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Antimicrobial and Cytotoxic Activities of Some Malaysian Flowering Plants

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Abstract – A total of 43 extracts from 21 species of Malaysian flowering plants were screened for antimicrobial and cytotoxic activities. Antimicrobial activity was tested against fungi, including yeast and candida, as well as Gram-positive and Gram-negative bacteria, and cytotoxicity was assayed using the CEM-SS and HT-29 cell lines. The methanol extracts of the roots and stems of *Plumeria acutifolia* showed the highest antimicrobial activity, i.e. against *Saccharomyces cerevisiae* and *Candida lipolytica*, and mostly moderate activity against the other microbes such as *Aspergillus ochraceous* and *Saccharomyces lipolytica*. *Phyllanthus emblica* also showed moderate activity especially that of the methanol extracts. The methanol extract of the roots of *Plumeria acutifolia* showed the strongest cytotoxic activity (CD_{50} = 3 μg/ml). Most of Zingiberaceae species gave negative results for antimicrobial activity and showed low cytotoxic activity.

Key words – Antimicrobial, cytotoxicity, plant extracts

Introduction

More than half of the 250,000 species of flowering plants of the world are found in the tropical rain forests but only a small fraction have been studied for their medicinal properties. The important contribution of tropical rain forest plants to medicine and to the well-being of humans is clearly documented (Soejarto *et al.*, 1991). Long before the development of modern medicine, the Malaysian people have used tropical forest plants in traditional medicine to treat various diseases such as fever, malaria, itch, cough, ulcers, wounds, bleeding during early pregnancy and after childbirth. The tropical forest of Malaysia is blessed with more than 15,000 species of medicinal plants (Burkill, 1966; Perry and Metzger, 1980; Taylor and Wong, 1987). Although extensive phytochemical surveys have been carried out on the flora of Malaysia (Goh *et al.*, 1993), only a few reports deal with screening for pharmacological activities (Ali *et al.*, 1995; Ali *et al.*, 1996a; Ali *et al.*, 1996b; Mackeen *et al.*, 1997a; Mackeen *et al.*, 1997b; Hock *et al.*, 1998; Mooi *et al.*, 1999). The diverse flora of the indigenous Malaysian plants serves as a good starting point for the screening of biologically active natural products. This paper reports the results of the screening of some Malaysian flowering plants for antimicrobial and cytotoxic activities.

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Materials and Methods

Plant materials – The plant materials were collected in the state of Selangor and identified by S. Anthonysamy and A. A. Rahman, Department of Biology, Universiti Putra Malaysia. Voucher specimens were deposited at the herbarium of the Biology Department. The plants collected were: **Acanthaceae**: *Lepidagathis longifolia* Wight; **Apocynaceae**: *Plumeria acutifolia* Poir; **Euphorbiaceae**: *Phyllanthus emblica* Linn., *Phyllanthus oxyphyllus* Miq.; **Gesneraceae**: *Cyrtandromoea grandis* Ridl., *Cyrtandromoea acuminata* Benth. & Hook. F.; **Labiatae**: *Leucas zeylanica* R. Br., *Hyptis brevipes* Poit.; **Liliaceae**: *Dracaena porteri* Baker; **Melastomaceae**: *Phyllagathis griffithii* King, *Sonerila heterostemon* Naud.; **Rubiaceae**: *Hedyotis verticillata* Lam., *Psychotria rostrata* Blume; **Zingiberaceae**: *Alpinia hookeriana* Val., *Alpinia mutica* Roxb., *Alpinia nutans* Rosc., *Amomum compactum* Soland, *Amomum gracile* Blume, *Costus mexicanus* Liebm., *Horn-*

stedtia leonurus Retz., and *Nicolaia speciosa* Horan.

Extraction – Approximately 200 g of the dried parts including the leaves, stems, fruits, and rhizomes were ground and soaked overnight in methanol. Several samples were successively soaked in n-hexane and chloroform before methanol. The extracts were filtered and evaporated under reduced pressure. This extraction procedure was repeated three times for each plant sample. The crude extracts obtained were used for testing.

Microorganisms – The test microorganisms were obtained from the culture collection of the Department of Pharmacognosy, University of Mansoura, Egypt. The cultures were originally purchased from the American Type Culture Collection (ATCC), Northern Regional Research Laboratories (NRRL) and College of Pharmacy, University of Iowa (UI). The bacterial and fungal stock cultures were maintained on nutrient agar (NA) and potato dextrose agar (PDA) slants respectively, which were stored at 4°C. The strains of microorganisms used were: **Gram-positive Bacteria:** *Bacillus cereus* (UI 1447) and *Bacillus megaterium* (ATCC 14581); **Gram-negative Bacteria**: *Escherichia coli* (UI 190494) and *Pseudomonas aeruginosa* (UI 60690); **Fungi**: *Aspergillus ochraceous* (ATCC 398), *Saccharomyces lipolytica* (ATTC 16617), *Saccharomyces cerevisiae* (NRRL 20381) and *Candida lipolytica* (ATTC 2075).

