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Brief Report

In vitro Antimicrobial Activity Evaluation of Mangrove Fruit (*Sonneratia caseolaris* L.) Extract

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ABSTRACT

Introduction: Mangrove fruit (*Sonneratia caseolaris* L) from Sonneratiaceae family known with local name "Pidada 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* ATTC9637, *Staphylococcus aureus* ATTC6538, and *Candida albicans* ATTC10231. **Results:** 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%. **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 Sonneratiaceae family, known as Mangrove with local name "Pidada Merah." This plant is foolproof found in coastal areas and estuaries where other plants difficult to grow.[1] Several studies have reported that almost all parts of the plant have pharmacological properties, including astringent, antiseptic,[2] analgesic, anti-inflammatory,[3] antimicrobial,[4,5,6] antidiabetic,[7] antioxidant.[8,9] Also used as a traditional cosmetic by the local community in Kalimantan island, Indonesia (especially Dayak tribe) called "*bedak dingin*".[10] This plant contains secondary metabolites such as flavonoids,[11] phenolics, terpenoids, steroids, and alkaloids.[3,12,13]

S. caseolaris is a plant to bear fruit and includes numerous small seeds are covered with flesh. It has bioactive components including Alkaloids, saponins, terpenoids, tannins, flavonoids, steroids, and derivates of benzene carboxylate,[8,12] and commonly consumed by the community in Java and Kalimantan island. The ripe fruit has a distinctive flavor and pharmacological properties similar to other parts. 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 anticolesterol.[14] and antidiabetic.[15] The fruit is also non-toxic and safe to consume routinely.[12] 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 Dendrology, Faculty of Forestry, Mulawarman University, Samarinda, East Kalimantan, Indonesia. The specimen was deposited at Pharmaceutical Research and Development Laboratory of FARMAKA 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* ATTC6538 (*S. aureus*), *Escherichia coli* ATTC9637 (*E. coli*), and *Candida albicans* ATTC10231 (*C. albicans*) were purchased from Sigma-Aldrich, Germany.

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

Extraction Process

The extraction process performed using a conventional method based on some the literature,[16,17,18] with slight modification. 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 got 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[4,17,19,20] 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 and silenced for a moment, 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 kill 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 0.025% 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

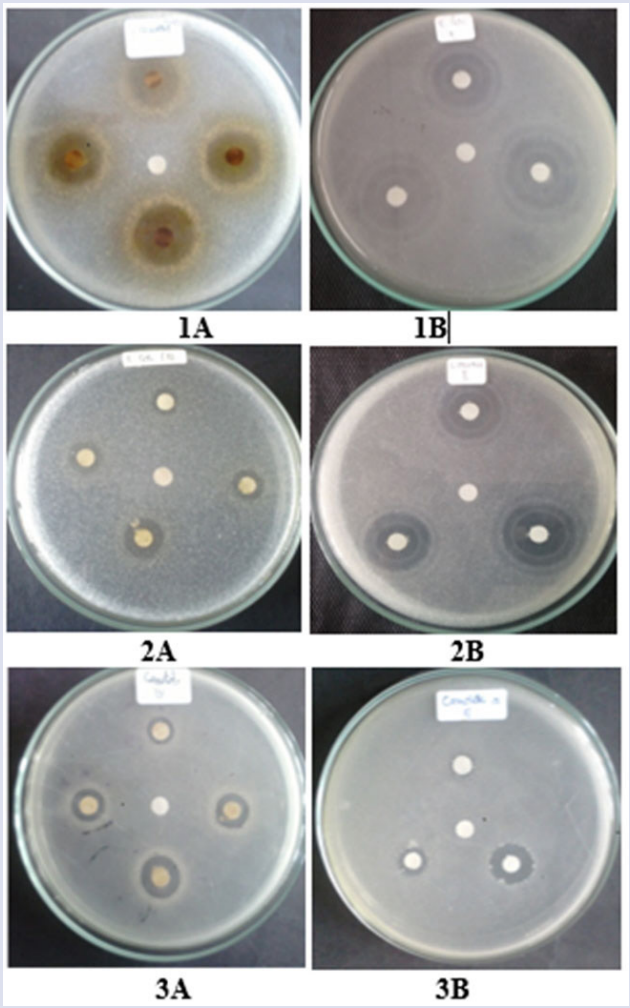

Figure 1: Antimicrobial activity test of Mangrove fruit extract (A) and positive control (B) against (1) *E. coli*, (2) *S. aureus*, and (3) *C. albicans.*

