ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS AND ISOLATION AND CHARACTERIZATION OF ALKALOIDS FROM *Samanea saman* (Jacq.) Merr. LEAVES

By

SUNDIS MAHDI SAHIB AJAM

Thesis submitted in fulfillment of

requirements for the degree

of Doctor Philosophy

June 2011

DEDICATION

To my late father …….

ACKNOWLEDGEMENTS

In the name of God, Most Gracious, Most Merciful

First of all, I would like to thank Almighty Allah for giving me patience and determination to complete this doctoral study.

I would like to thank my supervisor Professor Baharuddin Salleh for his guidance, time, moral support, and valuable feedback throughout my research and preparation of this thesis. I definitely learned a lot from him while conducting this research. Thanks a lot, Professor Baharuddin!

The present work would have never been accomplished without the inspiration, cooperation, keen and continued interest of my co-supervisor Professor Suliman Al-Khalil from the Faculty of Pharmacy, University of Jordan, Amman, to whom my humble thanks are due.

I would like to express my gratitude to my other co-supervisor, Associate Professor Shaida Fariza Sulaiman for her comments on my research.

I warmly thank Puan Siti Nurdijati for her valuable advice and friendly help.

My thanks also go to the academic editor Dr. Ghayth al-Shaibani for his editing and valuable comments on my dissertation.

I would also like to thank everybody in our laboratory at the School of Biological Sciences, Universiti Sains Malaysia (USM) for being friendly and helpful all the time. My thanks also go to Mr. Senan Al-Hakeem for his help and time in typing format to certain parts of the final draft, to the laboratory assistant at USM Mr. Kamurdin Mohd Maidin, and also to USM library staff members for their support and time. I truly thank Mr. Hayder Al-Salman, my Ph.D. colleague for his help in statistical analysis.

Of course, I would like to express here my sincere appreciation and thanks to the entire technical and non-technical staff at Faculty of Pharmacy, University of Jordan, especially the laboratory assistant Mr. Ismail Abaza who greatly helped me during my practical work over there.

I also wish to express never ending thanks to my friend Miss Estabraq al-Qaissi at the Education College, Baghdad University, Ibn al-Haytham for her help, love and trust.

I am indebted to my lovely mother for her continuous prayers and moral support. I would like to especially thank my children for their love, support and understanding in listening to my complaints all the time. My beloved children, I really appreciate it.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µg	Microgram (10^{-3} gram)
µl	Microliter(10^{-3} ml)
µm	Micrometer (10^{-3} mm)
α	Alpha
β	Beta
ϑ	Gama
%	Percentage
δ	Chemical shift in ppm
˚C	Degree centigrade
^{13}C-NMR	Carbon NMR
^{1}H-NMR	Proton NMR
ANOVA	Analysis of variance
$Bi(NO_2)_3$	Bismuth nitrate
BRI	Building related illness
CFU	Colony forming unit
$CHCl_3$	Chloroform
cm	Centimeter
Cosy	Correlated spectroscopy
Dept	Distortionless Enhancement by polarization transfer
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

e.g.	Example
EPA	Environmental Protection Agency
EtOAc	Ethyl acetate
$FeCl_3$	Ferric chloride
g	Gram
GC-MS	Gas Chromatography–Mass spectrometry
h	Hour
H_2O	Water
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
$HgCl_2$	Mercury chloride
HVAC	Heat, ventilation and air conditioning
Hz	Hertz
IAQ	Indoor air quality
IR	Infrared Spectrum
J	Coupling constant in Hz
kg	Kilogram
KBr	Potassium bromide
KI	Potassium iodide
KOH	Potassium hydroxide
m	Multiplet
m	Meter

MeOH	Methanol
MIC	Minimum Inhibitory Concentration
ml	Milliliter
mm	Millimeter
mp	Melting point
mg	Milligram
MS	Mass Spectrometry
NA	Nutrient Agar
Na_2SO_4	Anhydrous sodium sulfate
NH_4OH	Ammonium hydroxide
nm	Nanometer
NMR	Nuclear Magnetic resonance
PDA	Potato Dextrose Agar
pH	Hydrogen number
ppm	Part per million
R_f	Retention factor
RNA	Ribonucleic acid
s	Singlet
S.D	Standard deviation
SBS	Sick Building Syndrome
spp.	Species
SPSS	Statistical package for social science
TLC	Thin Layer Chromatography

US	United States
UV	Ultraviolet light
v/v	Volume/Volume
Vmax	Wave length maxima in cm^{-1}
VOCs	Volatile organic compounds
WHO	World Health Organization
ZOI	Zone of inhibition

Aktiviti antimikrob ekstrak tumbuhan dan pemencilan serta pencirian alkaloid daripada daun *Samanea saman* (Jacq.) Merr.

ABSTRAK

Akibat daripada kesan-kesan yang berbahaya terhadap penghuni dan persekitaran, keracunan residu, dan sifat karsinogen daripada biosid tiruan, maka biosid berasal daripada tumbuhan mungkin merupakan alternatif yang lebih selamat untuk digunakan dalam pengawalan bakteria dan kulat bioaerosol yang menyebabkan sindrom bangunan sakit dan kerosakan bangunan. Oleh itu, kajian ini bertujuan untuk menyaring flora tempatan terhadap bakteria and kulat yang telah dipencilkan daripada bangunan tertutup yang sakit, pengecaman tumbuhan yang mempunyai aktiviti antimikrob yang tinggi, melakukan penfraksian serta mengenalpasti sebatian dengan aktiviti antimikrob tertinggi daripada ekstrak methanol (80% MeOH) daun *Samanea saman*.

