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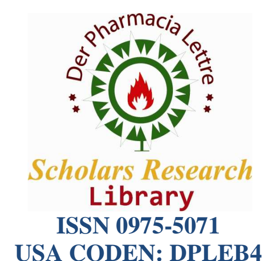


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# Isolation of Endophytic Fungi from Cortex, Leaf, and Pericarp of Mangosteen (*Garcinia mangostana* L.) and Testing of the Antimicrobial Activity

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## ABSTRACT

*A research on antimicrobial activity testing of the endophytic fungi which was symbiosis with mangosteen (Garcinia mangostana L.) has been conducted. About fourteen isolates of fungi from mangosteen have been isolated. Each fungal isolates were fermented in liquid medium of soybean extract broth for one week on a rotary shaker incubator at temperature of 25-27 °C and speed of 90 rpm. Each fermentation of fungal isolates were tested their antimicrobial activity against microbes Staphylococcus aureus, Escherichia coli and Candida albicans with the agar diffusion method. There were two fungal isolates that active against Escherichia coli and three fungal isolates that active against Staphylococcus aureus.*

**Keywords**: *Garcinia mangostana* L., endophytic fungi, mangosteen, antimicrobial activity

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## INTRODUCTION

Searches for the sources of bioactive compounds continue to be done along with the many new diseases are emerging. One source of bioactive compounds derived from microbes is endophytic microbes. Endophytic microbes can produce bioactive compounds that are potential to be developed into drugs. One of plant that is used to treat diarrhea, dysentery, and ulcers is mangosteen. The mangosteen fruit is used to treat diarrhea, dysentery, and ulcers [1]. Its skin is used to treat constipation, respiratory disorders, skin infections, and anti-inflammatory. The roots are used to cope with the irregular menstruation [2]. The purpose of this study was to test the antimicrobial activity of the compounds isolated from endophytic microbes of mangosteen.

## MATERIALS AND METHODS

### Test drug and chemicals

The leaves, bark and pericarp of the mangosteen (*Garcinia mangostana* L.) were obtained from Lubuk Alung, West Sumatra, Indonesia. All other chemicals used were of analytical grade.

### Isolation of endophytic fungus

Each fresh sample was washed with running water. Then each part of the plant (leaves, cortex, and pericarp) was cut to a size of 1 x 1 $cm^2$. Furthermore, in the laminar air flow cabinet, each organ of the plant was disinfected of their surfaces by immersing the organ by successively in 70 % ethanol for 30 seconds, a solution of 5 % sodium hypochlorite for 5 minutes, 70 % ethanol for 30 seconds and rinse with distilled water for 3 minutes. After that the samples were dried over sterile wipes [3, 4].

Each sample was planted using tweezers into Petri dishes containing Potato Dextrose Agar (PDA) by splitting parts of the plant and put in prone position. Each Petri dish was planted 2-3 slices of the sample, incubated at temperature

of 25-27 $^{o}$C for 5-7 days. The fungus that grows gradually purified one by one. Colonies that have a different form with each other colonies can be considered different colonies [5].

**Fermentation of isolated fungi and antimicrobial activity test of liquid fermentation**
Medium for fungal antibiotic production was the soybean extract broth liquid medium with a composition of glucose (1.2 %), calcium carbonate (0.1 %), 20 mL soybean extract (100 g soy bean sprouts in 1000 mL of distilled water) and added distilled water up to 100 % [6]. Each medium was heated to boiling and sterilized by autoclaving at 121 $^{o}$C for 15 minutes. Each of 1-2 ose fungal isolates that has been purified in previous experiments was inoculated on the liquid medium and then fermented. The fermentation process was done in a volume of 250 mL Erlenmeyer flask. Incubation for 72-120 hours at a temperature of 25-27 $^{o}$C on a rotary shaker incubator for fungus at a speed of 90 rpm [6]. Then the fermentation culture solution was centrifuged at a speed of 5000 rpm for 15 minutes. Supernatant was tested its antimicrobial activity against microbes using paper disc method. Paper discs dipped in the supernatant and planted in the medium NA containing bacteria and the medium PDA containing fungi. Then incubation was at a temperature of 37 $^{o}$C for 18-24 hours. Barriers to growth were observed and measured in diameter by using a caliper. While the results of centrifugation of endophytic fungi were washed with distilled water 3 times and dried by wind, so that obtained the weight of biomass [7].

**Characterization of endophytic fungus isolates**
Characterization of the fungal isolates was conducted against isolates that showed significant antibacterial activity. Observations were made macroscopically, a form colonies, color, and surface [7].

## RESULTS AND DISCUSSION

In the process of isolation of endophytic fungi, it was used direct planting method in which pieces of plant organs which have been disinfected its surface were affixed to potato dextrose agar (PDA) for fungi in the position of parts of the surface of the sample sticks to the media. This method was chosen because it was more practical and faster process [8]. Fungi that grow in media was purified and fermented to determine its ability to produce antimicrobial compounds. In this study, it was obtained the endophytic fungi that have not been purified (Fig. 1) and 12 isolates of endophytic fungi (Fig. 2). The profile of inhibition zone caused by fermentation liquid of endophytic fungi against testing bacteria was presented in Fig. 3. The antimicrobial test results were presented in Table 1. The pH medium and biomass of endophytic fungi were presented in Table 2.

