

Screening Antibacterial Potency of Metabolite Endophytic Fungi Mangosteen (*Garcinia mangostana* L.) Leaf

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Abstract—Mangosteen (*Garcinia mangostana* L.) is a tropical plant which fruit peel is widely used as an antioxidant, anti-diarrhea, anti-inflammatory, antitumor, and as an antibacterial. The previous study found the antibacterial activities of extract and metabolites endophytic fungi of mangosteen rind. In this research endophytic fungi was isolated from leaf of mangosteen. This research was aimed to explore utilization of mangosteen leaf and to screen antibacterial potency of the mangosteen leaf endophytic fungi metabolites.

Endophytic fungi were isolated from leaf of mangosteen in the Malt Extract Agar (MEA) medium in order to get 2 colonies of endophytic fungi. Screening of potential antibacterial metabolites was assessed using diffusion method and bioautography, obtained results of the antibacterial activity against *Escherichia coli* (Ec) and *Staphylococcus aureus* (Sa) on the metabolites endophytic fungi of leaf. Macroscopic and microscopic characteristics from fungi isolate which has antibacterial potency, was observed and fermented into Potatoes Dextrose Yeast (PDY) medium for 14 days. At 14th day, biomass and supernatant were separated and carried out separation by liquid-liquid extraction.

The supernatant and biomass were fractionated using n-hexane, ethyl acetate, and water. Each fraction was eluted to several mobile phase and tested its antibacterial activity against Ec and Sa. The result showed that there is a potential antibacterial activity of endophytic fungi metabolites leaf ED2 against Sa. Bioautography result was observed that the compound has antibacterial activity and is supposed as flavonoid compounds. It was supposed that endophytic fungi ED2 was a group of Trichoderma.

Keywords—mangosteen (*Garcinia mangostana* L.); endophytic fungi; antibacterial activity; *Escherichia coli*; *Staphylococcus aureus*

I. INTRODUCTION

Infectious diseases are health problem caused by pathogenic microorganisms. Antibiotics are potent treatment to infectious diseases. The use of antibiotics in the community is quite large, especially in self medication; which action is taken to address health problems by using drugs that can be used without medical supervision. Antibiotics are one of the drugs which is often used as self treatment. The use of antibiotics that are not accompanied by a rational use would lead to the microbes become resistant. This situation was attempted with some research to find new sources of natural or synthetic antimicrobial compounds. Antimicrobial compounds from natural sources can be obtained from plants and endophytic. Compounds from plants/ endophytic obtained by extracting certain parts of the plants/ endophytic with a solvent which can attract the active compounds in the form of secondary metabolites. Endophytic microbes are microbes that grow in the tissues of plants and produce bioactive compounds that act, among others, as anti-microorganism, anti-malaria, anti-cancer and also play a role in plant growth itself^[1]. Production secondary bioactive metabolites from parts of the plants many times are not efficient due to time and materials spent; while from endophytic microbes isolated from these plants and then cultured secondary metabolites can be easier to obtain^[2].

From Tan and Zou, among endophytic microbes to its host occurs evolutionary genetic exchange so that endophytic microbes can produce compounds that are similar or have the same characteristic with the host plants^[3]. Several studies also found the same compound to compound existing activities on the host plant, such as endophytic fungi *Alternaria sp.* isolated

from *Catharanthus roseus* phloem produce anticancer vinblastine [4], endophytic fungi *Fusarium oxysporum* isolated from the *Catharanthus roseus* phloem produce vincristine compound [5] and Yang *et al.* also identified vincristine in endophytic fungi isolated from leaves of *Catharanthus roseus*[6]. Pavithra, Satish and Ananda isolated and proven the antimicrobial activity of *Ocimum sanctum* L. endophytic fungi, where this plant's secondary metabolites also have antimicrobial activity[7].

Mangosteen (*Garcinia mangostana* L.) is one of the plants that is known has antimicrobial activity. Putra[8], proven the chloroform fraction and ethyl acetate fraction of methanol extract of mangosteen rind have antibacterial activity against *L. mesenteroides* and *L. plantarum*; while research on endophytic fungi mangosteen rind was done by Elfina, Martina and Roza was proven their antimicrobial activity against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*[9]. The antimicrobial activity of the mangosteen rind and its endophytic fungi was expected to be found also in other parts of this plant. As the previous hypothesis the same bioactive compound was isolated from endophytic from other parts of the plant; i.e from the leaves which is produced during the years compared to mangosteen fruit which is depend on seasonal time. The purpose of this study was to isolate endophytic fungi from the leaves of mangosteen (*Garcinia mangostana* L.) and screen its antibacterial potency of endophytic fungi and its metabolite so that expectations can be used as one source of the active compounds as antimicrobial.

