

## Antimicrobial Activity of Selected Malaysian Plants

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### ABSTRAK

Ekstrak metanol daripada 17 tumbuhan Malaysia telah disaring terhadap kulat yang meliputi yis dan kandida serta bakteria gram positif dan negatif. Aktiviti antimikrob terdapat didalam ekstrak dari tumbuhan *Borreria latifolia*, *B. setidens*, *Hedyotis diffusa*, *H. nudicaulis*, *Morinda elliptica*, *M. umbellata* (*Rubiaceae*), *Sida rhombifolia* (*Malvaceae*) dan *Vitex ovata* (*Verbenaceae*). Ekstrak dari *S. rhombifolia* dan *B. latifolia* telah menunjukkan aktiviti yang luar biasa terhadap *Candida albicans*, *Ca. intermedia*, *Aspergillus ochraceous*, *Cunninghamella elegans* dan *Pseudomonas aeruginosa*. Walaubagaimanapun, hanya aktiviti yang serdehana melawan *Bacillus cereus*, *B. megaterium* dan *Candida lipolytica* yang diperhati. Aktiviti antimikrob tidak terdapat daripada ekstrak tumbuhan *Calophyllum inophyllum* (*Guttiferae*), *Entada sp.* (*Leguminosae*), *Eclipta alba* (*Compositae*), *Dendrotrophe umbellata* (*Santalaceae*), *Cardiospermum halicacabum* (*Sapindaceae*), *Glochidion lutescens*, dan *Euphorbia prunifolia* (*Euphorbiaceae*).

### ABSTRACT

Methanolic extracts of seventeen Malaysian plants were screened against fungi, including yeast and candida, as well as gram negative and gram positive bacteria. Antimicrobial activities were present in extracts prepared from *Borreria latifolia*, *B. setidens*, *Hedyotis diffusa*, *H. nudicaulis*, *Morinda elliptica*, *M. umbellata* (*Rubiaceae*), *Sida rhombifolia* (*Malvaceae*) and *Vitex ovata* (*Verbenaceae*). Extracts from *S. rhombifolia* and *B. latifolia* showed exceptionally high activity against *Candida albicans*, *Ca. intermedia*, *Aspergillus ochraceous*, *Cunninghamella elegans* and *Pseudomonas aeruginosa*. However, only moderate activity was observed against *Bacillus cereus*, *Ba. megaterium* and *Ca. lipolytica*. Extracts from *Calophyllum inophyllum* (*Guttiferae*), *Entada sp.* (*Leguminosae*), *Eclipta alba* (*Compositae*), *Dendrotrophe umbellata* (*Santalaceae*), *Cardiospermum halicacabum* (*Sapindaceae*), *Glochidion lutescens* and *Euphorbia prunifolia* (*Euphorbiaceae*) gave negative results.

### INTRODUCTION

The tropical forest of Malaysia is blessed with more than 10,000 species of medicinal plants (Burkill 1930; Ginlette and Burkill 1930; Perry and Metzger 1980; Taylor and Wong 1987). Nakashini *et al.* (1965) reported the first screening of biological activity of indigenous Malaysian plants. Recently, Universiti Pertanian Malaysia has embarked on a

systematic screening of medicinal plants for antimicrobial, cytotoxic and antiviral activities. This paper reports the results of the screening of some Malaysian plants for antimicrobial activity. Filter paper disc diffusion and tube dilution methods were used for qualitative and quantitative evaluations of the antimicrobial activity against bacteria, fungi and candida.

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## MATERIALS AND METHODS

### Plants

Plant material was collected from the state of Selangor and identified by R. Kiew and S. Anthonysamy, Department of Biology, Universiti Pertanian Malaysia. Voucher specimens are deposited in the herbarium of the Biology Department. The plants included:

- Compositae:** *Eclipta alba*  
**Euphorbiaceae:** *Euphorbia prunifolia*, *Glochidion lutescens*  
**Guttiferae:** *Calophyllum inophyllum*  
**Leguminosae:** *Entada* sp.  
**Malvaceae:** *Sida rhombifolia*  
**Rubiaceae:** *Borreria latifolia*, *B. setidens*, *Hedyotis diffusa*, *H. nudicaulis*, *H. verticillata*, *Morinda citrifolia*, *M. elliptica*, *M. umbellata*  
**Santalaceae:** *Dendrotrophe umbellata*  
**Sapindaceae:** *Cardiospermum halicacabum*  
**Verbenaceae:** *Vitex ovata*

### Plant Extracts

Approximately 200 g of plant material was soaked overnight in methanol. The extracts were decanted and transferred into the rotary evaporator where evaporation occurred under reduced pressure. This extraction procedure was repeated three times for each plant sample. The dark residues obtained were used for the screening programme.

### Chemicals

Standard streptomycin sulphate antimicrobial discs (30 µg/disc), nystatin powder from which 50 µg/ml solution was prepared for disc impregnation and nutrient broth were used.

