

Isolation of Endophytic Fungi from Stem of Andaleh (*Morus macroura* Miq.) That Produce Antimicrobial Compound

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ABSTRACT

Antibiotic resistance is a serious problem that needs to be dealt with immediately. One of solution can be issued is find and develop new potential antimicrobial derived from natural resources. Stem of Andaleh plant (*Morus macroura* Miq.) is potential candidate of natural resources which has antimicrobial activity, but it was an endangered species, so using this plant as antibiotic source is not wise. We can use endophytic fungi from Andaleh's plant since they are known have the ability to produce similar antibiotic compound. So, the research aiming is to find potential candidate of endophytic fungi that have ability to produce antimicrobial compound. Stem sample of Andaleh are from Batusangkar. Stem surface sterilization was using NaOCl 0.5%. The isolation of endophytic fungi from Andaleh stem was using PDA containing Chloramphenicol 500mg/l. Antimicrobial activity assay was conducted by agar dilution method using 3 microbial agent i.e. *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. There are 7 pure isolate of endophytic fungi from Andaleh stem. All of isolate, have antimicrobial activity against *E. coli* and *S. aureus*, but none of them have inhibitory effect to *C. albicans*. Antimicrobial activity index against *E. coli* ranging from 2.51 to 5.71 which the highest index was from isolate 1. Meanwhile antimicrobial activity index against *S. aureus* ranging from 1.35 to 4.07 which the highest index was from isolate 2. So, it can be concluded that endophytic fungi from stem of Andaleh are potential to produce antimicrobial compound and can be developed to a new antibiotic source

Keywords: Andaleh stem, antimicrobial activity, endophytic fungi.

1. INTRODUCTION

Antibiotics is a therapeutic agent that is widely used to treat infectious disease. Nowadays, antibiotic misused had lead the development of pathogen resistance to antibiotic. This condition makes people have to pay more medical costs than before, longer hospital treatment and increase mortality. The urgently needs to solve this problem are control the regulation of antibiotic prescription, change patient behaviour and find some new antibiotics.

Medicinal plants are good sources for biological active compounds compare with other synthetic antibiotic sources. Several plant species have been used for traditional medicine and have been studied for their pharmaceutical content (1). Medicinal plants may inhabited by some fungi that are able to produce active metabolite similar to their host (2).

The potential antimicrobial compounds (antibiotics) may be derived from natural resources, i.g Andaleh plant (*Morus macroura* Miq.). Andaleh plant were known use as medicines for leukaemia, antitumor and antibacterial (3,4). Although Andaleh is potential candidate as antimicrobial,

but it is an endangered plant species, so direct exploration from this plant is not wise.

Since the endophytic microbes are able to produce similar chemical compound to their host, it is possible to use them as antimicrobial sources (1,2,5,6). Bacteria and fungi are two kinds of endophytic microbes. Afifah *et al.* (2018) (7) has isolate endophytic bacteria from Andaleh plant, but endophytic fungi is not explore yet. So, we isolate the the endophytic fungi from Andaleh stem and test its antimicrobial activity

2. MATERIALS AND METHODS

2.1. Stem Tissue Sample Collection

Healthy (showing no visual disease) stem of *M. macroura* (Miq.), were obtained from the local plantation of Andaleh village, Batipuah district, Batu Sangkar. Stem sample was 5cm x 5cm in size including bark and wood.

2.2. Surface Sterilization and Isolation of Endophytic Fungi

The plant stem sample was sterilized following the surface sterilization procedure (8). Briefly, plant stem was first washed several times under running tap water, then washed in distilled water. After that, the plant sample was rinsing sequentially with 70% ethanol for 30 s, 0.5% sodium hypochlorite (NaOCl) for 2–3 min, 70% ethanol for 2 min, and then sterile distilled water 2–3 times. Plant sample were then dried with sterile filter papers aseptically.

After surface sterilization, the plant sample were cut into segments for 1x1x1 cm in size. Four segments were placed aseptically in petridishes containing PDA + chloramphenicol 500 mg/l. The petridishes then sealed and incubated at room temperature for 3–6 days until fungal hyphae growth through PDA.

