Tropical Biomedicine 22(2): 165–170 (2005)

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

Zaidan, M.R.S.[1], Noor Rain, A.[1], Badrul, A.R.[2], Adlin, A.[2], Norazah, A.[3] & Zakiah, I.[4]
[1]Bioassay Unit, Herbal Medicine Research Centre, Institute for Medical Research, Kuala Lumpur
[2]Phytochemistry Unit, Herbal Medicine Research Centre, Institute for Medical Research, Kuala Lumpur
[3]Bacteriology Unit, Institute for Medical Research, Kuala Lumpur
[4]Herbal Medicine Research Centre, Institute for Medical Research, Kuala Lumpur

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 antibacteria 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 plays 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 *Staphylo-coccus aureus* (MRSA), *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*.

MATERIAL AND METHODS

Bacteria and reagents

Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* (IMR K25/96), *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922, were obtained from the Bacteriology Unit, Institute for Medical Research Culture Collection Center (ICCC), Kuala Lumpur. All bacteria strains were maintained as stock strains in Microbank™Cryovials and kept at -80°C until used. Blood Agar and MacConkey Agar were obtained from the Bacteriology Unit, Institute for Medical Research. Blank Antibiotic Disc, Vancomycin 30µg, Ampicillin 10 µg, Oxacillin 1 µg and Amikacin 30 µg were purchased from Oxoid, UK.

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 1. Medicinal plants tested for antibacterial activity using disc diffusion method

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

* 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 in 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, (1.0×10^8 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

	Gram positive bacteria		Gram negative bacteria		
	MIC µg/disc				
Plant material (Crude extract)	*S. aureus* (ATCC 25923)	Methicillin Resistant *S. aureus* (Wild Type)	*E. coli* (ATCC 25922)	*K. pneumonia* (WHO 1995/4)	*P. aeruginosa* (ATCC 27853)
Andrographis paniculata (leaves), Water extract	1000 µg/disc (6mm ± 0.1)*	250 µg/disc (8mm ± 0.1)*	NA	NA	2 µg/disc (8mm ± 0.1)*
Vitex negundo (leaves) Ethanol Hot	NA	NA	NA	NA	NA
Morinda citrifolia (leaves) Methanol Hot	1000 µg/disc (7.3mm ± 0.1)*	NA	NA	NA	NA
Piper sarmentosum (leaves) Methanol Hot	2000 µg/disc (9mm)	1000 µg/disc (8mm)	NA	NA	2000 µg/disc (12mm)
Centella asiatica (leaves) Methanol Hot	1000 µg/disc (5mm)	1000 µg/disc (7mm)	NA	NA	NA
Positive control (standard antibacterial drug)	Vancomycin 30µg (16mm)	Oxacillin 1µg (17mm)	Amikacin 30µg (17mm)	Ampicillin 10µg (17mm)	Amikacin 30µg (17mm)
Negative control (DMSO)	NA	NA	NA	NA	NA

*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 for 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.

Acknowledgement. The authors wish to thank the Director, Institute for Medical Research, Kuala Lumpur for permission to publish this paper. This project received funding from the "Small Project Grant 2005, IMR/PK/05/013".

REFERENCES

Ahmad, I., Mehmood, Z. & Mohammad, F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology* **62**: 183-193.

Bauer, A.W., Kirby, W.M.M., Serris, J.C. & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology* **45**: 493-496.

Bhakuni, D.S., Dhar, M.L., Dhawan, B.N. & Mehrotra, B.N. (1969). Screening of Indian plants for biological activity, part II. *Indian Journal of Experimental Biology* **7**: 250-262.

Dimayuga, R.E. & Garcia, S.K. (1991). Antimicrobial screening of medicinal plants from Baja California Sur. Mexico. *Journal of Ethnopharmacology* **31**: 181-192.

Eloff, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants?. *Journal of Ethnopharmacology* **60**: 1-8.

Geyid, A., Abebe, D., Debella, A., Makonnen, Z., Aberra, F., Teka, F., Kebede, T., Urga, K., Yersaw, K., Biza, T., Mariam, B.H. & Guta, M. (2005). Screening of some medicinal plants of

Ethiopia for their anti-microbial properties and chemical profiles. *Journal of Ethnopharma-cology* (In Press).

Guerin-Faublee, V., Muller, M.L.D., Vigneulle, M. & Flandrois, J.P. (1996). Application of a modified disc diffusion technique to antimicrobial susceptibility testing of *Vibrio anguillarum* and *Aeromonas salmonicida* clinical isolates. *Veterinary Microbiology* **51**: 137-149.

HMRC (2002). Compendium of medicinal plants used in Malaysia, Volume I and II. *Herbal Medicine Research Centre Publications*.

Mitscher, L.A., Drake, S., Gollopudi, S.R. & Okwute, S.K. (1987). A modern look at folkloric use of anti-infective agents. *Journal of Natural Products* **50**: 1025-1040.

Rios, J.L. & Recio, M.C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology* **100**: 80-84.

Salvet, A., Antonnacci, L., Fortunado, R.H., Suarez, E.Y. & Godoy, H.M. (2001) Screening of some plants from northern Argentina for their anti-microbial activity. *Journal of Ethnopharmacology* **32**: 293-297.

WHO (2002). Traditional Medicine Strategy 2002-2005. *WHO Publications*. 1-6.

Zakaria, M. (1991). Isolation and characterization of active compounds from medicinal plants. *Asia Pacific Journal of Pharmacology* **6**: 15-20.