Determination of Minimum Inhibition Concentration (MIC) of *Shorea Bracteolate* for Antibacterial Study

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ABSTRACT

Antimicrobial activities of methanol extract from the bulk of Shorea bracteolate were investigated. Antibacterial activities were tested against Gram –ve and Gram +ve bacteria by using Escherichia Coli and Bacillus subtilis to determine the MIC (Minimum Inhibition Concentration) of the crude extract. The in-vitro bioassay of Shorea bracteolate against both bacteria revealed there were moderate inhibition zone occurred in the paper disc diffusion assay. Growth inhibitions were expressed by using different concentration (50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml). High concentration crude extraction revealed a significant growth inhibition zone compared to low concentration. In this paper we wish to report on the first time report on antibacterial activity of Shorea Bracteolate to be a new discovery of traditional medicine.

Keywords: *Shorea bracteolate*, antimicrobial, methanol extract, traditional medicine

Introduction

Many studies have reported that plants contain a wide variety of compound with beneficial health effects. Medicinal plants constitute an arsenal of chemicals that could exploited by human to prevent microbial invasion. Plants extract and products are used in treatment of bacterial, fungal and viral infections (Bruneton, 1999). Malaysian plants species of the family *Dipterocarpaceae* has been reported for their antimicrobial potentials which has been discovered in UiTM Forest Reserve Jengka (Wan Zuraida et al., 2008). Within this family, species of genus *Shorea bracteolate* also have been reported to possess antimicrobial potency. This genus produces a wide variety of natural products, including among other terpenoids, flavonoids, arylpropanoids and stibenoids oligomers. Many of the latter class of compounds, which form major polyphenolic constituents, show useful biological activities, such as chemo preventive, hepatoprotective, anti-inflammation, cytotoxic, inhibition of topoisomerase II, gastric ATPase, 5α-reductase, antibacterial, fungicide and anti-oxidative (Ito et al., 2003). This explain why this study was designed to evaluated the antimicrobial activity of polar extract (methanol extract) and compounds from *S. bracteolate*.

Material and Methods

Plant material

The stem bark of *Shorea bracteolate* was collected from UiTM Pahang Reserved Forest, Malaysia and identified by a FRIM botanist, Kamaruddin Salleh and a voucher specimen was deposited under the reference number D08/06/09.

Extraction and Isolation

The dried stem barks (2kg) of *S. bracteolate* were powdered and macerated in methanol room at room temperature for 48h. The filtrates were evaporated under vacuum by using rotary evaporator to yield a dark crude extract.

Test Microorganisms

Strains of Gram-negative bacteria, *Escherichia coli* and Gram-positive bacteria, *Bacillus subtilis* were obtained from Institute Medical Research (IMR), Kuala Lumpur. Cultures were maintained on slant agar at 4°C and sub cultured to maintain viability. A working culture was prepared by inoculating a single colony of culture into 50 mL of nutrient broth (NB) plated for further experiment. Cultures were agitated and incubated at 180 rpm and 37°C, respectively for 24 hours. Bacteria was sub-cultured to NB and incubated for 30 minutes. Minimal inhibition concentration (MIC) was performed by a serial dilution technique by adjusting the optical density to 0.1 at 600nm SHIMADZU UV-120-01 spectrometer. The turbidity was adjusted to 0.5 M according to the Mc Farland standard.

Disc Diffusion Method

The bioassay for bacterial strains was employed by disc diffusion method (Ergene et al. 2006). Filter paper discs (Whatman AA disk) of 6mm diameter were loaded with difference concentrated of crude extracts (50 mg/mL, 25mg/mL, 12.5 mg/mL and 6.25 mg/mL). Discs were completely dried and sterilized. 20µL of culture were spread on sterilized nutrient agar media: impregnated discs were placed on it and incubated for 24 hours at 37°C. Rifampicin discs (10µg) were used as a positive control and methanol was used as negative control. The diameter zone of inhibition was recorded after incubation. The experiment was performed in triplicates and average diameter of zone of inhibition was obtained.

