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Short Communication

A Preliminary Study on the Antimicrobial Properties of Several Plants Collected from Terengganu, Malaysia

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ABSTRACT

Ethanol extracts of six plants used by local residents of Terengganu as medicinal plants were selected and screened for antimicrobial activities using the disc diffusion method. All plant extracts were effective against *Staphylococcus aureus*. The root extracts of *Abutilon indicum* and *Melastoma malabathricum*, and the leaf extracts of *Piper betel* exhibited antimicrobial activity against all microorganisms tested, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa* and the yeast *Candida albicans*. The leaf extracts of *Molineria latifolia* and *Abutilon indicum* were also effective against *Escherichia coli* while the extract of roots of *Molineria latifolia*, the tuber of *Dioscorea daemona*, and the leaves of *Piper sarmentosum* and *Melastoma malabathricum* exhibited an activity against *Candida albicans*. The results obtained were comparable to commercially available antibiotics, antifungals and *Cassia alata* extracts at equivalent concentrations. In conclusion, the tested plants possess potential antimicrobial activity and require further studies.

Keywords: Antimicrobial activity, disc diffusion, medicinal plants, ethanol extracts

ABSTRAK

Ekstrak etanol dari enam jenis tumbuhan yang digunakan oleh para penduduk di negeri Terengganu sebagai tumbuhan perubatan telah dipilih dan disaring bagi mengenalpasti aktiviti antimikrob melalui teknik penyerapan cakera. Semua ekstrak dari tumbuhan-tumbuhan terpilih menunjukkan keberkesanan ke atas *Staphylococcus aureus*. Ekstrak akar dari pokok *Abutilon indicum* dan *Melastoma malabathricum* serta ekstrak daun *Piper betel* menunjukkan aktiviti antimikrob terhadap semua jenis mikroorganisma seperti *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa* dan *Candida albicans*. Manakala ekstrak daun *Molineria latifola* dan *Abutilon indicum* juga efektif terhadap *Escherichia coli,* ekstrak akar *Molineria latifola* dan daun *Dioscorea daemona*, *Piper sarmentosum* dan *Melastoma malabathricum* mempamerkan aktiviti menentang *Candida albicans*. Hasil ujikaji menunjukkan keputusan yang setara dengan antibiotik, antikulat dan ekstrak *Cassia alata* di pasaran komersial pada kadar konsentrasi yang sama. Kesimpulan, semua tumbuhan yang melalui proses ujikaji menunjukkan potensi bagi aktiviti antimikrob dan memerlukan kajian lanjut.

Kata kunci: Aktiviti antimikrob, penyerapan cakera, tumbuh-tumbuhan perubatan, ekstrak etanol

INTRODUCTION

Microorganisms including Gram positive and Gram negative bacteria, as well as yeasts have been known to cause various human infections (Matasyoh *et al.*, 2008). Although effective antimicrobials have been developed over the years, drug resistance has been increasingly reported (Chopra, 2007). These circumstances necessitated the development of new generation of antimicrobial drugs. Medicinal plants, including herbs, have been one of the sources of bioactive molecules which may become lead compounds for the manufacture of antimicrobial agents. Malaysia is one of the Asian countries blessed with many potential plants of medicinal benefit. Various plants available in the eastern part of Peninsular Malaysia, particularly in the state of Terengganu, have been used in Malay folklore medicine to treat various diseases. These plants include *Molineria latifolia* (Hypoxidaceae), *Abutilon indicum* (Malvaceae), *Melastoma malabathricum* (Melastomataceae), *Piper sarmentosum* (Piperaceae), *P. betel* (Piperaceae) and *Dioscorea daemona* (Dioscoreaceae), which are known respectively to the locals as *Lemba*, *Kembang lubuk*/*Segudai*, *Senduduk*, *Kadok*, *Sireh* and *Ubi gadong*.

