Inhibitory Activity of Plant Extracts against Microbes Isolated from Sick Building

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ABSTRACT: Due to the harmful effects of commercial biocides, plant-based biocides may offer a safer alternative for controlling microbes that cause sick building syndrome and building deterioration. The aim of the study was to discover a new source of bioactive extracts from plants. 104 methanolic-aqueous extracts obtained from different parts of 75 plant species, were screened for potential antimicrobial activity against one species of bacteria (*Bacillus subtilis*) and four filamentous fungi (*Aspergillus flavus, Aspergillus niger*, *Penicillium oxalicum* and *Cladosporium oxysporum*) isolated from enclosed sick buildings in Penang. The extracts were obtained from different parts of the plants in 80% MeOH. Antimicrobial activity was conducted using the disk-diffusion method. In the tested concentration of 100 mg/ml, two (1.9%) of 33 (31.3%) antibacterial extracts, both antibacterial and antifungal activities were observed. Minimum inhibitory concentration was subsequently determined for plant extracts showing antimicrobial activity. Among the 104 plant materials screened, the extracts from *Samanea saman* leaves and *Garcinia mangostana* pericarp showed the most promising antibacterial properties inhibiting *B. subtilis* with MIC of 0.078 mg/mL whereas *Cinnamomum zeylanicum* bark extract was the most active against fungi with minimum inhibitory concentrations (MIC) ranging from 1.25 - 2.5 mg/mL. Results of this research would be useful in preparing a more environment and occupant-friendly plant-based formulations for microbial bioaerosols management.

Keywords: Antimicrobial activity, inhibitory, plant extracts, minimum inhibitory concentration (MIC)

Introduction

Microbes, mainly fungi and bacteria; on building materials and indoor surfaces may cause indoor air quality problems (Zhu *et al*., 2003; Peat *et al*., 1998; Dales *et al*., 1991). Various species of fungi and bacteria have been isolated from indoor damaged building materials (Hyvärinen *et al*., 2002; Andersson *et al*., 1997). These organisms were found to cause odour, defacement and deterioration to the building materials (Alwakeel, 2008; Srikanth *et al*., 2008). Many of these microorganisms were associated with allergic and irritant reactions, infectious disease respiratory problems, hypersensitivity reactions, cancer and leukaemia on the building occupants (Green and Scarpino, 2002; Sorenson, 1999; Karol, 1991; Sanchez *et al.*, 1987).

Treating these microorganisms using active ingredients such as synthetic biocides has been restricted due to their harmful effects on the environments, residue problems and their carcinogenicity (Verma *et al*., 2008). Thus, it is essential to search for ways to control and cure the harmful effects of microbes in a eco-friendly manner. Biocides derived from plants are safer, more effective and environment-friendly alternatives for microbial control because they are rich in bioactive phytochemicals

such as alkaloids, flavonoids, terpenes, coumarins and saponins.

Bioactive phytochemical substances are usually found in various parts of the plant. Several studies have reported the antibacterial, antifungal, antiviral and insecticidal activity of plant extracts (Oliveira *et al*., 2007; Banso and Ngbede, 2006; Jbilou *et al*., 2006; Okigbo and Ogbonnaya, 2006; Kloucek *et al*., 2005; Leatemia and Isman, 2004; Goun *et al*., 2003; Rajbhandari *et al*., 2001; Fabry *et al*., 1998). This has led us to screen *in vitro* a large number of plants against bacteria and fungi isolated from enclosed air-conditioned buildings in Malaysia. We aimed to screen for a new source of plant-based formulations for remediation of microbial aerosols.

Materials and methods

Collection and preparation of plant material

104 plant samples belonging to 35 families were randomly collected from different localities of Penang, Malaysia except for *Cinnamomum zeylanicum* bark, *Capsicum frutescens* fruits, *C. annum* fruits, *Etlingera elatior* flowers, and *Garcinia mangostana* fruits. The latter types of plant samples were purchased from local sundry markets. All samples were washed under running tap water for 30 minutes and dried using a circulating hot oven air at 40 °C (Okwori *et al*., 2008).

