In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method

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Abstract. Medicinal plants have many traditional claims including the treatment of ailments of infectious origin. In the evaluation of traditional claims, scientific research is important. The objective of the study was to determine the presence of antibacterial activity in the crude extracts of some of the commonly used medicinal plants in Malaysia, Andrographis paniculata, Vitex negundo, Morinda citrifolia, Piper sarmentosum, and Centella asiatica. In this preliminary investigation, the leaves were used and the crude extracts were subjected to screening against five strains of bacteria species, Methicillin Resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Escherichia coli, using standard protocol of Disc Diffusion Method (DDM). The antibacterial activities were assessed by the presence or absence of inhibition zones and MIC values. M. citrifolia, P. sarmentosum and C. asiatica methanol extract and A. paniculata (water extract) have potential antibacterial activities to both gram positive S. aureus and Methicillin Resistant S. aureus (MRSA). None of the five plant extracts tested showed antibacterial activities to gram negative E. coli and K. pneumoniae, except for A. paniculata and P. sarmentosum which showed activity towards P. aeruginosa. A. paniculata being the most potent at MIC of 2 µg/disc. This finding forms a basis for further studies on screening of local medicinal plant extracts for antibacterial properties.

INTRODUCTION

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Zakaria, 1991). The discovery of medicinal plants in different parts of the world is important both to the agriculture and medicine sectors, in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits. Medicinal plants do play an important role in the treatment of ailments in Malaysia. The use of plant preparation for such purposes has been documented (Herbal Medicine Research Centre, 2002). More than hundred plant species in Malaysia are reported to have medicinal properties. Some of these plants are commonly used and have been used by people as folk medicine for hundreds of years (Herbal Medicine Research Centre, 2005). The control of bacteria infection has been remarkably effective since the discovery of antibacterial drugs. However some of the pathogens rapidly become resistant to many of the first discovered effective drugs. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics (WHO, 2002) has led to the search of new antibacterial agents in particular from medicinal plants. Higher plants have been shown to be a potential source for new anti-microbial agents (Mitscher et al., 1987). The screening of
plant extracts has been of great interest to scientist for the discovery of new drugs effective in the treatment of several diseases (Dimayuga & Garcia, 1991). A number of reports concerning the antibacteria screening of plant extracts of medicinal plants have appeared in the literatures (Salvat et al., 2001; Geyid et al., 2005). The present study was to screen the antibacterial activities of five local medicinal plant extracts; *Piper sarmentosum*, *Morinda citrifolia*, *Vitex negundo*, *Andrographis paniculata* and *Centella asiatica* against common bacteria species, methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*.

**MATERIAL AND METHODS**

**Bacteria and reagents**

Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*.

**Selection of Plant Material**

Five medicinal plant species were selected for the in vitro antibacteria screening. The names of these plants, their scientific and local names, parts used and traditional claims are presented in Table 1. These plants were collected and authenticated by Dr. Badrul Amini Rashid from the Phytochemistry Unit. The voucher specimens were kept at the Herbal Medicine Research Center. The plant

<table>
<thead>
<tr>
<th>No.</th>
<th>Scientific name and part of plant used in the study</th>
<th>Local name</th>
<th>Traditional claims*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Andrographis paniculata</em> (leaves), Water extract</td>
<td>Hemptedu bumi</td>
<td>The entire plant is claimed to be an antipyretic, an antiperiodic, an anti-inflammatory, an antibacterial, an antihelmintic and antiimmuno-suppressive.</td>
</tr>
<tr>
<td>2</td>
<td><em>Vitex negundo</em> (leaves) Ethanol Hot</td>
<td>Lemuni</td>
<td>The herb is recommended as an alternative and a tonic for skin disorders and the nervous system and can act as well as antibacterial.</td>
</tr>
<tr>
<td>3</td>
<td><em>Morinda citrifolia</em> (leaves) Methanol Hot</td>
<td>Mengkudu</td>
<td>The Malays use the heated leaves to threat coughs, splenomegaly, nausea, abdomina colic, fever and bacterial infection by placing them onto the infected area.</td>
</tr>
<tr>
<td>4</td>
<td><em>Piper sarmentosum</em> (leaves) Methanol Hot</td>
<td>Kaduk</td>
<td>The plant is believed to act as an antimalarial, to relieve fever, cough, influenza, pleurisy, asthma, abdominal pain, and bacterial infection.</td>
</tr>
<tr>
<td>5</td>
<td><em>Centella asiatica</em> (leaves) Methanol Hot</td>
<td></td>
<td>Act as an antibacterial and also recommended as an alternative for skin disorders and the nervous system.</td>
</tr>
</tbody>
</table>

* Most of the ethnomedical information has been taken from Compendium of medicinal plants used in Malaysia.
species were selected based on their traditional claims as having antibacterial properties.