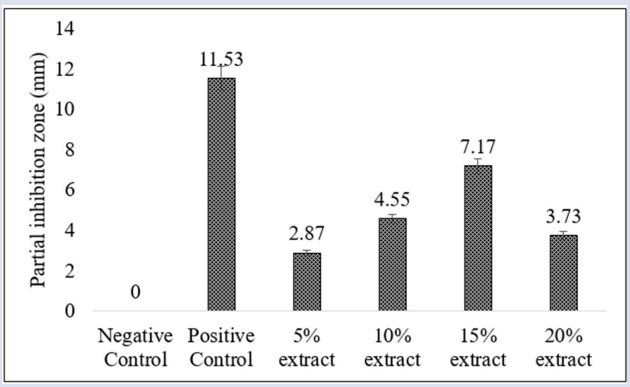

Figure 2: Antimicrobial activity of Mangrove fruit extract against *E. coli.*

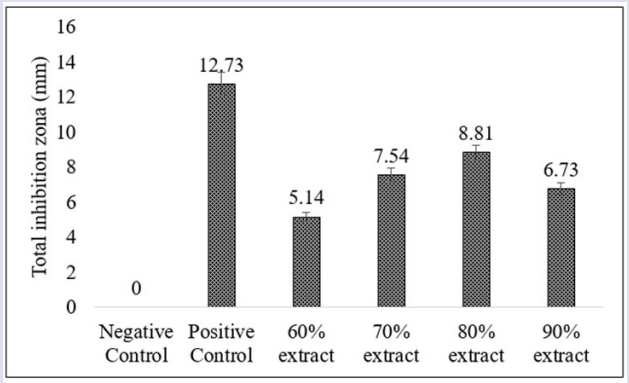

Figure 3: Antimicrobial activity of Mangrove fruit extract on *S. aureus*.

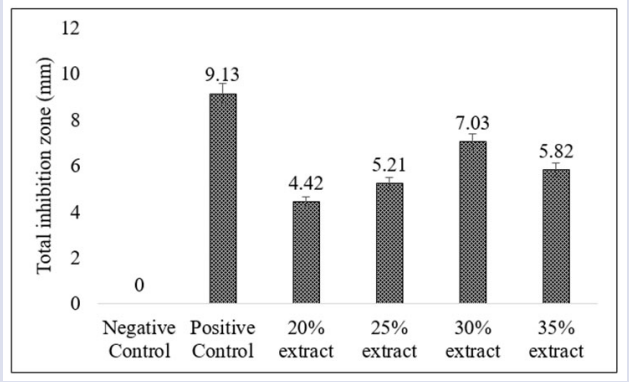

Figure 4: Antimicrobial activity of Mangrove fruit extract against *C. albicans*.

bacteria requires a high concentration compared to the yeast in providing the inhibition zone, where the cell wall of bacteria consists peptidoglycan which serves as additional protection from external influences. Also, it also helps to prevent rupture of the cells by osmotic pressure on the hypotonic environment. The cell walls of gram-positive bacteria are composed of a single layer of relatively thicker peptidoglycan, while the gram-negative bacteria consisting of two layers, namely the outer layer (contains lipopolysaccharide and protein) and the inner layer including the thin peptidoglycan. Also, differences in the ability of Mangrove fruit extracts inhibit or kill the growth of microbes may be caused by sensitivity to antimicrobial compounds contained in extracts, wherein the constituent is more sensitive to the yeast and the gram-negative bacteria compared to the gram-positive bacteria. Microbial growth influenced by several factors include (1) the osmotic pressure difference inside and outside the cell, (2) the cell membrane damage due to irritation, (3) changes in pH, and (4) the cell fluid diffusion.[17,19-22]

The decreased power of inhibition zone at the highest concentration of the possibility because of the rate of diffusion of active substances. In general, the diameter of inhibition zone tends to increase proportionately to the increasing level of the extract but does not occur in this extract. It is due to the growing concentration of the extract also enhance the viscosity of a sample which affects the absorption of the extract through the paper disc and diffusion of the extract on medium.

CONCLUSION

Based on the above results, the mangrove 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.

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CONFLICT OF INTEREST

We declared that we have no conflict of interest

ABBREVIATONS USED

S. caseolaris; Sonneratia caseolaris L, NA; nutrient agar, PDA; 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

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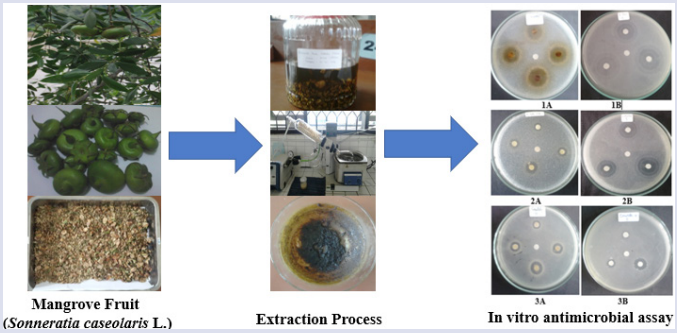
GRAPHICAL ABSTRACT



SUMMARY

- Mangrove fruit (*Sonneratia caseolaris* L) from Sonneratiaceae family known with local name "Pidada 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.

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