Molineria latifolia (*Lemba*) is a well known medicinal plant among villagers in Terengganu who use it for the treatment of kidney disorders, headaches, ulcers, cough and swellings. A*butilon indicum* is used to treat fever, ulcers, dysuria and snake bites (Prajapati and Kumar, 2005) and has also been claimed to possess analgesic activity (Ahmed *et al.*, 2000). In some areas in Terengganu, *A. indicum* is used by local residents to treat swollen skin and pre-stage cancers. *Melastoma malabathricum* is traditionally used as antiemetic and antispasmodic agents (Bhattacharjee, 2008). *P. sarmentosum* is used in traditional Asian medicines to treat diarrhea, dysentery, ulcers, and to prevent scarring from smallpox while *P. betel* leaves have been chewed and credited with many medicinal effects (*e.g.* digestive, stimulative, carminative and aphrodisiac) (Anonymous, 1992). Furthermore, betel juice is given to children for cough and administered to the eye for night blindness in adults. *D. daemona*, which is locally known as toxin tubers by villagers in Terengganu, has been used in the form of paste to stop nose bleeds and is applied on the face to treat pimples (Ong and Nordiana, 1999). This study is aimed to determine the potential antimicrobial properties of several plants used as traditional Malay remedies in Terengganu, one of the states in the eastern part of Peninsular Malaysia.

MATERIALS AND METHODS

Sampling of Plants

Ethnobotanical literatures were reviewed after getting the information from several village elders and certain species were selected for antimicrobial screening. Some plants were already known for their potential antimicrobial activities while others have not been reported or claimed to possess the said activity. The plants were collected from various areas within the state of Terengganu, Malaysia. *P. betel*, *D. daemona* and *M. latifolia* were collected at Batu Sawar Village, Lentang, Terengganu whereas *P. sarmentosum* and *A. indicum* were sampled at Rhu Rendang Village, Marang, Terengganu. *M. malabathricum* was collected from its natural habitat at Sentul Patah Village, Marang, Terengganu. The specimens obtained were identified at MARDI Jerangau, Hulu Terengganu by their Herbs Research Officer and Science Officer of the Biodiversity Unit of the Institute of Biosciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Table 1 provides a description of the scientific and local names, parts of the plants used and medicinal claims either by other researchers or traditional beliefs.

Table 1. Plants that were used in this study.

Plant species	Common name	Part used	Medicinal claims/References
Molineria latifolia (formerly known as *Cucurligo latifolia*)	*Lemba (Nyior Lemba)*	Leaves, roots	Treatment of kidney disorders (fruits), headache, ulcers, cough, and swellings (Anonymous, 1992).
Abutilon indicum	*Kembang Lubuk / Segudai*	Leaves, roots	Treatment of fever, ulcers, dysuria and snake bites (Prajapati and Kumar, 2005); analgesic potential (Ahmed *et al.,* 2000)
Piper betel	*Sireh*	Leaves and bark	Antimicrobial, gastroprotective, wound healing, hepatoprotective, antioxidant, treatment of night blindness, catarrh and diphtheria, gastric, purulent ulcers and lung disorders (Jayaweera, 1982; Ratnasooriya *et al.,* 1990); prevent halositosis (Ramji *et al.,* 2002); stop nose bleeds, *Piper betel* with coconut oil paste used to treat headaches (Ong and Norzalina, 1998)
Dioscorea daemona	*Ubi Gadong*	Tuber	A paste is applied on the face to treat pimples (Ong and Nordiana, 1999).
Piper sarmentosum	*Kaduk*	Leaves	Anti tuberculosis, anti plasmodial activities, treatment of diarrhea piles and ulcers, prevents smallpox scars Treatment of kidney stones and difficulty in urination (Ong and Norzalina, 1998)
Melastoma malabathricum	*Senduduk Bunga Ungu*	Leaves, roots	Bark and leaves used for skin troubles (Parajapati and Kumar, 2005); astringents and accelerate wound healing (Ong and Norzalina, 1998; Wiart *et al.,* 2004)

Preparation of Herbal Extracts

The respective plant's leaves, roots or tubers were oven dried at 50 °C for two weeks and the dried samples were grounded prior to extraction with ethanol. The extracts of *M. latifolia*, *A. indicum*, *M. malabathricum*, *P. sarmentosum*, *P. betel* and *D. daemona* were prepared by mixing the coarse ground samples with ethanol in the ratio of 1:3 (w/v). Each mixture obtained was first filtered using Whatman No. 1 filter paper followed by evaporation under reduced pressure to yield the crude extracts. All crude extracts obtained were stored at -18 °C prior to antimicrobial testing. Prior to use, the ethanol crude extracts of the respective plants were dissolved in dimethyl sulfoxide (DMSO) to final concentrations of 30 and 50 μg/mL followed by their sterilization via filtration using 0.45 mm Millipore filters.