Disc diffusion method – Antimicrobial activity of the plant extracts was tested by a modified disc difussion method (Bauer *et al.*, 1966). A lawn of microorganism was prepared by pipetting and evenly spreading 100 µl of inoculum (adjusted turbidometrically to 10^5~10^6 CFU/ml) onto agar set in petri dishes, using NA for the bacteria and PDA for fungi. Whatman No. 1 filter paper discs of 6 mm diameters were impregnated into the ethanol stock solutions of the plant extracts (100 mg/ml) and dried under sterile conditions to remove the ethanol. The dried discs were then placed on the previously inoculated agar surface. The plates were inverted and incubated for 24 h at 30°C. Antimicrobial activity was recorded by measuring the diameter (d.) of the clear inhibition zones around each discs.

Cytotoxicity assay – The CEM-SS (T-lymphoblastic leukaemia) and HT-29 (colon carcinoma) cell lines were obtained from the National Cancer Institute, Frederick, Maryland, USA. The cells were cultured in RPMI-1640 medium supplemented with 10% (v/v) of fetal calf serum (FCS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin as a complete growth medium (CGM). Cells were maintained in 25 cm^2 flasks with 10 ml of CGM in a CO_2 incubator at 37°C until attaining confluence. The cytotoxicity assay was performed in 96 flat bottom microwell plates. Briefly, 100 µl of exponentially growing cell suspension at the concentrations of 5×10^5 cells/ml were seeded into the wells in the presence of various concentration of test extracts. The plates were incubated for 72 hours in a CO_2 incubator at 37°C. After 72 hours, the fraction of surviving cells in treated population was determined relative to the untreated cell population by the colorimetric MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenil-tetrazolium bromide) method (Mosmann, 1983). The viability of cells was determined by measuring the absorbance at 550 nm (reference at 630 nm) of the amount of blue formazan crystals converted from tetrazolium salts by living cells. The concentration that killed cells by 50% (CD_{50}) was deduced from the absorbance vs concentration curve.

Results and Discussion

Table 1 shows the inhibition zone diameter (d. in mm) and CD_{50} (µg/ml) values of the 43 extracts from 21 species. Most of the extracts recorded weak (d. < 10 mm) to moderate activities (d. 10 to 15 mm) against the test microorganisms. Among all the extracts tested, only the methanol extract from roots and stems of *Plumeria acutifolia* were inhibitory against all the target microorganisms. They showed strong activity (d. > 15 mm) against *Saccharomyces cerevisiae* and *Candida lipolytica*. Moderate activity was shown by both extracts against *Aspergillus ochraceous* and *Saccharomyces lipolytica*, but only the root extract was active against *Bacillus megaterium* and *Escherichia coli*; and the stem extract against *Bacillus cereus*. However, these extracts showed weak activity against other test microorganisms.

Phyllanthus emblica also showed potential as antimicrobial agent since some its methanol extracts showed moderate activity such as against *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. However, most of its *n*-hexane extracts showed weak activity, except the stem extract against *Saccharomyces cerevisiae* and the fruit extract against *Bacillus cereus* and *Escherichia coli* which all showed