Results and Discussion

Escherichia coli (Gram-negative) and *Bacillus subtilis* (Gram-positive) were used to determine the antibacterial activities of *Shorea bracteolate* stem barks extract by disc diffusion method at different concentrations. Clear inhibition zone were found at 24 hours after incubation at 37°C (Figure 1). *S. bracteolate* extracts at higher Minimal inhibition concentration, MIC (25 mg/mL and 50 mg/mL) showed strong inhibition zone against *B. subtilis* and lower activity was found at lower MIC (6.25 mg/mL and 12.5 mg/mL). *E. coli* showed weak inhibition by *S. bracteolate* extracts at lower MIC from 6.25 mg/mL to 25mg/mL, whilst there were moderate inhibition zone at 50 mg/mL. Rifampicin act the positive control showed strong inhibition of both *E. coli* and *B.*

subtilis and it is contra to methanol as a negative control with gave no significant inhibition zone. The antibacterial activities of crude extracts are shown in Table 1. These data revealed that extracts of *S. bracteolate* exhibited significant antibacterial activity. In testing, inhibition zone increased with the increasing of concentration of crude extracts thus exhibiting concentration dependent activity.

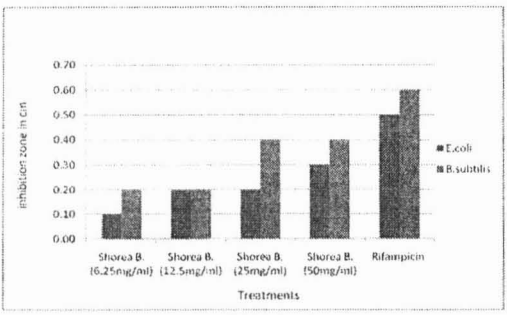

Figure 1. *In vitro* antibacterial activities of *S. bracteolate* at different concentration against *E. coli* and *B. subtilis* in disc diffusion assays. Data shown as mean (n=4).

Extract MIC conc/Microorganism	***E. coli***	***B. subtilis***
Shorea B. (6.25mg/ml)	0.1±0.00	0.2±0.1
Shorea B. (12.5mg/ml)	0.2±0.00	0.2±0.15
Shorea B. (25mg/ml)	0.2±0.06	0.4±0.1
Shorea B. (50mg/ml)	0.3±0.06	0.4±0.12
Rifampicin (+ve control)	0.5±0.00	0.6±0.06

Table 1. The antimicrobial activity (diameters of growth inhibition zones) of crude methanol extracts from different concentration of *S. bracteolate*

From the results obtained, it appears that the antibacterial action of the extracts is more pronounced on Gram-positive (*B. subtilis*) than on Gram-negative bacteria in most cases. These finding are correlate with the observations of previous screening of medicinal plants for antimicrobial activity, where most of the active plants extracts showed activity against Gram-positive strains only (Ali et al., 2001; Herrera et al., 1996; Kelmanson et al., 2000).

With the regard to the components responsible for the strong broad spectrum of antimicrobial activity shown, several compounds of distinct nature must be acting as antimicrobial agents in this plant. Majority of the *Dipterocarpaceae* species studied so far there are a large variety of active principles, including among other terpenoids, flavonoids, arypropanoids and oligomer resveratrol (Wan Zuraida et al., 2008). Resveratrol is naturally found in plants to protect them from disease, injury or fungal infection and it is called 'phytoalexins' (Sotheeswaran & Pasupathy, 1993). Resveratrol also has been shown to act as antioxidant. It is also has cardiovascular benefits by enhancing production of nitric oxide to keep arteries relaxed thus improve the blood flow (Ito et al., 2003).Resveratrol also shown to help in fight against cancer cell in body by stopping them to rapidly grow (Friedman, 2006).

Conclusion

The data from preliminary screening indicate that *Shorea bracteolate* species from UiTM Pahang Reserved Forest, Jengka show excellent antimicrobial activity. The antimicrobial activity of *S. bracteolate* may be attributed to the various phytochemical constituents present in the crude extract. The screening proved that various concentration of crude extracts give significant towards inhibition zone activity. Higher concentration might give better inhibition zone. In this respect, further investigation is ongoing.

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