by Nss Ambarwati
In vitro Antimicrobial Activity Evaluation of Mangrove Fruit (Sonneratia caseolaris L.) Extract

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ABSTRACT
Introduction: Mangrove fruit (Sonneratia caseolaris L.) from the Rhizophoraceae family known with local name "Pida da Merah" has bioactive components. The present study aimed to evaluate the antimicrobial activity of this plant. Method: The sample was macerated using methanol, the antimicrobial activity assay was performed using agar disc diffusion method against Escherichia coli ATCC8638, Staphylococcus aureus ATCC6538, and Candida albicans ATTC10231. Results: Antimicrobial activity of Mangrove fruit methanol extract was obtained the optimum concentration of S. aureus of 80%, E. coli of 15%, and C. albicans of 30%. Conclusion: based on the results, the sample has antimicrobial properties, and this is a preliminary data for further study. Key words: Sonneratia caseolaris L, Mangrove fruit, Antimicrobial activity, Agar disc diffusion method. Key Messages: Evaluation of antimicrobial activity of Mangrove fruit (Sonneratia caseolaris L) extract against Escherichia coli, Staphylococcus aureus, and Candida albicans using Agar disc diffusion method.

INTRODUCTION
Sonneratia caseolaris L. is one of the plants from the Rhizophoraceae family, known as Mangrove with local name "Pida da Merah". This plant is found along coastal areas and estuaries where other plants are difficult to grow. Several studies have reported that most parts of the plant have pharmacological properties, including astringent, antiseptic, analgesic, anti-inflammatory, antimicrobial, anti-diabetic, and antioxidant. Also used as a traditional cosmetic by the local community in Kalimantan island. It is also known as "badak daging" and has secondary metabolites such as flavonoids, phenolics, terpenoids, steroids, and alkaloids[1,2,3]. S. caseolaris is a tree that bear fruit and includes numerous small seeds are covered with a thin layer. This plant has been used in traditional medicine. The ripe fruit has a distinctive flavor and pharmacological properties similar to other plants. On the other hand, the study of bioactive compounds from Mangrove fruit is still limited. The previous research has been reported that the fruit extract has a pharmacological activity as anticoagulant, antioxidant, and anti-diabetic. The fruit is also non-toxic and safe to consume routinely. However, research antimicrobial active constituent on Mangrove fruit has not been reported.

Antimicrobial activity assay on Mangrove fruit extract using the gram-negative bacteria of Escherichia coli, the gram-positive bacteria of Staphylococcus aureus, and the yeast of Candida albicans conducted with agar disc diffusion method. This study aimed to explore as antimicrobial properties, in particular against potential human pathogens.

SUBJECTS AND METHODS
Plant Material
A sample of Mangrove fruit (S. caseolaris) was collected from Muara Badak, Kutai Kertanegara, East Kalimantan, Indonesia, and was identified at Laboratory of Botany, Faculty of Forestry, Mulawarman University, Samarinda, East Kalimantan, Indonesia. The specimen was deposited at Pharmaceutical Research and Development Laboratory of FARMKA TROPIS, Faculty of Pharmacy, Mulawarman University.

Materials and General Equipment
The chemical material was used including chloramphenicol, metronidazole, Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were purchased from Sigma-Aldrich, Germany. Staphylococcus aureus ATCC6538 (S. aureus), Escherichia coli ATCC8638 (E. coli), and Candida albicans ATTC10231 (C. albicans) were purchased from Sigma-Aldrich, Germany.

Aqua demineralization (Aqua DM), methanol, and NaCl 0.9% dilution were obtained from PT SmartLab Indonesia, Indonesia. The equipment was utilized, such as rotary evaporator (Stuart), Waterbath (Whatman), autoclave (Tomy SX-700), Oven (Mammet), incubator (Mammet), Petri Dishes (Normax), Laminar Air Flow (LAF) (Nusarie NU-126-400 E), Vernier calipers, and maceration equipment.

