

Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract

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Abstract

Macaranga bancana is one of the Indonesian medicinal plants that is empirically used to treat infectious disease. The aim of this study was to evaluate anti-bacterial and anti-fungal activities of *M. bancana* against human pathogenic microbes, *i.e.*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The dried leaves of *M. bancana* were extracted using cold extraction method with n-hexane, dichloromethane, ethylacetate, methanol, and ethanol solvents. The evaluation of anti-microbial activity from these extracts at the concentration of 1000 µg/ml was conducted using microdilution method. Amoxicillin, cephadroxil, and ketoconazole were used as positive controls. All extracts showed 100% inhibitory activity against *S. aureus*, with the exception of ethanol extract which was 72.8%. Nevertheless, methanol and ethanol extracts showed 100% inhibitory activity against *E. coli*, while the lowest activity was shown by n-hexane extract (45.2%). All extracts exhibited 100% inhibitory activity against *C. albicans*. In conclusion, leaves extract of *M. bancana* is a potential source of anti-bacterial and anti-fungal agents.

Keywords: anti-bacterial, anti-fungal, *M. bancana*, microdilution

Introduction

The irrational use of antibiotics cause many pathogenic microbes to adapt to their environment and become resistant to synthetic antibiotic drugs. This has encouraged the development of novel anti-microbial substances derived from natural products.¹ *M. bancana* plant, a member of *Euphorbiaceae* family, is widely found in Indragiri Hulu, Province of Riau, Indonesia. Talak Mamak tribe from Indragiri Hulu empirically use the leaves and fruits of

this plant to treat diarrhea. Previous studies showed that various plants from the same genus, such as *M. gigantea*, *M. pruinosa*, *M. tanarius* and *M. triloba* had strong anti-microbial activity against *S. aureus*.^{2,3} Salah *et al* reported that *M. monandra* possessed anti-fungal activity.⁴ Nevertheless, the anti-microbial activity from *M. bancana* had not been previously reported.

Therefore, this study was conducted to

evaluate the anti-bacterial and anti-fungal activities of *M. bancana* against human pathogenic microbes, i.e., *E. coli*, *S. aureus*, and *C. albicans*.

Methods

Extract preparation

M. bancana leaves were collected from Indragiri Hulu, Province of Riau, in March 2018. Plant determination was conducted at Department of Biology, Faculty of Mathematics and Natural Sciences, University of Riau. The leaves were cleaned, air-dried, and powdered. Subsequently, 20 g of the leaves powder were cold extracted using n-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), methanol and ethanol for 3 x 24 hours, respectively. The filtrate was collected and concentrated using rotary evaporator.

Anti-microbial activity

The anti-microbial activities of the extracts against *S. aureus*, *E. coli* ATCC 35218, and *C. albicans* ATCC 10231 were conducted using microdilution method.⁵ The extracts were dissolved in dimethyl sulfoxide (DMSO) in the concentration of 1000 µg/ml. Amoxsan®, cefadroxil and ketokenazole at 1000 µg/ml were used as positive control, while DMSO was used as negative control. Precultures of the tested microorganisms were made by inoculating 25 ml of nutrient broth medium for bacteria (WP medium for fungus) and incubated for 18 hours at 37 °C for bacteria (4 days at room temperature for fungus). Subsequently, the cell density was adjusted to 107 CFU/ml.⁶ Microbial suspension of 20 µl was distributed in each well containing extract, nutrient broth, and resazurin. The plate was incubated at 37 °C for 24 h for the bacteria or 4 days for the fungus and the optical density of each was measured by using microplate reader. The experiment was run in three replicates.

Data analysis

The inhibition activity was calculated using the formula:

$$\% \text{ growth} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

$$\% \text{ inhibition} = 100 - \% \text{ growth}$$

Results and Discussion

In this study, anti-bacterial and anti-fungal activities of *M. bancana* leaves extracts were evaluated against three pathogenic microbes (*S. aureus*, *E. Coli*, and *C. albicans*) using microdilution method. This method was used because it has several advantages, e.g., can be used to analyzed several different samples at one time, requires small amount of samples, and has high sensitivity.⁷

In this assay, the resazurin was added as microbial growth indicator. The addition of resazurin in the assay can predict the presence of visual anti-microbial activity (qualitative) through colour change. A compound which has anti-microbial activity changes from pink to blue, while those which does not have activity or weak activity stays pink. This reaction is resulted from the activity of oxyreductase in the microbe that converts blue resazurin into pink resofurin.⁵ Furthermore, the anti-microbial activity can be determined by measuring the optical density to observe the inhibition growth.

The anti-microbial activity from various extracts of *M.bancana* leaves is presented in the Table 1. All extracts showed high inhibitory with 100% inhibition, except ethanol extract (72.8%). Nevertheless, methanol and ethanol extracts showed 100% inhibitory activity against *E. coli*, while the lowest activity was shown by n-hexane extract (45.2%). All extracts exhibited 100% inhibitory activity against *C. albicans*.

Table 1. Anti-Microbial Activity from Various Extracts of *M. bancana* Leaves

Sample (1000 µg/ml)	Inhibition (%)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
n-hexane extract	100	45.2	100
EtOAc extract	100	72.1	100
DCM extract	100	69.4	100
Methanol extract	100	100	100
Ethanol extract	72.8	100	100
Amoxsan®	100	100	-
Cefadroxil	100	100	-
Ketoconazole	-	-	100

All extracts exhibited high inhibition activity toward Gram-positive bacteria. These results are in accordance with previous study by Othman *et al* showing that Gram-negative microorganisms are typically more resistant to antimicrobial agents than Gram-positive bacteria.⁸ This has long been explained by the presence of an outer membrane permeability barrier in Gram-negative bacteria, which limits the access of the antimicrobial agents to their targets in the bacterial cells. Furthermore, all extracts showed high inhibition activity against *C. albicans* with 100% inhibition, similar with ketoconazole as positive control. Strong anti-fungal activity of the extract is caused by the activity of compounds that prevent the synthesis of ergosterol by inhibiting the P450 enzyme. This will cause the depletion of ergosterol, which can lead to a change in membrane permeability and cell membrane damage.⁹ Nevertheless, the further investigation is needed to investigate the mode of action of the extracts towards the fungal.

Conclusion

Leaves extract of *M. bancana* is a potential source of anti-bacterial and anti-fungal agents.

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Conflict of interest

None declared.

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