp* isolates from mangrove plant cultures were also identified (Mukhlis, et al., 2018).

Observations on isolate 2 were macroscopically in the form of yellowish green colonies, irregular colony shape, spread, smooth colony surface like flour. Microscopic observations in the form of many conidia in series. This fungus has thin-walled conidiophores. These characteristics are thought to be the same as those of the flavus fungus. It is based on Amaliyah's (2017) statement that colonies of Aspergillus flavus generally grow rapidly and reach a diameter of 6-7 cm in 10-14 days. This mold has a yellow initial color which will turn greenish yellow or brown with an inverted golden color or colorless while the old colonies have a dark green color. Gandjar (2000), explained that the typical conidia head is round, then splits into several columns, and is yellowish green to yellowish dark green. Conidiophores are hyaline, leathery, and can reach a length of 1.0 mm (some are up to 2.5 mm). Vesicles are round to semi-spherical, conidia are round to semi-spherical (Sumampouw, 2019). Khalimah and Ainy (2019), have also isolated 3 isolates from mangroves and have even identified secondary metabolites from *Aspergillus sp* isolates as flavonoids, tannins and saponins.

The results of endophytic fungi isolates from mangrove fruit were obtained after antibacterial testing was performed against *Staphylococcus aureus* and *Escherichia coli*. The results showed that there were inhibition zones of isolates 1 and 2 of endophytic fungi of mangrove fruit against the growth of *Staphylococcus aureus* and *Escherichia coli*. In isolate 1, endophytic fungi of mangrove fruit which was examined on *Staphylococcus aureus* test bacteria had an average inhibition zone diameter of 9.75 mm and for Escherichia coli had an average inhibition zone diameter of 10.06 mm. For isolates 2 endophytic fungi of mangrove fruit tested on *Staphylococcus aureus* had an average inhibition zone of 13 mm and for *Escherichia coli* had an average inhibition zone of 10.03. These data indicate that endophytic fungi that grow on mangrove fruit (*Sonneratia alba*) can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. The endophytic fungi activity of mangrove fruit is in line with the activity of mangrove plant extracts as antibacterial against several bacteria, including *Staphylococcus aureus* and *Escherichia coli* (Dwilestari, et al., 2015). This antibacterial potential is caused by the content of secondary metabolites present in plants such as steroids, terpenoids, phenolics, and alkaloids (Rollando, 2019; Kumala, 2014; Hidayat, et al., 2016). Endophytic fungi isolated from other coastal plants such as mangrove roots (Rhizophora apiculate) also produced 2 isolates of black fungus and white fungus that have potential as antibacterial *Staphylococcus aureus* and *Escherichia coli* (Kartika, et al., 2014). Posangi and Bara (2014) found 2 isolates identified as *Aspergillus sp.* and *Acremonium sp* which functions as an antibacterial for *Staphylococcus aureus* and *Escherichia coli*. Mukhlis, et al., (2018) found 3 types of isolates, which are *Fusarium sp, Penicillium sp* and *Aspergillus sp* which have the potential to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*.

Isolate 1 was identified as Aspergillus niger (colored black) and isolate 2 was identified as Aspergillus niger (colored white/beige). The results of the measurement of the diameter of the inhibition zone of isolates 1 and 2 of endophytic fungi against the growth of *Staphylococcus aureus* and *Escherichia coli* were analyzed by SPSS. The normality test shows a sig value of 0.024 which means that there is data that is not normally distributed. The homogeneity test shows a sig value of 0.045 <0.05, which means the data is not homogeneous. Hence, the next analysis was performed by the Kruskal Wallis non-parametric test and continued with Mann Whitney. Kruskal Wallis

test showed a sig value of 0.005 <0.05, which means that there was a difference in the effect of isolate 1 and isolate 2 in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli*. Further analysis with Mann Whitney showed differences in the effectiveness of endophytic fungi isolates as antibacterial as follows: The effectiveness of isolate 1 against *Staphylococcus aureus* and *Escherichia coli* was not significantly different, the effectiveness of isolate 1 and isolate 2 was not significantly different against *Escherichia coli* and isolate 2 was more effective than isolate 1 in inhibit the growth of Staphylococcus aureus. The effectiveness of isolate 2 which was identified as Aspergillus flavus was better than isolate 1 which was identified as Aspergillus niger in line with research (Kasi, et al., 2015) which concluded that white mycelium endophytic fungi had a better antibacterial effect than black mycelium endophytic fungi and positive controls.

The findings of endophytic fungi isolates in this study have the potential for further testing of pharmacological activities. The discovery of endophytic fungi isolates can further develop the use of *Sonseratia alba* without having to harvest this mangrove species every time.

4. CONCLUSION

Mangrove fruit (*Sonneratia alba*) produced two isolates of endophytic fungi, which were Isolate 1 suspected of Aspergillus niger and Isolate 2 suspected of Aspergillus flavus. Isolate 1 and isolate 2 of endophytic fungi from Mangrove Fruit (*Sonneratia alba*) have the potential as antibacterial against the growth of *Staphylococcus aureus* and *Escherichia coli*. Isolate 2 was more effective than isolate 1 in inhibiting the growth of Staphylococcus aureus. It is recommended to continue this research to test the pharmacological and microbiological activities of isolates 1 and 2 in vivo.

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