The dried plant parts were then ground to powder using an electric mill. For some plants, more than one plant parts were used. Thus, from the 75 plant species used, 104 powdered materials were obtained. The powdered materials were kept in airtight containers at 4° C until used. The botanical identification was made at the Herbarium, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. More detailed information about the plant materials are presented in **TABLE 1**.

TABLE 1: List of plant samples used

Plant species	Growth habit	Family	Plant parts used
Acacia mangium	Tree	Fabaceae	Leaves and fruits
Adenanthera pavonica	Semi-deciduous tree	Fabaceae	Leaves, wood and seeds
Alstonia angustifolia	Tree	Apocyanceae	Leaves and stem bark
Amaranthus spinosus	Herb	Amaranthaceae	Whole plant
Andira inermis	Tree	Fabaceae	Leaves and stem bark
Argyreia mollis	Climber	Convolvulaceae	Leaves
Asystasia gangetica	Herb	Acanthaceae	Whole plant
Averrhoa carambola	Tree	Oxalidaceae	Leaves and fruits
Averrhoa bilimbi	Tree	Oxalidaceae	Leaves and fruits
Avicennia alba	Tree	Acanthaceae	Leaves
Avicennia officinalis	Tree	Acanthaceae	Leaves
Azadirachta indica	Tree	Meliaceae	Leaves
Bidens pilosa	Herb	Asteraceae	Whole plant
Callistemon viminalis	Shrub or small tree	Myrtaceae	Leaves
Capsicum frutescens	Herb	Solanaceae	Fruits
Capsicum annuum	Herb	Solanaceae	Fruits
Carica papaya	Shrub or small tree	Caricaceae	Young leaves and seeds
Cassia alata	Shrub or small tree	Fabaceae	Leaves
Casuarina equisetifolia	Tree	Casuarinaceae	Leaves and fruits
Celosia argentea	Herb	Amaranthaceae	Whole plant
Cinnamomum altissimum	Shrub or small tree	Lauraceae	Leaves
Cinnamomum zeylanicum	Shrub or small tree	Lauraceae	Stem bark
Cleome viscosa	Herb	Capparaceae	Whole plant