II. MATERIALS AND METHODS

A. Plant Material

Fresh leaves of mangosteen (*Garcinia mangostana* L.) were obtained from the area of Malang. The plant was identified and sample documented at Botany Laboratory Faculty of Pharmacy, Widya Mandala Catholic University Surabaya Indonesia.

B. Material Media

Malt Extract Agar, Potatoes Dextrose Yeast, Mueller Hinton Broth, Sodium hypochlorite, and Sodium chloride.

C. Microorganism

Escherichia coli ATCC 8739 and *Staphylococcus aureus* ATCC 6538.

D. Methods

This study was an experimental study with endophytic fungi isolated from mangosteen leaves. Leaves were cultured to Malt Extract Agar (MEA)

in order to obtain fungi colonies. Colonies isolated to pure colonies obtained were tested for antibacterial activity against *Escherichia coli* (Ec) and *Staphylococcus aureus* (Sa) by diffusion method and the area of growth inhibition diameter (GID) was observed. Endophytic fungi from each of the leaves that have been observed wide diameter inhibition was characterized and fermented in Potatoes Dextrose Yeast (PDY) medium for 14 days. Filtrate was separated from the biomass, and then fractionated with n-hexane and ethyl acetate. Each fraction will be tested its antibacterial potency against Ec and Sa to determine the most active fraction.

Variables in this research consist of independent variable, dependent variable and controlled variable. The independent variable was used preparation of plant and endophytic fungi, bacterial test; the dependent variable was covers an area of growth inhibition diameter; while the controlled variables was include processing the growth of endophyte, fermentation method, and time of incubation.

E. Macroscopic and Microscopic Observation of Leaves of Mangosteen (*Garcinia mangostana* L.)

Macroscopic and microscopic observation was done by observing the characteristics of leaves and adapted to the characteristics of the leaves of the mangosteen.

F. Isolation of Endophytic Fungi Cultures of the Leaves of Mangosteen (*Garcinia mangostana* L.)

Fresh leaf of mangosteen was cleaned under running water and then cut into smaller pieces approximately the length/width of 3 cm. Pieces of leaf were soaked in 70% ethanol for 2 minutes then followed by immersion in a solution of 5.3% sodium hypochlorite for 5 minutes and soaked in 70% ethanol for 1 minute[10]. Pieces of leaf were dried with sterile filter paper for a few minutes, then each pieces of leaf were placed on the MEA medium and incubated at room temperature for 2-14 days.

G. Purification Culture of Endophytic Fungi from Leaves of Mangosteen (*Garcinia mangostana* L.) and Antibacterial Potency Assay

Endophytic fungi that grew on the MEA medium was observed macroscopic and separated to the MEA medium repeatedly to obtain pure colony. The fungi then screen its antibiotic potency by inoculated endophytic fungi to Sa and Ec. Endophytic fungi that has the largest area of growth inhibition was an endophytic fungi that has the greatest antimicrobial activity and selected for further fermentation process.

H. Fermentation Endophytic Fungi

Pure colonies endophytic fungi were inoculated into Erlenmeyer containing PDY medium and incubated at room temperature for 14 days. At 14th day, it was centrifuged at 3000 rpm for 20 minutes to separate the supernatant/ filtrate and biomass. The supernatant and biomass were separated and carried out separation by liquid-liquid extraction. The supernatant and biomass were fractionated using n-hexane, ethyl acetate, and water.

I. Bioautography

Mueller Hinton Broth was inoculated with 1.5×10^6 CFU/ml bacteria. Each fraction of a filtrate/ supernatant and biomass spotted on TLC plates as much as 20 μ L to silica gel F254 plates and eluted with n-hexane:ethyl acetate (2:8) mobile phase. The plates were sprayed with bacterial suspension, incubated at 37°C for 24 hours and observed of bacterial growth inhibition area. The plate was sprayed with 1% solution of triphenyl tetrazolium chloride. The existence of growth inhibition areas would be observed as colorless area in the spot contrast to the red stain on the plate.

