Antimicrobial activity of endophytic fungi from Andalas (Morus macroura Miq.) plant

D Handayani*, D H Putri, M Oktaviani and Rahwani
Departement of Biology, Faculty of Mathematics and Science (FMIPA), Universitas Negeri Padang. Indonesia
*dezihandayani3252@gmail.com

Abstract. Many of recent study were conducted to find and investigate new antimicrobial substance to overcome the bacterial resistance problem. We can explore antimicrobial substance from medicine plant including Andalas (Morus macroura Miq.), but this plant is an endangered species. So, we can use endophytic fungi from this plant since they were known have the ability to produce similar antimicrobial compound. The aim of this study is to find some endophytic fungi from Andalas plant that have the ability to inhibit pathogen growth. Part (roots and leaves) of Andalas plant sample are from Andaleh village, Tanah Datar district, West Sumatera. Part of plant surface sterilization was using NaOCl 0.5%. Isolation of endophytic fungi from Andaleh plant was using PDA containing Chloramphenichol 500mg/l. Antimicrobial activity assay was conducted by agar dilution method. This study using 3 microbial agent i.e. Escherichia coli, Staphylococcus aureus and Candida albicans. We found 19 pure isolates of endophytic fungi from roots sample meanwhile 9 pure isolates from leaves. Most of those endophytic fungi (24 of 28 isolates) have the ability to inhibit E.coli and S. aureus growth, but none of them have the same ability to inhibit C. albicans. The highest average inhibition zone against E. coli was 47.1 mm and 51.7 mm against S. aureus. Meanwhile the lowest average inhibition zone against E. coli and S. aureus was 13.6 mm. Based on this study, we can conclude that endophytic fungi from Andalas plant are potential source to develop antimicrobial agent.

1. Introduction
Nowadays, many pathogenic bacteria has become resistant to commercial drugs given. World Health Organization (WHO) has declared that antimicrobial resistance (AMR) is one of the top 10 global public health threats facing humanity. Some factors were responsible to the development of pathogenic bacteria resistance. These factors are inappropriate and extensive use of antibiotics, poor hygienic conditions, increased number of immunocompromised patient, continuous movement of travelers and delay in diagnosis of infections [1]. This problem would decrease the successful of infectious desease treatment. Major surgery and cancer chemoteraphy also would be at increased risk, [2].

There are several ways to overcome the bacterial resistance problem such as, thighten antibiotic prescribing rules, change patient behavior, and conduct research related to the discovery of new microbial substance that have the ability to inhibit pathogens growth. Many studies has found that endophytic microorganisms isolated from medicinal plant are the potential resources of antimicrobial compounds [3,4,5].
Andalas plant (*Morus macroura* Miq), were known as medicinal plant because the use of its root barks to threat rheumatism, diabetes and arthritis in Chinese herbal medicine. Andalas plant posses the novel substance used as antibacterial, antitumor and leukemia drugs [6,7]. Seven isolate of endophytic fungi isolated from Andalas stem were reported able to inhibit *E. coli*, and *S. aureus* growth [8]. The average of inhibition zone against *E. coli* and *S. aureus* were 52.9 mm and 46.3 mm respectively. So, we assumed that Andalas plant is potential source of antimicrobial substance either from plants parts or from endophytic fungi that lives in Andalas plant tissue.

Due to the importance of finding and develop new antimicrobial substance to overcome antibiotic resistance problems, objective of this study is to find endophytic fungi from Andalas roots and leaves that able to inhibit pathogens growth.

2. Material and Methods

2.1 Plant samples

Roots and leaves sample of Andalas plant were collected from Andaleh Village, Tanah Datar District, West Sumatera, Indonesia. Healthy roots and leaves sample were cut-off from Andalas plant and immediately processed in the Microbiology Laboratorium at Universitas Negeri Padang.

2.2 Isolation of endophytic fungi

Epiphytic microorganisms that may be present on the surface of the samples were eliminate by the methods describe by [ ]. Each sample were washed several time under running tap water and then immersed in 70% ethanol for 30 s, in 0.5% sodium hypochlorite for 2-3 min and in 70% ethanol for 2 min. After that, the sample was rinse 2-3 times with sterile distillated water.

After surface sterilization was completed, the sample then cut into 0.5 cm$^2$ in size and transferred aseptically in petridishes containing potato dextrose agar (PDA) medium supplemented by 500mg/L chloramphenicol to suppress bacterial growth. The petridishes were incubated for 20 day at room temperature and checked daily to investigate endophytic fungal growth. All different morphology fungal found were isolate, purified and maintained in PDA medium for futher investigating.