Clitoria fairchildiana	Tree	Fabaceae	Leaves
Cordia dichotoma	Tree	Boraginaceae	Leaves and fruits
Croton hirtus	Herb	Euphorbiaceae	Aerial part
Delonix regia	Tree	Facaceae	Leaves and stem bark
Eclipta prostrata	Herb	Asteraceae	Whole plant
Eryngium foetidum	Herb	Apiaceae	Whole plant
Erythrina variegata	Tree	Fabaceae	Leaves and stem bark
Etlingera elatior	Herb	Zingiberaceae	Flowers
Eupatorium odoratum	Shrub	Asteraceae	Aerial part
Euphorbia hirta	Herb	Euphorbiaceae	Whole plant
Fragrea fragrans	Tree	Loganiaceae	Leaves
Filicium decipiens	Tree	Sapindaceae	Leaves and stem bark
Garcinia mangostana	Tree	Cluisaceae	Peel fruits
Gomphrena globosa	Herb	Amaranthaceae	Whole plant
Hyptis suaveolens	Herb	Lamiaceae	Aerial part
Ipomoea pes-caprae	Herb	Convolvulaceae	Leaves
Justicia betonica	Herb	Acanthaceae	Leaves
Lagerstroemia speciosa	Tree	Lythraceae	Leaves
Lawsonia inermis	Shrub or small tree	Lythraceae	Leaves
Lantana camara	Shrub	Verbenaceae	Aerial part
Lumnitzera racemosa	Shrub or small tree	Combertaceae	Leaves
Melaleuca alternifolia	Tree	Myrtaceae	Leaves and stem bark
Melaleuca leucadendra	Tree	Myrtaceae	Leaves
Mikania cordata	Herb	Asteraceae	Whole plant
Milletia pinnate	Tree	Fabaceae	Leaves and stem bark
Mimusops elengi	Tree	Sapotaceae	Leaves and stem bark
Morinda elliptica	Small tree	Rubiaceae	Leaves and fruits
Muraya paniculata	Shrub or small tree	Rutaceae	Leaves
Myristica fragrans	Tree	Myristicaceae	Leaves, mace, shell and kernell
Ocimum sanctum	Herb	Lamiaceae	Leaves
Oxalis corniculata	Herb	Oxalidaceae	Whole plant
Passiflora foetida	Herb	Passifloraceae	Leaves
Peltophorum pterocarpum	Tree	Fabaceae	Stem bark
Phyllanthus emblica	Tree	Euphorbiaceae	Leaves
Pluchea indica	Herb / Subshrub/ Shrub	Asteraceae	Leaves
Plumeria obtusa	Tree	Apocynaceae	Flowers, bark, leaves and wood
Polyalthia longifolia	Tree	Annonaceae	Leaves and stem bark
Portulaca oleraceae	Herb	Portulacea	Aerial part
Pterocarpus indicus	Tree	Fabaceae	Leaves and stem bark
Punica granatum	Tree or shrub	Puniaceae	Leaves
Ricinus communis	Tree, shrub, subshrub	Euphorbiaceae	Aerial part
Samanea saman	Tree	Fabaceae	Leaves and stem bark
Sonneratia ovata	Small tree	Lythraceae	Leaves
Stachytarpheta indica	Herb	Verbenaceae	Leaves
Swietenia macrophylla	Tree	Meliaceae	Leaves
Syzygium grande	Tree	Myrtaceae	Leaves and stem bark
Syzygium samarangense	Tree	Myrtaceae	Leaves
Terminalia catappa	Tree	Combretaceae	Stem bark
Tetracera indica	Shrub	Dilleniaceae	Leaves
Thespesia populnea	Tree	Malvaceae	Leaves
Triphasia trifolia	Shrub	Rutaceae	Leaves and fruits
Vitex pubescens	Tree	Verbenaceae	Leaves and stem bark

Preparation of extracts

About 30 g of each of the powdered plant materials were extracted with 80% MeOH by a Soxhlet extractor until the colour of the final drop of the extract became colourless. The extracts were filtered through Whatman (No.1) filter paper with the aid of water jet suction pressure and then concentrated using a rotary Buchi vacuum evaporator at 40 °C.

Microorganisms

Four species of fungi and one species of bacteria isolated during a series of indoor samplings in air-conditioned sick buildings (Wardah *et al*., 2011) were obtained from the culture collection unit of the Plant Pathology Laboratory, School of Biological Sciences, Universiti Sains Malaysia. The microorganisms were identified as *Bacillus subtilis* (BP1BA), *Aspergillus flavus* (AP22BA), *A. niger* (AP29BA), *Penicillium oxalicum* (PP1BA) and *Cladosporium oxysporum* (CPIBA) and kept in 15% glycerol at -86°C (Salleh and Sulaiman, 1984). The work cultures of bacteria and fungi were maintained at 4°C on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) slants, respectively.

Bacterial suspension preparation

A pure culture of the bacterium from agar slant was streaked on the surface of NA and incubated at air-conditioned room temperature (28 ± 2°C) for 48 h. The test bacterium was transferred to a test tube containing 5 ml of sterile distilled water until the turbidity reached 0.5 McFarland (10^8 CFU/mL) standards.

Spore suspension preparation

Pure cultures of the fungal strains from agar slants were sub-cultured onto PDA and incubated under the same condition as for bacterium for 5 - 10 days. The Petri dishes were flooded with 8-10 mL of sterile distilled water and the spores were scraped using a sterile spatula (Mahesh and Satish, 2008). Then, the spore suspensions were adjusted to a final concentration of 1×10^4 spore/ ml using a haemocytometer.