**Extraction Process**

The extraction process performed using a conventional method based on some literature, with slight modifications. Briefly, the dried fruit (420 g) was macerated (three times) using 2 L methanol for 24 h and was filtered to separate the extract solution and residue. The extract solution was evaporated to get dry extract. The extract was dry-stored (44.15 g) in sterile Eppendorf at a cold temperature until further used.

**Antimicrobial Assay**

An antimicrobial test using Agar disc diffusion method against E. coli, S. aureus, and C. albicans. Briefly, the bacterial and yeast suspension were diluted at 1:40 and 1:20, respectively. The dilution suspension of 0.02 mL was mixed with 10 mL of medium NA and PDA in Petri, homogenized, and waited to form the semi-solid medium. The paper discs (with a diameter of 6 mm, Whatman) were dipped into the test solution extracts andincubated for 48 h, then laid on the surface of the solid medium NA and PDA, and incubated at 37°C for 24 h. The negative control using distilled water, whereas the positive control using chloramphenicol (antibacterial) and metronidazole (antifungal). The antimicrobial activity was calculated by the area of total/partial inhibition zone.

**RESULTS**

Antimicrobials are compounds that used to inhibit or destroy microbial growth. Microbes were employed in this study including gram-positive bacteria of S. aureus, gram-negative bacteria of E. coli, and the yeast of C. albicans using agar disc diffusion method. The antimicrobial activity of fruit extracts Mangrove indicated by their inhibition zone which is an area of bright or turbid influenced by the extracts that are not covered by the microbes. Further zones are measured in diameter are formed by using a micrometer screw.

The concentration of fruit extracts mangrove was utilized in this study include S. aureus (60%, 70%, 80% and 90%), E. coli (5%, 10%, 15% and 20%), and 0.02% chloramphenicol a positive control. Whereas C. albicans was used at 20%, 25%, 30% and 35%, and 0.15% metronidazole as a positive control (can be seen in Figure 1).

As can be seen in Figure 2, showed the antimicrobial activity of Mangrove fruit extract against the bacteria of E. coli, which was characterized by the total partial inhibition zone around the paper disc. The highest total/ partial inhibition zone was at a concentration of 15%, while at 20% has decreased.

In Figure 3 demonstrated the antimicrobial activity of mangrove fruit extracts against S. aureus which was characterized by the partial inhibition zone at a concentration from 10% to 50% and the total inhibition zone at a level from 60% to 90%. The highest and optimum the total inhibition zone was at 80%.

The antimicrobial activity of Mangrove fruit extract to C. albicans yeast presented in Figure 4 and marked by the total inhibition zone around the paper disc. The highest and optimum of antimicrobial activity was demonstrated at 30%.

**DISCUSSION**

Differences in the ability of the Mangrove fruit extract in providing antimicrobial activity based on the difference of the cell wall structure, where the yeast has no cell wall, while opposite in the bacteria. In the...
CONCLUSION
Based on the above results, the mango fruit extracts have antimicrobial activity; mainly the compound is more sensitive to C. albicans and S. aureus compared to E. coli. The results of this study are preliminary data, and further research focused on the identification and isolation of compounds responsible as an antimicrobial.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
We declared that we have no conflict of interest.

ABBREVIATIONS USED
S. caseolaris, Sonneratia caseolaris L., NA; nutrient agar, FDA; potato dextrose agar, LAF; laminar air flow, S. aureus: Staphylococcus aureus ATTC6538, E. coli: Escherichia coli ATTC9637, C. albicans: Candida albicans ATTC10231, °C, degree Celsius

REFERENCES
Ahmad et al.: Antimicrobial Activity of Sonneratia casuarinfol Extract


SUMMARY

- Mangrove fruit (Sonneratia caseolaris L.) from Sonnerataceae family known with local name “Piloia Merah”.
- Antimicrobial activity of Mangrove fruit methanol extract were obtained the optimum concentration of S. aureus of 80%, E. coli of 15%, and C. albicans of 30%.
- Mangrove fruit (Sonneratia caseolaris L.) has antimicrobial properties and this is a preliminary data for further study.

GRAPHICAL ABSTRACT

Mangrove Fruit
(Sonneratia caseolaris L.)

Extraction Process

In vitro antimicrobial assay

ABOUT AUTHORS

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