2.3 Antimicrobial assay

Pure isolate of endophytic fungi were subjected to an antimicrobial assay using agar plug diffusion methods [ ]. Agar plug diffusion provide us a fast selection method with satisfactory qualitative result. Three types of human pathogens were used for antimicrobial assay. These microbes were *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Medium assay for bacteria was Nutrient agar and PDA for *C. albicans*.

Each pure isolate of endophytic fungi was cultivated in petridish containing PDA medium for 7 days and then the fungal micellium were cut from medium (5 mm diameter). The micellium then transferred into new petridish previously spread by pathogens bacteria and *C.albicans* (turbidity adjusted to 0.5 McFarland Standard). The petridish were incubated at 37°C for 24-48 hours. 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

After isolation and purification of fungal colonies has grown from Andalas plant sample, we found 19 pure isolate of endophytic fungi from roots and 9 pure isolate from leaves. Picture 1 showed some of those isolate that have distinct morphology characteristics of colony.
Figure 1. Morphology of some endophytic fungi from roots (top) and from leaves (bottom)

The antimicrobial activity of endophytic fungi isolated from Andalas roots was shown in Table 1. From the table we can see that most of fungal isolate (15 of 19) from Andalas roots, were able to produce inhibition zone at least against 1 pathogen. The average inhibition zone against *E. coli* were ranging from 17,6 mm to 47,1 mm meanwhile 13,6 mm to 43,2 mm against *S. aureus*. None of all isolate were able to inhibit *C. albicans* growth.

Table 1. Antimicrobial activity of endophytic fungi isolated from Andalas roots

| Isolate Code | Average of Inhibition Zone (mm) |
|--------------|---------------------------------|
|              | *E. coli* | *S. aureus* | *C. albicans* |
| CEA1         | 26,2      | -            | -             |
| CEA2         | 24,2      | -            | -             |
| CEA3         | -         | -            | -             |
| CEA4         | 17,6      | 13,6         | -             |
| CEA5         | 34,5      | -            | -             |
| CEA6         | -         | 17,9         | -             |
| CEA7         | -         | 43,2         | -             |
| CEA8         | 24,2      | -            | -             |
| CEA9         | -         | -            | -             |
| CEA10        | 47,1      | 32,7         | -             |
| CEA11        | -         | 34,7         | -             |
| CEA12        | 37,7      | 17,9         | -             |
| CEA13        | 45,2      | 37,3         | -             |
| CEA14        | 25,9      | -            | -             |
| CEA15        | -         | 18,7         | -             |
| CEA16        | 32,6      | 19,0         | -             |
| CEA17        | -         | -            | -             |
| CEA18        | -         | -            | -             |
| CEA19        | 26,4      | -            | -             |
All of endophytic fungal isolated from Andalas leaves were able to suppress the growth of *E. coli* and *S. aureus*. The average inhibition zone were ranging from 24.6 mm to 45.8 mm against *E. coli* and from 21.2 mm to 51.7 mm against *S. aureus*. Data was shown at Table 2.

| Isolate Code | Average of Zone Inhibition (mm) |
|--------------|---------------------------------|
|              | *E. coli* | *S. aureus* | *C. albicans* |
| CED1         | 29.5      | 23.3        | -             |
| CED2         | 29.1      | 23.5        | -             |
| CED3         | 39.4      | 51.7        | -             |
| CED4         | 41.4      | 32.2        | -             |
| CED5         | 37.1      | 32.5        | -             |
| CED6         | 45.8      | 48.7        | -             |
| CED7         | 44.2      | 35.7        | -             |
| CED8         | 24.6      | 21.2        | -             |
| CED9         | 37.6      | 28.0        | -             |

4. Discussion
Endophytic fungi are fungi that lives in plant tissue without harming the plant host [9]. Endophytic fungi plays important rule in relationship with plant host. They can produce compound that promote vegetative growth, competitiveness and protection of the plant against pathogens and herbivores [10]. Searching and identifying endophytics microorganism is important since, they are able to produce secondary metabolites that similar to their host medicinal properties [3].