Antimicrobial activity test

Plant extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 100 mg/ml and then sterilized by filtering through a Millipore 0.2 µm filters. Antimicrobial tests were then carried out by disc diffusion method (Murray *et al*., 1995) with a slight modification in the volume and in the concentration of the extracts. About 0.1 mL of suspension containing 10^8 CFU/ml of bacterium and 10^4 spore /mL of fungi were evenly spread using sterilized cotton swab or L-shaped glass spreader on NA and PDA plates, respectively. The agar plates were then allowed to dry in a laminar flow hood.

Sterilized (autoclave at 121°C for 15 minutes) Whatman AA discs (6 mm in diameter) were placed on the plates and 20 µL of each extract at 100 mg/mL concentration was pipetted aseptically onto the discs. The quantity of each extract was 2 mg/disc. Discs prepared with only the corresponding volume of DMSO were used as a negative control. The discs were left to dry at room temperature before they were placed on the surface of the inoculated agar.

The inoculated plates were incubated at room temperature for 48 h for the bacterium and 3-5 days for fungi. Each extract was tested against each organism in triplicates. The cultures were examined for areas of no growth around the disc (zone of inhibition). The microorganisms that were susceptible to antimicrobial agents were inhibited at a distance from the disc whereas the resistant strains grew

up to the edge of disc. Measurement of the inhibition zones around the discs were done using rulers and expressed in millimeter (mm) unit. Based on the antimicrobial sensitivity test results, extracts that produced an inhibition zone greater than 6 mm in the disc diffusion test were separated and further examined for the minimum inhibitory concentration (MIC) values.

Minimum inhibitory concentration (MIC) test of crude extracts

Serial dilutions of each extract were prepared by reconstituting the extract in 10 % dimethylsulfoxide (10 mL of DMSO in 90 ml of sterile distilled water), followed by diluting it in sterile distilled water to reach the concentrations of 100, 75, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390 mg/mL. For *S. saman* leaves extract, it was dissolved either in 10 % DMSO or sterilized distilled water. The MIC values of the crude extracts were determined by the agar dilution method (EUCAST Definitive Document, 2000; Mukherjee, 2002) with a slight modification in the concentration of the extracts and volume of microbial suspension.

The growth media i.e. NA for bacterium and PDA for fungi were prepared and then allowed to cool to 50°C. About 18 ml of the molten agar was added to test tubes which contained 2 ml of different concentrations of the crude extracts and the control (10 % DMSO or distilled water in case of *S. saman* leaves extract), thoroughly mixed and poured into pre-labelled sterile Petri dishes on a level surface. The final concentrations of the tested samples were 10, 7.5, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039 mg/mL respectively. For the control, the final concentration was 1 %. Additional Petri dishes containing only the growth media were prepared in the same way to compare the growth of the respective organisms.

The plates were dried at an air-conditioned room (28 ± 2°C). Exactly 20 µL of each suspension was applied and spread onto the surface of the agar plates. The plates were incubated at the air-conditioned room temperature for 48 h for the bacterium and for 72 h for the fungi. The lowest concentration of an antimicrobial agent at which there was no visible growth of a microorganism after the incubation was taken as MIC (Banso and Adeyemo, 2006; Falahati *et al*., 2005; EUCAST Definitive Document, 2000). All the tests were carried out in triplicates.

Statistic Analysis

The data were expressed as the mean ± standard deviation. The differences between the means of groups were separated by Duncan's Multiple Comparison Test. Significance level of 0.05 was applied. The statistical analysis was evaluated by one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) for windows.

Results

The results of the antibacterial and antifungal activity screening of 104 extracts from different organs of the 75 investigated plants were fabulated in **TABLE 2** and **TABLE 3**. More plant extracts were active against bacterium than against fungi. Of 33 (31.3 %) antibacterial extracts, only two (1.9 %) extracts were active against fungi.

Extract of *C. zeylanicum* showed activity against all of the five test organisms. The active extracts showed antimicrobial activity with the diameters of inhibition zone ranging from (7.00 ± 0.00 mm) to (24.6 ± 0.57 mm). There were significant

differences in the antimicrobial activities of plant extracts.