2.3. Purification, Selection and Preservation of Endophytic Fungi

Each fungal colony that showed different morphology, then transfer into new petridish containing PDA (without antibiotic) repeatedly to obtain pure fungal isolate. The pure isolate of endophytic fungi were then transferred separately to PDA slants and maintained at room temperature till further use.

2.4. Antimicrobial Activity Assay by Agar Plug Diffusion Method

Three tests microorganisms used for the antimicrobial assay. They are including *Escherichia coli* (Gram - bacteria), *Staphylococcus aureus* (Gram + bacteria) and *Candida albicans* (yeast). Antimicrobial activity of pure isolates of endophytes was tested by agar plug diffusion method [10] using NA medium for *E. coli* and *S. aureus* and PDA medium for *C. albicans*. All the overnight culture (turbidity adjusted to 0.5 McFarland Standard) of test microbes were inoculated into

NA or PDA plates using sterile cotton swab. About 5 mm size fungal colony was made using sterile cork borer were placed onto each inoculated test microbes and incubate at room temperature for 24–48 h. The antimicrobial activities were assessed by the presence or absence of inhibition zone and the average diameter of inhibition zone measured in millimeter

3. RESULT AND DISCUSSION

Medicinal plant, such as Andaleh plant is one of host candidate to obtain the endophytic fungi that have the ability to produce antimicrobial compound. Endophytic fungi are one of the most promising groups of antimicrobial compound producers that play important

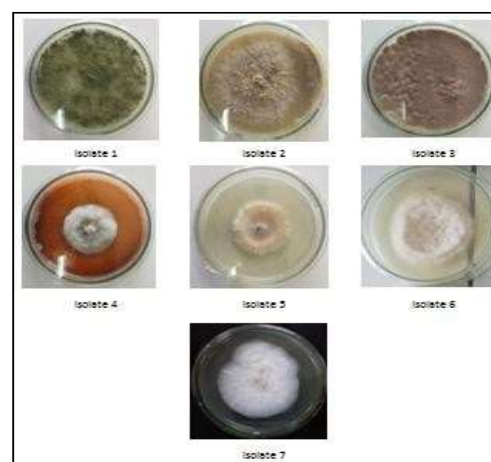


Figure 1. Endophytic fungal from Andaleh plant stem

In this recent study, we found seven isolate of endophytic fungi (Figure 1) and named as Isolate 1, 2, 3, 4, 5, 6, and 7. Each of isolate showed the difference of morphological feature. Isolate 4 produced dark red pigment that diffuse through PDA medium, meanwhile isolate 6 produce light yellow pigment. They also have different microscopic feature (Data was not shown here). Identification of each isolate was not conducted yet.

Tabel 1. Average inhibition zone develop by endophytic fungi from Andaleh plant stem against the test microorganism

Number of isolate	Average of inhibition zone (mm)		
	E.coli	S.aureus	C.albicans
1	38,9	32,0	-
2	40,8	47,7	-
3	31,1	23,9	-
4	50,9	35,1	-
5	31,2	17,7	-
6	53,0	39,4	-
7	43,6	33,3	-

After each isolate tested for antimicrobial activity, we found that all of isolate have the ability to inhibit *E. coli* and *S. aureus*, but none of them develop clear zone against *C. albicans* (Tabel 1.) All of endophytic fungi showed strong inhibitory effect toward *E. coli* and *S. aureus* with inhibition zone ranging from 16.7 – 53.0 mm. The ability of endophytic fungi to inhibit both Gram Positive and Negative bacteria indicated that these fungi are promising candidate for novel antibiotic sources, although the inhibitory effect toward Gram Negative bacteria was more effective than toward Gram Positive bacteria in general. Several study also found that many endophytic fungi able to inhibit *E. coli* and *S. aureus*, but most of them have

4. CONCLUSION

Seven isolate of endophytic fungi were recorded from Andaleh stem sample. All of isolate able to inhibit the growth of *E. coli* and *S. aureus*, but none of them able to inhibit *C. albicans*. The antimicrobial activity develop by these endophytic fungi was strong because the inhibition zone was large. So we can say that endophytic fungi from Andaleh stem are potential candidate for novel antibiotic source..

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