**Preparation of plant extract**

Dried ground leaves of *P. sarmentosum*, *M. citrifolia* and *C. asiatica* were exhaustively extracted with methanol (MeOH, Analytical Grade, BDH Laboratory Supplies) in a soxhlet apparatus for approximately 12 hours. The resulting MeOH extract was filtered through Whatman paper No.1 and concentrated under reduced pressure at 45°C using the Buchi Rotavapor R-200 to obtain a crude residue (23.5%). Dried ground leaves of *V. negundo* was macerated in Ethanol (EtOH) overnight at ambient temperature while the *A. paniculata* was extracted with water for approximately 12 hours. Further extraction procedures were similar to that of *P. sarmentosum*, *M. citrifolia*, and *C. asiatica* to obtain a crude residue of 8.8%.

**Plant extracts dilution and preparation of impregnated disc**

Plant extracts were diluted in DMSO in a serial two fold dilution across a 96-well plate starting from 200 mg/ml. The concentration was then further diluted to 16 fold in water correspondingly. Twenty microliter from each of the well was then used to impregnate a blank sterilized disc (Oxoid, UK). The final concentration used for the test were from 1 mg/disc to 0.002 mg/disc. The impregnated discs were dried at 37°C incubator for 18 to 24 hours and immediately used for the sensitivity test.

**Bacteria Culture**

Prior to sensitivity testing, each of the bacteria strains were cultured onto blood agar plate and incubated for 18 to 24 hours at 37°C. A single colony was then cultured in 5 ml Mueller Hinton Broth for 4 hours at 37°C. The density of bacteria culture required for the test was adjusted to 0.5 McFarland standard, (1.0 x 10^8 CFU/ml) measured using the Turbidometer (Oxoid, UK).

**Disc Diffusion Method**

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Bauer et al. (1966) to assess the presence of antibacterial activities of the plant extracts. A bacteria culture (which has been adjusted to 0.5 McFarland standard), was used to lawn Muller Hinton agar plates evenly using a sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with a series of plant extracts were placed on the Mueller-Hinton agar surface. Each test plate comprises of six discs. One positive control, which is a standard commercial antibiotic disc, one negative control, and four treated discs. The standard antibiotic discs were Vancomycin 30 µg and Ampicillin 10 µg for *S. aureus* and *K. pneumoniae* respectively, Amikacin 30 µg was for *P. aeruginosa* and *E. coli* while Oxacillin 1 µg was for Methicillin Resistant *S. aureus* (MRSA). The negative control was DMSO (100%). Besides the controls, each plate had four treated discs placed about equidistance to each other. The plate was then incubated at 37°C for 18 to 24 hours depending on the species of bacteria used in the test. After the incubation, the plates were examined for inhibition zone. The inhibition zone were then measured using calipers and recorded. The test were repeated three times to ensure reliability.

**Minimum Inhibition Concentration Determination**

Minimum Inhibition Concentrations (MIC’s) was determined using Inhibitory Concentrations in Diffusion (ICD) method (Guerin-Faublee et al., 1996). It is done by carrying out the diffusion test with twelve discs of different concentration of the plant extracts similar to the concentration used in the sensitivity tests against the five bacteria strains mention earlier (Guerin-Faublee et al., 1996). The lowest concentration that inhibit the growth of bacteria were noted and considered as the MIC value for each of the bacteria strain.
RESULT

The antimicrobial activities of all the plant extracts against the five bacteria strains examined were assessed by the presence or absence of inhibition zones and MIC values. The MIC values and the inhibition zones of the five plant extracts tested for antibacterial activity are given in Table 2. *M. citrifolia*, *P. sarmentosum* and *C. asiatica* methanol extract and *A. paniculata* (water extract) have potential antibacterial activities to both gram positive *S. aureus* ATCC 25923 and Methicillin Resistant *S. aureus* (MRSA). None of the five plant extracts tested showed antibacterial activities to gram negative *E. coli* ATCC 25922 and *K. pneumoniae* (IMR K25/96), except for activity of *A. paniculata* (water extract) and *P. sarmentosum* (methanol extract) showed activity towards *P. aeruginosa* ATCC 27853. *A. paniculata* being the most potent at MIC of 2µg/disc. *V. negundo*, ethanol extract showed no activities to any of the bacteria species tested.