Table 1. Antimicrobial and cytotoxic activities of plant extracts

Species	Part	Extract*	Target microorganism** Inhibition zone (mm)†							Cytotoxicity CD_{50} (mg/ml)	
			Bc	Bm	Ec	Pa	Ao	Sl	Sc	Cl	CEM-SS cell
Lepidagathis longifolia	Root	M	<7‡	7	9	<7	<7	8	8	9	30
Plumeria acutifolia	Root	M	9	12	10	9	14	10	22	18	3
	Stem	M	11	9	8	8	11	14	24	19	10
Phyllanthus emblica	Leaf	M	11	10	12	10	14	9	8	9	10
	Stem	M	10	9	10	11	9	<7	8	10	10
	Fruit	M	10	11	11	10	8	<7	8	12	10
	Leaf	H	<7	8	8	7	<7	7	<7	8	30
	Stem	H	8	8	9	<7	<7	8	10	9	>30
	Fruit	H	10	9	11	<7	<7	<7	8	9	>30
Phyllanthus oxyphyllus	Stem	M	8	8	10	9	<7	10	<7	10	30
	Stem	C	<7	8	8	9	<7	9	7	8	30
Cyrtandromoea grandis	Root	M	<7	8	8	7	<7	<7	8	<7	30
	Leaf	M	<7	7	8	7	<7	<7	7	7	>30
Cytrandromoea acumina	Leaf	M	<7	10	9	<7	7	<7	8	<7	>30
ta	Root	M	7	7	8	8	7	<7	11	9	30
Leucas zeylanica	Leaf	M	7	8	<7	<7	7	<7	<7	7	30
	Root	M	7	<7	<7	7	<7	<7	8	13	30
Hyptis brevipes	Root	H	8	<7	<7	9	<7	<7	7	8	>30
	Stem	M	8	<7	<7	10	<7	<7	7	12	30
	Stem	H	<7	<7	<7	8	<7	<7	<7	9	>30
	Root	M	8	8	7	7	<7	<7	<7	<7	>30
Dracaena porteri	Leaf	M	<7	7	8	<7	<7	<7	<7	7	>30
Phyllagathis griffithii	Stem	M	7	<7	<7	<7	<7	<7	7	<7	>30
	Leaf	M	8	8	9	7	<7	<7	<7	8	30
Sonerila heterostemon	Root	M	7	8	8	<7	7	<7	<7	<7	30
Hedyotis verticillata	Leaf	M	<7	8	7	7	<7	<7	<7	7	10
	Leaf	M	8	8	10	9	<7	<7	8	7	30
Psychotria rostrata	Rhizome	M	<7	7	<7	7	<7	<7	<7	7	30
Alpinia hookeriana	Rhizome	C	9	<7	7	8	<7	10	11	11	>30
	Rhizome	M	7	<7	<7	8	<7	<7	<7	<7	10
Alpinia mutica	Rhizome	C	7	7	<7	<7	<7	11	9	8	30
	Rhizome	M	<7	7	<7	7	<7	<7	<7	<7	30
Alpinia nutans	Rhizome	C	<7	8	7	<7	<7	<7	<7	<7	>30
	Rhizome	M	<7	<7	7	<7	<7	<7	<7	<7	30
Amomum compactum	Rhizome	C	8	7	<7	8	<7	<7	7	7	30
	Rhizome	M	<7	<7	<7	7	<7	<7	<7	<7	10
Amomum gracile	Rhizome	C	7	7	<7	<7	<7	<7	11	<7	30
Costus mexicanus	Rhizome	M	<7	<7	7	7	<7	<7	7	<7	>30
	Rhizome	C	<7	7	<7	8	<7	<7	<7	<7	>30
Hornstedtia leonurus	Rhizome	M	<7	<7	<7	7	<7	<7	7	<7	30
	Rhizome	C	10	7	<7	<7	<7	<7	7	7	30
Nicolaia speciosa	Rhizome	M	<7	8	7	8	<7	<7	7	<7	30
	Rhizome	C	<7	7	<7	7	<7	<7	<7	<7	>30
Cislatin											5.5
Doxorubicin											0.1

*Extracts: M = methanol; C = chloroform; H = n-hexane

**Microorganism: *Bc = Bacillus cereus; Bm = Bacillus megaterium; Ec = Escherichia coli; Pa = Pseudomonas aeruginosa; Ao = Aspergillus ochraceous; Sl = Saccharomyces lipolytica;; Sc = Saccharomyces cerevisiae; Cl = Candida lipolytica.*

†Standard deviation of all extracts was <0.3, ‡No activity: <7 mm

moderate activity. In this study, most of the Zingiberaceae species were inactive in both assays with only moderate activity being shown by the chloroform extracts of *Alpinia hookeriana* against *Saccharomyces lipolytica*, *Saccharomyces cerevisiae* and *Candida lipolytica*; *Alpinia mutica* against *Saccharomyces lipolytica*; *Amomum gracile* against *Saccharomyces cerevisiae*; and *Hornstedtia leonurus* against *Bacillus cereus*.

From the 29 (67%) extracts showing cytotoxic activity against CEM-SS cell line, only the methanol extract from roots of *Plumeria acutifolia* exhibited strong activity with CD_{50} a 3 μg/ml. The cytotoxicity of this extract against CEM-SS cells was stronger that the standard cytotoxic agent Cisplatin (CD_{50}: 5.5 μg/ml) but was much less potent than doxorubicin (CD_{50}: 0.1 μg/ml). Furthermore, the extracts of *Plumeria acutifolia* were only cytotoxic toward CEM-SS leukaemic cells but not HT-29 cells (CD_{50}: > 30 μg/ml; data not shown). Mild cytotoxicity (CD_{50} = 10 μg/ml) was shown by the methanol extracts from stems of *Plumeria acutifolia*, leaves of *Hedyotis verticillata*, and rhizomes of *Alpinia mutica* and *Amomom gracile*. However, 21 (49%) extracts showed low cytotoxic activity (CD_{50} = 30 μg/ml).

The strong antimicrobial and cytotoxic activities of *Plumeria acutifolia* extracts that were observed in our study complements previous reports on the antimicrobial, antimutagenic and pharmacological activities of *Plumeria acutifolia* (Muir and Hoe, 1982; Garcia and Garcia, 1988; Guevarra *et al.*, 1995). The presence of iridoids with a five-membered lactone ring at C-8, viz. plumericin and its derivatives, has been reported as being characteristic of the *Plumeria* genus (Abe *et al.*, 1988). These cyclopentanopyran terpenes show a wide range of biological activities such as antimicrobial, antitumour, antifeedant and algicidal activities and therefore, are most likely responsible for the significant biological activities of *Plumeria acutifolia* (Coppen and Cobb, 1983; Parkes and Pattenden, 1988, refs. cited therein)

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