Microorganisms

The microorganisms used in this preliminary study were the American Type Culture Collection (ATCC)-type strains of the following bacteria: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 35218, *Bacillus cereus* ATCC 14579 and *Pseudomonas aeruginosa* ATCC 27853. The yeast *Candida albicans* IMR C504 that was used was provided by the Institute of Medical Research, Kuala Lumpur, Malaysia.

Screening of Antimicrobial Activity Using the Disc Diffusion Method

The disc diffusion method described by Somchit *et al.* (2002) with slight modification was used to measure the ability of an antimicrobial agent to inhibit bacterial growth *in vitro*. Bacterial strains were cultured overnight at 37 °C in Mueller Hinton Broth (MHB, Oxoid) whereas *C. albicans* to be used as inoculum was cultured at 28 °C for 72 hours in Potato Dextrose Broth (PDB, Oxoid). A final inoculum using 100 μL of suspension containing 10^8 cfu/mL of bacteria and 10^4 cfu/mL of the yeast *C. albicans* were spread on Mueller Hinton Agar and Potato Dextrose Agar media, respectively. The paper discs of 6 mm in diameter were impregnated with either 30 μg/mL or 50 μg/mL of the plant extracts reconstituted in DMSO and were placed on the inoculated agar.

Negative controls were prepared using DMSO while commercially available antibiotics were used as positive controls. The antibiotics used were trimethoprim (30 μg/mL), ceftazidime (30 μg/mL), cefotaxime (30 μg/mL), cetriaxone (30 μg/mL), amikacin (30 μg/mL), fluconazole (25 μg/mL) and metronidazole (25 μg/mL), all of which were purchased from Oxoid Ltd. UK. *Cassia alata* extracts, which have been reported to possess antimicrobial activity (Somchit *et al.*, 2002), was also used for comparative purposes at concentrations of 30 and 50 μg/mL. Duplicated readings were recorded.

The test plates were incubated at 37 °C for 24 hours for bacteria and 28 °C for 72 hours for yeast. The antimicrobial activity of all plant extracts were assessed by the presence or absence of inhibition zone and the diameter of the zone were measured by means of triplicate. The range of inhibition zone diameter was assessed by using the interpretative chart of zone sizes for rapidly growing bacteria by applying the modified Kirby-Bauer technique (NCCLS, 2001).

RESULTS AND DISCUSSION

This preliminary study was aimed to identify new plants with antimicrobial and antifungal properties. Six plant species traditionally used by the local residents in Terengganu, Malaysia were selected. The selection of plants was supported by previous studies, and their findings were compared with the present findings (Table 1). The plant extracts were tested against five types of microorganisms by using the disc diffusion sensitivity test.

The present study demonstrated the antimicrobial potential of ethanol extracts of several plants available in Terengganu, Malaysia (Table 2). The root extracts of *Molineria latifolia* were more effective than the leaf extracts as indicated by the former's ability to inhibit the growth of *C. albicans* and *P. aeruginosa* at all concentrations, whereas effective against *E. coli* only at a concentration of 30 μg/mL. The leaf extracts were effective only against *S. aureus* and *E. coli* at all concentrations, whilst only effective at the highest concentration against *C. albicans*. The root extracts of *A. butilon* were also more effective than the leaf extracts as the root extracts were able to inhibit the growth of all tested microorganisms at all concentrations whereas the leaf extracts only inhibited *S. aureus*, *E. coli* and *B. cereus*. The leaf extracts of *P. betel* was effective against all microorganisms at all concentrations tested. Based on the inhibition zones obtained, the *P. betel* leaf extract can be a potential candidate for the treatment of *C. albicans* and *S. aureus* as the extract gave the largest inhibition zone compared to other plant extracts and was comparable with commercial antimicrobes (Table 3).

The root extracts of *M. malabathricum* also exhibited antimicrobial activity against all tested microorganisms at all concentrations used as compared to its leaf extracts, which were only effective against *S. aureus* and *C. albicans* at all concentrations used and against *B. cereus* at the highest concentration used. This is in contrast to an earlier report by Wiart *et al.* (2004) who found no antimicrobial activity with 10 μg methanol extract of *M. malabathricum* leaves. Nevertheless, our assessment of the antimicrobial potential of *M. malabathricum* included both the leaf as well as root, and utilized ethanol instead of methanol extracts.