J. Thin Layer Chromatography (TLC) Screening Bioactive Compounds

Determination of compounds that have antibacterial activity against Sa and Ec was done by spraying some specific reagents to the compound to TLC of the extract and fractions; for example FeCl₃, AlCl₃, Dragendorff, and Liberman Burchard. Observations were also made on the bioautography spot test results which were give positive result as areas of growth inhibition.

III. RESULT AND DISCUSSION

A. Macroscopic and Microscopic of Leaves and Endophytic Fungi Cultures of Mmangosteen (*Garcinia mangostana* L.)

Macroscopic and microscopic observation revealed that materials which were used have the same characteristics as mangosteen (*Garcinia mangostana* L.) (Fig. 1).

Endophytic fungi was isolated from leaf begin to be observed growth in the 2nd day of incubation and the longer will be more widespread. Each of these fungi has different incubation period so as to easier the purification of the culture of fungi observations made every day and if the observation has been able to differentiate fungi then continue the purification process without waiting for the time of incubation for 14 days. Isolates endophytic fungi that grow on MEA was observed macroscopic and microscopic (Fig.2, Table 1, Table 2).

Table 1. Macroscopic Endophytic fungi isolated from Mangosteen leaf

Code	Macroscopic
ED1	Filamentous colony orange diameter \pm 9 cm (Fig 2., A)
ED2	Filamentous colony Grey diameter \pm 6 cm (Fig2., B)



Fig 1. Hypha of an Endophyte in epidermal cells of Mangosteen leaf.

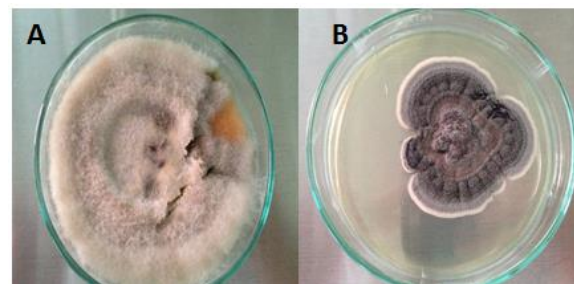


Fig 2. Endophytic fungi isolated from Mangosteen leaf

B. Assay for Antibacterial Activities Against *Staphylococcus aureus* and *Escherichia coli*

Screening of antibacterial activity showed that ED2 isolate has antibacterial activity against *Staph. aureus* but not against *E. coli*. This antibacterial activity can be observed due to the presence of compounds that have antibacterial activity against *Staph. aureus* and *E. coli* diffuses from endophytic fungi isolates to medium and inhibit the growth of bacteria so that the assay remains colorless clear medium in the surroundings (Table 3).

Table 2. Microscopic Endophytic fungi isolated from Mangosteen leaf

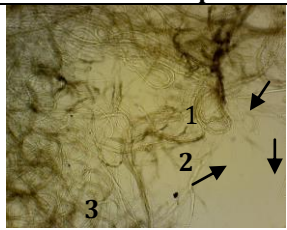
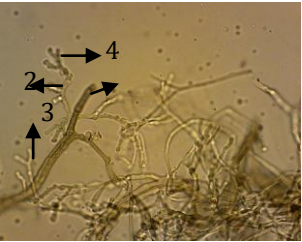
Code	Microscopic
ED1	 <p>1 = vegetative hyphae 2 = fertile hyphae 3 = macroconidia</p>
ED2	 <p>1 = vegetative hyphae 2 = fertile hyphae 3 = phialide 4 = macroconidia</p>

Table 3. Result screening antibacterial potency of endophytic fungi isolated from Mangosteen leaf

Code	Antibacterial potency	
	<i>Staph. aureus</i>	<i>E. coli</i>
ED1	-	-
ED2	+	-

(-) = no activity, (+) = activity

Bioautography with TLC (indicate compounds that can inhibit the growth of *Staph. aureus*. Results of screening such compounds with $FeCl_3$ blue black coloration, this color indicates phenol group in the metabolites. Screening with $AlCl_3$ yellow coloration, this color indicates the flavonoids.

IV. CONCLUSION

Mangosteen leaf was obtained 2 kinds of endophytic fungi; which were 1 have the potential as a producer of antibacterial compounds against *Staph. aureus*; which were predicted to be the class of compounds is flavonoids. Endophytic fungi isolates that have characteristic of macroscopic and microscopic is supposed a group of Trichoderma.

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