### Microorganisms

The microorganisms were obtained from the culture collection of the Department of Pharmacognosy, University of Mansoura, Egypt (which had originally come from the American Type Culture Collection, ATCC or Northern Regional Research Laboratories, NRRL), and the College of Pharmacy, University of Iowa, USA. The stock cultures were grown on Sabouraud-Dextrose Broth (SDB) for 24 hours at 28°C at which time the cells were harvested by centrifugation (4°C, 2000 rpm, 3 min.). The cells were washed and suspended in sterile 0.9 %

saline solution to give a final concentration of between 10<sup>5</sup>-10<sup>6</sup> CFU/ml (Colony Forming Unit) using a haemocytometer.

The microbial strains were: **Bacteria:** *Bacillus cereus* NRRL 14591B (UI-1447), *Ba. megaterium* ATCC 14581, *Pseudomonas aeruginosa* UI-60690; **Fungi:** *Aspergillus ochraceus* NRRL 398, *A. niger* ATCC 11394, *Cunninghamella elegans* ATCC 9244; **Candida and Yeast:** *Candida lipolytica* ATCC 2075, *Ca. albicans* ATCC 10231, *Ca. intermedia* ATCC 5159 and *Sacchromyces cerevisiae* NRRL 2034.

### Assay for Antibacterial Activity

#### Disc Diffusion Method

Antibacterial activity of the plant extracts was tested using disc diffusion method according to Bauer *et al.* (1966). The discs were prepared by impregnating them with ethanolic solution of each extract (50 mg/ml). They were then evenly spaced on the agar surface previously inoculated with the suspension of each microorganism (10<sup>5</sup> - 10<sup>6</sup> CFU/ml) to be tested. Standard discs of nystatin (50 µg/disc) and streptomycin sulphate (30 µg/disc) were used as positive controls. The plates were incubated at 37°C for 24 hours and the antimicrobial activity was recorded by measuring the width of the clear inhibition zones around each disc.

#### Minimum Inhibition Concentration

The effectiveness of antibacterial activity of the plant extracts was quantified using the tube dilution method according to Hufford *et al.* (1975). Plant extracts at different concentrations (30-1000 mg/ml) were added into 5 ml broth. Cultures containing between 10<sup>5</sup>-10<sup>6</sup> CFU were inoculated in 5 ml broth tubes and incubated for 24 hours at 37°C. Nystatin (50 mg/ml) and streptomycin sulphate (30 mg/ml) were used as standard antibiotics for comparison with the activities of the plant extracts against microbial species. For antifungal and anticandida activities, the SDB was used. Minimum inhibitory concentration (MIC, mg/ml) was recorded at the highest dilution which was free from microbial growth.

## RESULTS AND DISCUSSION

The filter paper disc diffusion method is a very convenient and rapid method for screening of antimicrobial activity from plant extracts (Bauer *et al.* 1966). The formation of inhibition zone is observed as a result of the diffusion of antimi-

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TABLE 1  
Inhibition zone (mm) of the impregnated disc against bacteria, fungi and candida

Microbes	G <sub>1</sub> (w)	G <sub>2</sub> (w)	G <sub>9</sub> (l-t)	G <sub>10</sub> (l-s)	G <sub>12</sub> (w)	G <sub>13</sub> (l-t)	G <sub>20</sub> (r)	G <sub>21</sub> (r)	S	N
A	18	13	13	14	20	19	12	11	25	n
B	21	22	20	20	-	20	20	17	30	n
C	-	-	15	16	18	-	-	-	25	n
D	-	-	25	25	8	9	8	12	n	28
E	19	20	8	8	12	11	13	16	13	22
F	8	-	25	24	15	14	21	15	29	22
G	10	-	12	13	8	11	-	-	n	20
H	17	8	20	20	13	17	13	12	n	26
I	18	19	21	19	9	11	19	13	n	25

**Bacteria :** A *Bacillus cereus* UI-1477 (NRRL 14591B), B. *Pseudomonas aeruginosa* UI 60690, and C. *Ba. megaterium* ATCC 14581. Fungi : D *Aspergillus ochraceus* NRRL398, E. *A. niger* ATCC11391, and F. *Cunninghamella elegans* ATCC 9244. Candida: G. *Candida lipolytica* ATCC 2075, H. *Ca. albicans* ATCC 10231, and I. *Ca. intermedia* ATCC 5159.

**Plant extract:** G<sub>1</sub> = *Hedyotis nudicaulis*, G<sub>2</sub> = *H. diffusa*, G<sub>9</sub> = *Sida rhombifolia*, G<sub>10</sub> = *Borreria latifolia*, G<sub>12</sub> = *B. setidens*, G<sub>13</sub> = *Vitex ovata*, G<sub>20</sub> = *Morinda elliptica*, G<sub>21</sub> = *M. umbellata*.