In our study, 28 endophytic fungi were isolated from roots and leaves of *M. macroura* and they antimicrobial activity was evaluated. A total of 24 (85.7%) strain showed that they posses antimicrobial activity at least against 1 of the 3 types of pathogens tested. This percentage of these endophytic fungi that have antimicrobial activity was exceeding the results from similar studies. For example, the percentage of endophytic fungi that have antimicrobial activity from medicinal plant *Indigofera suffruticosa* only 33.6% [11]. The result reported by other author even less than 10% [12]. It means endophytic fungi from *M. macroura* roots and leaves revealing enormous capacity of production secondary bioactive metabolites.

The average inhibition zone of endophytic fungi from this study also showed strong antimicrobial activity. They produce inhibition zone with large diameter (ranging from 13.7 to 51.7 mm) with 21 (75%) of isolate have inhibition zone more than 20 mm. The size of inhibition zone against pathgens indicates their antimicrobial activity strength. The antimicrobial activity of most endophytic fungi isolated form Andalas plant roots and three was classified as strong and very strong according to zone of inhibition (ZOI) classified. The inhibition of zone were classified in four intensities corresponding to ZOI diameters: >20 mm, very strong; 10-20 mm, strong, 5-10 mm, medium; and <5 mm, no response [13]. From this study, we can conclude that endophytic fungi isolated from roots and leaves of Andalas plant were potential as a new candidates of antibiotic compound.

References
[1] von Nussbaum F, Brands M, Hinzen B, Weigand S and Habich D 2006 Antibacterial natural products in medicinal chemistry-exodus or revival? Angew Chem. 45, 5072-5129. doi 10.1002/anie.200600350
[2] World Health Organization 2020 Antibiotic resistance. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance.
[3] Kaul S, Gupta S, Ahmed M, Dhar MK 2012 Endophytic fungi from medicinal plant: a treasure
hut for bioactive metabolites. Phtyochen Rev. Springer Science+Business Media Dordrecht. DOI 10.1007/s11101-012-9260-6

[4] Marcellano JP, Collanto AS and Fuentes RG 2017 Antibacterial Activity of Endophytic Fungi Isolated from the Bark of Cinnamomum mercadoi. Pharmacogn J. 9(3): 405-409

[5] Garcia A, Rhoden SA, Bernardi-Wenzel J, Orlandelli RC, Azevedo JL and Pamphile JA 2012 Antimicrobial activity of crude extracts of endophytic fungi isolated from medicinal plant Sapindus saponaria L. App Pharm Sci J. 2 (10) 035-040

[6] Fajrina A, Idris M, Mansyurdin and Surya NW 2012 Penggandaan kromosom dan pertumbuhan somaklonal Andalas (Morus macroura Miq. var. macroura) yang diperlakukan dengan kolkisin. Jurnal Biologi Universitas Andalas 1(1); 23-26

[7] Jasmansyah, Hernandi S, Hakim E and Syah YM. 2014 Aktivitas senyawa aktif anti kanker leukemia dari Spesies Morus macroura Miq. (Tanaman Andalas) secara in vitro. Prosiding Seminar Nasional Ilmu Pengetahuan dan Teknologi (IPTEK) Universitas Jenderal Achmad Yani 29-30 April 2014. Bandung.

[8] Handayani D, Putri DH, Farma SA, Annisa N, Oktaviani M and Rahwani 2020 Isolation of endophytic fungi from stem of Andaleh (Morus macroura Miq.) that produce antimicrobial compound Advances in Biological Sciences Research 10:43-45. Atlantis Press.

[9] Schulz BC and Boyle 2005 The endophyte continuum Mycol Res 109:661-686

[10] Porras-Alfaro A and Bayman P 2011 Hidden fungi, emergent properties: endophytes and microbiomes Annu Rev Phytopathol 49:291–315. Doi: 10.1146/annurev-phyto-080508-081831

[11] dos Santos IP, da Silva LCN, da Silva MV, de Araujo JM, da Silva Calvacanti M and de Menezes Lima VL 2015 Antibacterial activity of endophytic fungi from leaves of Indigofera suffruticosa Miller (Fabaceae) Front. Microbiol 6: 350 https://doi.org/10.3389/fmicb.2015.00350

[12] Gong LJ and Guo SH 2009 Endophytic fungi from Dracaena cambodiana and Aquilaria sinensis and their antimicrobial activity Afr J Biotechnol 8: 731–736 Doi: 10.5897/AJB2009.000-9124

[13] David WW and Stout TR 1971 Disc plate method of microbiological antibiotic assay. I. Factors influencing variability and error Appl Microbiol 22, 659-665