The maximum zone of inhibition (24.6 ± 0.57 mm) against *B. subtilis* was given by *S. saman* (leaves) extract and the minimum zone of inhibition (7.00 ± 0.00 mm) against the similar bacterium was shown by *Averrhoa bilimbi* (fruit), *Etlingera elatior*, (flower) and *Myristica fragrans* (mace) extracts. Of the fungi tested, *P. oxalicum* was the most susceptible one against the *S. Saman* (leaves) extract with an inhibition zone of 10.67 ± 0.57 mm; whereas *C. Zeylanicum* (bark) extract showed an equal activity against *P. oxalicum* and *A. flavus*. *A. niger* exhibited the lowest degree of sensitivity towards both extracts. The results clearly indicated that the antimicrobial activity varied with species of the plants and plant parts used.

TABLE 2: Diameter of inhibition zones of the effective crude extracts from plants against *Bacillus subtilis*

Plant species	Plant part used	Diameter of inhibition zone (mm) *
		Bacillus subtilis
Acacia mangium	LF	8.00 ± 0.00^{bc}
	FR	10.67 ± 0.57^{ghi}
Alstonia angustifolia	LF	9.33 ± 0.57^{def}
Averrhoa bilimbi	FR	7.00 ± 0.00^{a}
Callistemon viminalis	LF	14.67 ± 0.57^{m}
Casuarina equisetifolia	LF	8.00 ± 0.00^{bc}
	FR	10.00 ± 0.00^{fgh}
Cinnamomum altissimum	LF	8.33 ± 0.57^{c}
Cinnamomum zeylanicum	SB	8.00 ± 0.00^{bc}
Delonix regia	SB	12.67 ± 0.57^{k}
Etlingera elatior	FL	7.00 ± 0.00^{a}
Flicium decipiens	LF	9.67 ± 0.57^{efg}
	SB	10.67 ± 0.57^{ghi}
Garcinia mangostana	PF	14.33 ± 1.15^{lm}
Lawsonia inermis	LF	11.67 ± 1.15^{j}
Melaleuca leucadendra	LF	9.00 ± 0.00^{cde}
Mimusops elengi	LF	10.67 ± 0.57^{ghi}
	SB	11.67 ± 0.57^{J}
Myristica fragrans	MA	7.00 ± 0.00^{a}
	SH	11.33 ± 0.57^{ij}
Peltophorum pterocarpum	SB	13.67 ± 0.57^{l}
Phyllanthus emblica	LF	8.67 ± 0.57^{cd}
Polyalthia longifolia	SB	7.33 ± 0.57^{ab}
Punica granatum	LF	10.00 ± 0.00^{fgh}
Samanea saman	LF	24.67 ± 0.57^{p}
	SB	19.67 ± 0.57^{o}
Swietenia macrophylla	LF	10.00 ± 0.00^{fgh}
Syzygium grande	LF	8.67 ± 0.57^{cd}
	SB	17.33 ± 0.57^{n}
Syzygium samarangense	LF	9.67 ± 0.57^{efg}
Terminalia catappa	SB	11.00 ± 0.00^{hij}
Tetracera indica	LF	10.33 ± 0.57^{gh}
Thespesia populnea	LF	8.00 ± 0.57^{c}

Annotation:* Inhibition zone including the diameter of disc paper (6 mm); - refers to no inhibition zone.
Values are mean inhibition (mm) ± S.D. of three replicates.
Values followed by different letters are significantly different at $P < 0.05$ levels.
Note: control (DMSO) did not show any activity.
LF: leaf; SB: Stem bark; FR: Fruit; FL: Flower; PF: Pericarp fruit; MA: Mace; SH: Shell.