**Plant part:** (w) = whole plant, (l) = leaf, (t) = twig, (r) root, (s) = stem.

**Antibiotic:** S= Streptomycin sulphate (30 µg/ml), N = Nystatin (50 µg/ml).

- : no activity

n: not tested

TABLE 2  
Minimum inhibition concentration (MIC, µg/ml) of plant extract inhibiting the growth of bacteria, fungi and candida

Microbes	G <sub>1</sub> (w)	G <sub>2</sub> (w)	G <sub>9</sub> (l-t)	G <sub>10</sub> (l-s)	G <sub>12</sub> (w)	G <sub>13</sub> (l-t)	G <sub>20</sub> (r)	G <sub>21</sub> (r)	S	N
A	62	125	125	125	62	62	250	250	31	n
B	62	62	62	62	500	125	62	125	31	n
C	500	250	125	125	125	-	-	-	31	n
D	500	500	62	62	250	250	250	125	n	31
E	62	62	250	250	125	125	125	125	n	31
F	500	500	62	62	250	250	125	250	n	62
G	250	500	125	125	250	250	500	500	n	31
H	125	500	62	62	125	125	125	125	n	31
I	62	62	62	62	250	250	62	125	n	31

**Bacteria :** A *Bacillus cereus* UI-1477 (NRRL 14591B), B. *Pseudomonas aeruginosa* UI 60690, and C. *Ba. megaterium* ATCC 14581. Fungi : D *Aspergillus ochraceus* NRRL398, E. *A. niger* ATCC11391, and F. *Cunninghamella elegans* ATCC 9244. Candida: G. *Candida lipolytica* ATCC 2075, H. *Ca. albicans* ATCC 10231, and I. *Ca. intermedia* ATCC 5159.

**Plant extract:** G<sub>1</sub> = *Hedyotis nudicaulis*, G<sub>2</sub> = *H. diffusa*, G<sub>9</sub> = *Sida rhombifolia*, G<sub>10</sub> = *Borreria latifolia*, G<sub>12</sub> = *B. setidens*, G<sub>13</sub> = *Vitex ovata*, G<sub>20</sub> = *Morinda elliptica*, G<sub>21</sub> = *M. umbellata*.

**Plant part:** (w) = whole plant, (l) = leaf, (t) = twig, (r) root, (s) = stem.

**Antibiotic:** S= Streptomycin sulphate (30 µg/ml), N = Nystatin (50 µg/ml).

- : no activity

n: not tested

crobal compounds from the filter paper. The effectiveness of compounds found in the extract was quantified further by measuring the minimum inhibition concentration that inhibited the growth of microbes compared with the standard antibiotics (Hufford *et al.* 1975). Table 1 shows the size of the inhibition zone of streptomycin to be between 25 and 30 mm where the MIC value was 31 µg/ml when tested against *Bacillus cereus*, *Ba. megaterium* and *Pseudomonas aeruginosa*. In the case of fungi and candida, an inhibition zone of 22-28 mm was measured with MIC value of 31 µg/ml when nystatin was used. However, the MIC value for *Candida elegans* was 62 µg/ml (Table 1 and 2). In this study, the highest antimicrobial activity was observed at 50% level of the standard antibiotics (62 mg/ml for plant extracts and 31 µg/ml for standards). Plant extracts which gave MIC values of between 125 and 250 µg/ml were considered to possess moderate activity while those with MIC value of more than 250 µg/ml were considered to possess a weak activity.

Out of the seventeen extracts screened, only eight exhibited pronounced antimicrobial activities against the tested microbes (Table 1). Extracts from *Sida rhombifolia* and *Borreria latifolia* produced an inhibition zone in all the microbes and possessed exceptionally high activity against *P. aeruginosa*, *Aspergillus ochraceus*, *Cunninghamella elegans*, *Candida intermedia* and *Ca. albicans* (MIC value of 62 µg/ml), moderate activity against *Ba. cereus*, *Ba. megaterium* and *Ca. lipolytica* and weak activity against *A. niger*.

*Hedyotis nudicaulis* and *H. diffusa* showed high activity against *P. aeruginosa*, *Ba. cereus*, *Ca. albicans* and *A. niger* but only moderate activity against yeast (Table 1 and 2), the MIC values for both extracts being 62 µg/ml with the *H. diffusa* extract possessing MIC value of 125 µg/ml against *Ba. cereus*. The MIC value of streptomycin and nystatin against these microbes was 31 µg/ml. Unfortunately these two plant extracts failed to inhibit *Ba. megaterium* and *A. ochraceus*. Extract of *H. diffusa* also failed to inhibit *Ca. elegans* and *Ca. lipolytica*. Higher concentrations of *H. diffusa* and *H. nudicaulis* extracts were required to inhibit these two microbes (MIC value of 500 µg/ml).

Except for *Ba. megaterium*, all tested microbes were inhibited by the *B. setidens* extracts. The extract was highly active against *Ba. cereus*

and moderately active against *Ba. megaterium*, *A. niger* and *Ca. albicans*. Petroleum ether extract of *Vitex ovata* showed good antimicrobial activity against all tested species with the exception of *Ba. megaterium*.

Among the Rubiaceae species, *Morinda elliptica* exhibited high activity against fungi especially *A. niger*, *A. ochraceus*, *Ca. elegans*: moderate activity was observed against *Ca. intermedia*. Moderate antibacterial activity was shown by the extracts of *M. elliptica* and *M. umbellata* but the former was only active against *A. niger*.

Several of these plant extracts have potential as new antibiotics, in particular those from *S. rhombifolia* and *B. latifolia* which displayed inhibition against all the microbes tested.

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