**Fig. 1: Endophytic fungi that has not been purified**

**Fig. 2: Isolates of endophytic fungi**

**Fig. 3: The profile of inhibition zone caused by fermentation liquid of endophytic fungi against testing bacteria**

**Table 1: Antimicrobial test results of endophytic fungi**

| No | Code of fungal isolates | Genus | Diameter of inhibitions | | |
|---|---|---|---|---|---|
| | | | *E.coli* (mm) | *S.aureus* (mm) | *C.albicans* (mm) |
| 1. | J-$H_1$ | *Curvularis* sp | - | 9 | - |
| 2. | J-$H_2$ | *Aspergillus niger* | - | - | - |
| 3. | J-$G_1$ | *Candida* sp | 10 | - | - |
| 4. | J-$G_2$ | *Candida* sp | - | 11 | - |
| 5. | J-$P_1$ | *Cladosporium* sp | - | - | - |
| 6. | J-$P_2$ | *Candida* sp | - | - | - |
| 7. | J-$T_1$ | *Candida* sp | 10 | 13 | - |
| 8. | J-$T_2$ | *Cladosporium* sp | - | - | - |
| 9. | J-$U_1$ | *Cladosporium* sp | - | - | - |
| 10. | J-$U_2$ | *Cladosporium* sp | - | - | - |
| 11. | J-$K_5$ 1 | *Cladosporium* sp | - | - | - |
| 12. | J-$K_5$ 2 | *Aspergillus* sp | - | - | - |

**Table 2: pH medium and biomass of endophytic fungi after cultivation**

| Code of fungal isolates | pH medium | Biomass (gram) |
|---|---|---|
| J-$G_1$ | 6.70 | 0.909 |
| J-$G_2$ | 6.75 | 0.875 |
| J-$H_1$ | 6.92 | 0.768 |
| J-$H_2$ | 6.85 | 0.586 |
| J-$P_1$ | 6.70 | 0.483 |
| J-$P_2$ | 6.82 | 0.508 |
| J-$T_1$ | 6.72 | 0.728 |
| J-$T_2$ | 6.75 | 0.506 |
| J-$U_1$ | 6.90 | 0.456 |
| J-$U_2$ | 6.85 | 0.496 |
| J-$K_5$1 | 6.74 | 0.785 |
| J-$K_5$2 | 6.60 | 0.763 |

wo fungal isolates were active against *Escherichia coli* i.e. isolates with code J-G1 and J-T1 with a diameter of 10 mm. Three fungal isolates were active against *Staphylococcus aureus* i.e. isolates J-T1 with inhibitory diameter 13 mm, isolates J-H1 inhibitory diameter 9 mm, and isolates J-G2 with diameter of 11 mm. While there were no activity of fungal isolates toward the fungus *Candida albicans*.

In testing of the antimicrobial activity of the antimicrobial compounds produced by the endophytic microbes against the fungus *Candida albicans*, there were no inhibition due to *Candida albicans* including eukaryotic microorganisms, whereas pathogenic microbes tests used were Gram-positive and Gram-negative microbes including prokaryotic microorganism. The structure of the cell wall components was differ in the eukaryotic especially *Candida albicans*. In the eukaryotic, cell wall structural components contain chitin, cellulose or glucans, whereas the prokaryotic cell wall component contain peptidoglycan. In addition, metabolite produced was not suitable for eukaryotic microorganisms because the secondary metabolites produced cannot damage the cells of *Candida albicans* [9]. The limitations of the antifungal drug discovery over the antibacterial drugs this was due to the fungus associated with complex cell structure [10].

The activity of endophytic fungi in general was greater against Gram-positive bacteria such as *Staphylococcus aureus* bacteria than Gram-negative *Escherichia coli.* This was due to the structure of the cell wall of Gram-negative bacteria were complicated and also the external lipopolysaccharide layer was found in Gram-negative bacteria. This resistance was also associated with the permeability and pore bacterial cell wall. The pores of the cell wall of Gram-negative bacteria were highly selective for molecules that can get into the cell [11]. There were several isolates that do not have antimicrobial activity and the possibility of these isolates have other activities such as poison, giving color or pigment, growth agents, and pesticides [12].

There was influence of pH changes toward the filtrate of endophytic fungi. A decrease in the pH of the filtrate of endophytic fungi showed the role on the production of antimicrobial which can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* [6]. The addition of biomass of each endophytic microbe also has an influence on the growth of *Escherichia coli* and *Staphylococcus aureus*. The results showed that the antimicrobial synthesized in its infancy. Initial growth seen when a microbial cell inoculated on nutrient agar was enlarging the size, volume and weight of the cell. The cells continue to divide exponentially/quickly. As long as conditions allow, the growth and division of cells takes up a number of established cell population. The more cells that grow, the antimicrobial activity was increasing along with the metabolites excreted in the form of antimicrobial compounds out of the cell [7].

## CONCLUSION

About twelve isolates of fungi from leaves, bark, and fruit peel of mangosteen (*Garcinia mangostana* L.) have been isolated. There were two fungal isolates that active against *Escherichia coli* and three fungal isolates that active against *Staphylococcus aureus*. There was no fungal isolate that active against *Candida albicans*.

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