DISCUSSION

The present study was to carry out a preliminary investigation on the antibacterial activity of some local medicinal plants. The most susceptible bacteria to the plant extract preparations were gram positive *S. aureus*, MRSA and gram negative *P. aeruginosa*. *A. paniculata* water extract being the most potent towards *P. aeruginosa* with presence of activity at 2µg/disc. A study has indicated that experiments with presence of activity at concentration of 100 µg/disc for extracts and 10 µg/disc for isolated compounds demonstrated a potential activities for antibacteria (Rios & Recio, 2005). *V. negundo* ethanol extract has no antibacterial activity to any of the bacteria species tested. This is probably

### Table 2. *In vitro* antibacterial activity of the plant extract

<table>
<thead>
<tr>
<th>Plant material (Crude extract)</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em> (ATCC 25923)</td>
<td>Methicillin Resistant <em>S. aureus</em> (Wild Type)</td>
<td><em>E. coli</em> (ATCC 25922)</td>
</tr>
<tr>
<td><em>Andrographis paniculata</em> (leaves), Water extract</td>
<td>1000 µg/disc (6mm ± 0.1)*</td>
<td>250 µg/disc (8mm ± 0.1)*</td>
<td>NA</td>
</tr>
<tr>
<td><em>Vitex negundo</em> (leaves) Ethanol Hot</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><em>Morinda citrifolia</em> (leaves) Methanol Hot</td>
<td>1000 µg/disc (7.3mm ± 0.1)*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><em>Piper sarmentosum</em> (leaves) Methanol Hot</td>
<td>2000 µg/disc (9mm)</td>
<td>1000 µg/disc (8mm)</td>
<td>NA</td>
</tr>
<tr>
<td><em>Centella asiatica</em> (leaves) Methanol Hot</td>
<td>1000 µg/disc (5mm)</td>
<td>1000 µg/disc (7mm)</td>
<td>NA</td>
</tr>
<tr>
<td>Positive control (standard antibacterial drug)</td>
<td>Vancomycin 30µg (16mm)</td>
<td>Oxacillin 1µg (17mm)</td>
<td>Amikacin 30µg (17mm)</td>
</tr>
<tr>
<td>Negative control (DMSO)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Parenthesis indicate the inhibition zone and standard deviation

NA = No activity
due to the preparation of the extract in ethanol. It is reported that ethanol extract of some medicinal plants lack antibacterial activities (Bhakuni et al., 1969). This observation has also been indicated by other study showing that ethanol is not a good solvent for extraction of antimicrobial substances from medicinal plants (Ahmad et al., 1998; Eloff, 1998). The antibacteria activities of these plants are particularly noteworthy considering the importance of these organism.

*S. aureus* causes infections including superficial skin lesion, localized abscesses, and food poisoning. While MRSA infections most often occur in patients in hospitals and are rarely seen among the general public. Although it is usually harmless, it may occasionally get into the body (example through breaks in the skin such as abrasions, cuts, wounds, surgical incisions or indwelling catheters) and cause infections. These infections can be treated with standard drug such as Vancomycin and Oxacillin respectively. But not all strains of the two bacteria species can be successfully treated by these two drugs. Since *A. paniculata* and *P. sarmentosum* showed potential activities to these bacteria, further evaluation on these plants are needed.

The two bacteria strains that were not susceptible to the plant extracts were *E. coli* and *K. pneumoniae*. These could be due to several possible reasons, the distinctive feature of gram-negative bacteria is the presence of a double membrane surrounding each bacterial cells. Although all bacteria have an inner cell membrane, gram-negative bacteria have a unique outer membrane. This outer membrane excludes certain drugs and antibiotics from penetrating the cell, partially accounting for why gram-negative bacteria are generally more resistant to antibiotics than other gram-positive bacteria. This could be the beginning for further research on the screening approach by taking into consideration the extracts preparation and the mechanism of action.

Although the nature and number of active components involved in each extract are not clear, however they are promising. All these medicinal plant extracts tested have traditional claims for antibacteria activity and this findings are in line with their indication as therapeutic properties for antibacterial as claims. This finding can form the basis for further studies to prepare an optimize preparation of the herbal extract to further evaluate them against a wider range of bacteria strains.

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**REFERENCES**