TABLE 3: Diameter of inhibition zones of the effective crude extracts from plants against fungi

Plant species	Plant Part used	Diameter of inhibition zone(mm) *			
		Aspergillus flavus	***Aspergillus niger***	***Cladosporium oxysporum***	***Penicillium oxalicum***
Cinnamomum zeylanicum	SB	10.33 ± 0.57^{c}	8.67 ± 0.57^{a}	10.0 ± 0.00^{bc}	10.33 ± 0.57^{c}
Samanea saman	LF	-	8.67 ± 1.15^{a}	9.00 ± 0.00^{ab}	10.67 ± 0.57^{c}

*Annotation:*Inhibition zone including the diameter of disc paper (6mm); - refers to no inhibition zone.*
Values are mean inhibition (mm) ± S.D. of three replicates.
Values followed by different letters are significantly different at P< 0.05 levels.
LF: leaf; SB: Stem bark

Antimicrobial effects, expressed as MICs, of active crude extracts against the tested microorganisms are listed in **TABLE 4** and **TABLE 5**.

The strongest activity (MIC 0.078 mg/mL) was observed in the extracts of *S. saman* (leaves) and *Garcinia mangostana* (pericarp) followed by *Callistemon viminalis* (leaf), *Peltophorum pterocarpum* (bark), *S. saman* (bark) and *Syzygium grande* (bark), which inhibited *B. subtilis* with MIC of 0.312 mg/mL. Extract of *Cinnamomum altissimum* (leaves), *Etlingera elatior* (flowers), *Myristica fragrans* (mace), *Polyalthia longifolia* (bark) and *Thespesia populnea* (leaves) were the least active, showing an MIC of 10 mg/mL. All fungi were susceptible to the extract of *C. zeylanicum* (bark) (MIC 1.25 - 2.5 mg/mL) while *S. saman* (leaves) extract was active against *C. oxysporum*, *P. oxalicum*, and *A. niger* with MICs of 5, 5, 7.5 mg/mL, respectively.

TABLE 4: Minimum inhibitory concentration of active plant extracts against *Bacillus subtilis*

Plant species	Plant part used	MIC(mg/ml)*
		Bacillus subtilis
Acacia mangium	LF	7.5
	FR	2.5
Alstonia angustifolia	LF	7.5
Averrhoa bilimbi	FR	5.0
Callistemon viminalis	LF	0.312
Casuarina equisetifolia	LF	7.5
	FR	5.0
Cinnamomum altissimum	LF	10
Cinnamomum zeylanicum	SB	7.5
Delonix regia	SB	2.5
Etlingera elatior	FL	10.0
Filicium decipiens	LF	2.5

	SB	2.5
Garcinia mangostana	PF	0.078
Lawsonia inermis	LF	2.5
Melaleuca leucadendra	LF	5.0
Mimusops elengi	LF	1.25
	SB	1.25
Myristica fragrans	MA	10.0
	SH	5.0
Peltophorum pterocarpum	SB	0.312
Phyllanthus emblica	LF	5.0
Polyalthia longifolia	SP	10.0
Punica granatum	LF	5.0
Samanea saman	LF	0.078
	SB	0.312
Swietenia macrophylla	LF	2.5
Syzygium grande	LF	2.5
	SB	0.312
Syzygium samarangense	LF	2.5
Terminalia catappa	SB	1.25
Tetracera indica	LF	1.25
Thespesia populnea	LF	10.0

*Annotation:*Refers to minimum inhibitory concentration of active plants extracts*
Note: control (1%DMSO) did not show any activity.
LF: leaf; SB: Stem bark; FR: Fruit; FL: Flower; PF: Pericarp fruit; MA: Mace; SH: Shell.

TABLE 5: Minimum inhibitory concentration of active plants against fungi

Plant species	**Plant Part used**	**Diameter of inhibition zone(mm) ***			
		Aspergillus flavus	***Aspergillus niger***	***Cladosporium oxysporum***	***Penicillium oxalicum***
Cinnamomum zeylanicum	SB	1.25	2.5	1.25	1.25
Samanea saman	LF	-	7.5	5	5

Annotation: Minimum inhibitory concentration of active plants extracts*
Note: control (1% DMSO) did not show any activity.
LF: leaf; SB: Stem bark