The tuber extracts of *D. daemona* were effective at all concentrations against *S. aureus*, *B. cereus* and *C. albicans*, and against *E. coli* only at the highest concentration. There have been studies to show that some species of *Dioscorea* have antioxidant activities and anti inflammatory activities, which can be linked to the antiphlogistic effects of the steroidal saponins (Araghiniknam *et al.,* 1996). Antimicrobial activity against Gram positive bacteria and Gram negative *Escherichia coli* has also been reported for *Dioscoreae sylvatica* and *Dioscorea dregeanna* (Kelmanson, 2000).

Based on the diameter of the inhibition zone, it is believed that the root extracts of *M. latifolia*, the leaf extracts of *P. betel* and *M. malabthricum* and the root extracts of *M. malabathricum* could be developed as potential antifungal agents against *C. albicans*. Both of the stated plants, as well as tuber extracts of *D. daemona* could also possibly be developed as an agent to fight *S. aureus* infection.

CONCLUSION

All tested plant specimens displayed a spectrum of antimicrobial activity against several microorganisms with the leaf extract of *P. betel* being the most effective. These preliminary results proved and supported previous findings and also provided new information on the potential antimicrobial activity of several plants that have not been investigated before, such as *M. latifolia*. The study on plants as a source of antimicrobial agent is crucial to gain new lead compounds for the development of new drugs for the treatment of various types of infectious diseases.

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Table 2. Inhibition zones produced by plant extracts.

Plant specimen	Source of extracts	*S. aureus*		*E. coli*		*B. cereus*		*C. albicans*		*P. aeruginosa*	
		Diameter of inhibition zone (mm)									
		30 µg	50 µg	30 µg	50 µg	30 µg	50 µg	30 µg	50 µg	30 µg	50 µg
Molineria latifolia	Leaves	11 ±1.7	12.5±0.5	5±2.1	7.5±4.5	-	-	-	7±3.6	-	-
	Roots	7.5 ±0.5	11.5±0.9	-	5.5±4.5	-	-	13±0.0	16±3.6	5±2.3	7.5±4.1
Abutilon indicum	Leaves	6 ± 0.0	6±4.0	6±3.5	6±3.8	6±1.2	6±1.2	-	-	-	-
	Roots	8 ± 0.6	9.5±6.1	9±0.0	11±3.6	6±3.5	7±2.0	7±0.0	8±0.9	7±0.0	8±2.1
Piper betel	Leaves	16± 1.6	22.5±4.6	9.5±2.9	13.5±5.4	7.5±2.4	8±4.0	26±6.3	29±8.2	8±3.4	12.5±6.0
D. daemona	Tubers	12.5± 3.0	15± 7.7	-	7±6.4	6.5±3.6	8±3.8	5±10.7	1±11.6	-	-
P. sarmentosum	Leaves	5± 2.9	8.5±6.9	-	-	-	-	10.5±9.6	11.5±11.7	-	-
M. malabathricum	Leaves	12.5±3.0	15±2.0	-	-	-	6.5±2.3	13.5±3.0	15±4.4	-	5±1.7
	Roots	10.5±4.6	14.5±3.0	8.5±7.5	10±5.3	6±3.9	8±4.6	13±1.0	15±7.2	5.5±2.8	7.5±4.1

* Concentrations of plant extracts were used as 30 µg and 50 µg plant extracts/100 mL DMSO (±S.D.)

Table 3. Inhibition zones of commercial antibiotics and *Cassia alata* extracts against the test microorganisms used in this study.

Test microorganisms	**Diameter of inhibition zone (mm)**							
	TMP	**CFZ**	**CFT**	**CTA**	**AKC**	**MND**	**FCN**	**CA**
Staphylococcus aureus	24±3.5	8±2.83	25±4.24	14±0.0	20±5.66	-	-	10±0.0
Escherichia coli	25±6.4	33±4.24	35±0.0	25±5.66	25±4.24	-	-	-
Bacillus cereus	30±0.0	27±4.24	30±0.0	30±0.0	20±0.0	-	-	6±0.0
Pseudomonas aeruginosa	14±0.0	28±3.54	24±8.49	-	26±5.66	-	-	-
Candida albicans	-	-	-	-	-	10±0.0	10±0.0	8±4.2

Notes: Trimethoprim - TMP; Ceftazidime - CFZ; Cefotaxime - CFT; Cetriaxone - CTA; Amikacin - AKC; Metronidazole - MND; Fluconazole - FCN; *Cassia alata* extracts– CA; all antimicrobials were used at a concentration of 30 µg/mL

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