Discussion

Much attention has recently been directed toward plant extracts that would be of great use for the improvement of air quality. Plants appear to be more acceptable and less hazardous than synthetic compounds. In the present investigation, the methanolic extract of 104 plant materials were screened for their antimicrobial activity against the more common microorganisms in the indoor environment. Of 33 antibacterial extracts, two of them were found active against fungi. Motiejunaite and Peciulyte (2004) reported the antimicrobial activity of *Pinus sylvestris* oil against airborne microorganisms (8 fungi, 2 yeast like fungi, yeast and bacteria) isolated from the human environment. Huang *et al.* (2010) also used *Melaleuca alternifolia* as disinfecting media for inactivation of

common environment fungal spores on HVAC filters who found that 50% and 40% of *A. niger* and *Rhizopus stolonifer* spores, respectively were inactivated over a period of 60 minutes.

In this study the active crude extracts showed varying levels of activity against the test organisms. Similar observations were made by Joshi *et al*. (2009) and Alanis *et al*. (2005). Parekh and Chanda (2007) reported that the variation of antimicrobial activity among different crude extracts may be attributed to the phytochemical properties and differences among species.

Antimicrobial activity of *G. mangostana* has been reported (Chomnawang *et al.*, 2005; Voravuthikunchai and Kitpipit, 2005; Vishnu Priya *et al.*, 2010). Xanthones and its derivatives isolated from its pericarp were active against a variety of microorganisms including *B. subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Fusarium oxysporum* f. sp. *vasinfectum, Alternaria tenius, Drechslera oryaze, Candida albicans* and *Mycobacterium tuberculosis* (Sundaram *et al.*, 1983; Gopalakrishnan *et al.*, 1997; Suksamrarn *et al.*, 2003; Kaomongkolit *et al*., 2009).A crude extract of *S. saman* leaves collected in India possessed antimicrobial activity against *P. aeruginosa, S. paratyphi* A, *S. paratyphi* B, *S. typhi, S. typhimurium, Shigella boydii, S. flexneri, S. sonnei, Escherichia coli, Streptococcus faecalis, Citrobacter* sp., *Klebsiella* sp., *S. aureus* and *C. albicans* (Prasad *et al.*, 2008; Raghavendra *et al.*, 2008). Out of the different fractions obtained from the methanolic extract of *S. saman* leaves, only alkaloid fraction showed *in vitro* activity against three phytopathogenic *Xanthomonas* pathovars and 14 human pathogenic bacteria (Raghavendra *et al*., 2008). Based on the present results as well as the above-mentioned results, *S. saman* leaves extract was selected for detailed studies and so far three antifungal and antibacterial constituents have been isolated, two pithecolobines and one new quaternary alkaloid (Ajam, 2011).

Several studies reported the inhibitory activity of *C. zeylanicum* bark extract against *Alternaria solani, Curvularia lunata, Streptococcus mutans, S. aureus, C. albicans, Saccharomyces cerevisiae* and *B. subtilis* (Gupta *et al*., 2008; Aneja *et al*., 2009; Mishra *et al*., 2009). The major constituents possessing antimicrobial activity in its bark were found to be *cinnamaldehyde* and *eugenol* (Joshi *et al*., 2009; Mishra *et al*., 2009). In addition, other compounds with fungicidal property were reported to be present in its bark and leaves (Montes and Carvajal, 1998; Delespaul *et al*., 2000; Ranasinghe *et al*., 2002).

Comparing with reported literatures other effective extracts may look promising for detailed studies. Bioassay-guided research may yield new and more potent compounds in these plants. It is estimated, that if inhibition is obtained by 1-10 mg/ml, it needs further investigation (Valsaraj *et al*., 1997). It should be emphasised that the investigated plants with no antimicrobial activity should not be considered as completely inactive. It is possible that the active chemical constituents were not soluble in methanol and water (Krishnaraju *et al*., 2005). Therefore, further detailed studies need to be carried out by extracting these samples with other solvents and evaluation of their potential effectiveness as the antimicrobial agents.

Conclusion

In summary, the present investigation together with previous results are an important step in developing plant-based microbial biocides which are eco-friendly

in managing airborne aerosols and subsequently to be developed commercially.

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