Pada dasarnya kajian ini dibahagikan kepada dua bahagian. Bahagian 1 melibatkan penyaringan 104 ekstrak daripada 75 spesies tumbuhan yang diambil di sekitar negeri Pulau Pinang. Bahagian II menumpukan kepada pemencilan dan pemencilan alkaloid daun *S. saman.* Ekstrak diperolehi daripada bahagian tumbuhan yang berbeza dalam 80% MeOH. Aktiviti antimikrob dilakukan menggunakan kaedah cakera-penyerapan terhadap satu spesies bakteria (*Bacillus subtilis*) dan empat kulat berfilamen (*Aspergillus flavus, A. niger, Penicillium oxalicum,* dan *Cladosporium oxysporum*) yang dipencilkan daripada bangunan tertutup yang sakit di Pulau Pinang. Dalam pengujian menggunakan kepekatan 100 mg/ml, 33 (31.3%) ekstrak tumbuhan menunjukkan aktiviti antibakteria dan 2 (1.9%) daripada ekstrak tersebut menunjukkan aktiviti antikulat. Kepekatan perencatan minimum (MIC)

ditentukan terhadap ekstrak tumbuhan yang menunjukkan aktiviti antimikrob. Antara 104 bahagian tumbuhan yang disaring, ekstrak methanol daun *S. saman* dan perikarp *Garcinia mangostana* menunjukkan sifat antibakteria, iaitu merencatkan pertumbuhan *B. subtilis* dengan nilai MIC 0.078 mg/ml, manakala ekstrak kulit *Cinnamomum zeylanicum* pula paling aktif terhadap kulat dengan MIC dalam julat 1.25 - 2.5 mg/ml.

Ekstrak daun *S. saman* telah dipisahkan menjadi fraksi alkaloid dan bukan alkaloid dan seterusnya diikuti dengan penentuan aktiviti antimikrob. Fraksi alkaloid menunjukkan spektrum aktiviti yang luas terhadap bacteria dan kulat yang diuji. Teknik kromatografi yang dilakukan berulang kali telah berjaya memencil dan mencirikan tiga alkaloid iaitu pitekolobina A ($C_{22}H_{46}N_4O_2$) dan pitekolobina B ($C_{22}H_{46}N_4O$), serta satu alkaloid kuarterner C ($C_{22}H_{48}N_4O_2$). Alkaloid C dilaporkan di sini untuk pertama kali yang berkemungkinan sebagai satu sebatian novel dan secara tentatif dinamakan sebagai samosina. Ketiga-tiga alkaloid tersebut dicamkan menggunakan kaedah spektroskopi dan membandingkannya dengan rujukan yang pernah diterbitkan. Alkaloid A dan C menunjukkan aktiviti antimikrob yang signifikan secara *in vitro* terhadap bakteria dan kulat. Nilai MIC sebatian-sebatian tersebut terhadap *B. subtilis* dan *P. oxalicum* adalah dalam julat 0.019 hingga 0.625 mg/ml, manakala alkaloid B menunjukkan kesan perencatan hanya terhadap *B. subtilis* dengan nilai MIC 0.312 mg/ml. Diharap keputusan-keputusan yang diperolehi dalam penyelidikan ini dapat memberi manfaat dalam penyediaan sebatian berasal daripada tumbuhan yang lebih mesra penghuni dan alam sekitar bagi mengawal mikrob bioaerosol yang menyebabkan sindrom bangunan sakit dan kerosakan bangunan.

Antimicrobial activity of plant extracts and isolation and characterization of alkaloids from *Samanea saman* (Jacq.) Merr. leaves

ABSTRACT

Due to the harmful effects on the occupants and environment, residual toxicity, and carcinogenic nature of synthetic biocides, plant-based biocides may offer safer alternatives for controlling bacterial and fungal bioaerosols that cause sick building syndromes and building deterioration. Therefore, the purpose of this study was to screen local flora for antimicrobial activity against bacteria and fungi isolated from sick enclosed buildings, fractionate and identify the compounds with the highest antimicrobial activities from the methanolic (80% MeOH) extract of *Samanea saman* leaves.

Basically, the present study was divided into two parts. Part I involved the screening of 104 plants extracts obtained from 75 different plant species collected around the State of Penang. Part II concentrated on the isolation and characterization of alkaloids of the leaves of *S. saman.* The extracts were obtained from different parts of the plants in 80% MeOH. Antimicrobial activity was conducted by using the disk-diffusion method against one species of bacteria (*Bacillus subtilis*) and four filamentous fungi (*Aspergillus flavus*, *A. niger*, *Penicillium oxalicum,* and *Cladosporium oxysporum*) isolated from sick enclosed buildings in Penang Island. In the tested concentration of 100 mg/ml, 33 (31.3%) plant extracts showed antibacterial and 2 (1.9%) of them exhibited antifungal activities. Minimum inhibitory concentration (MIC) was determined for plant extracts that recorded antimicrobial activities. Among 104 plant materials screened, the methanolic

extracts of *S. saman* leaves and *Garcinia mangostana* pericarp showed the most promising antibacterial activity inhibiting *B. subtilis* with an MIC of 0.078 mg/ml, whereas *Cinnamomum zeylanicum* bark extract was the most active against fungi with MICs values in the range of from 1.25 - 2.5 mg/ml.

The *S. saman* leaves extract was fractionated into alkaloid and non-alkaloid fractions and the antimicrobial activity was further determined. The alkaloid fractions showed a broad spectrum of activities against bacteria and fungi tested. Extensive chromatography resulted in the isolation and characterization of three alkaloids identified as pithecolobine A ($C_{22}H_{46}N_4O_2$) and pithecolobine B ($C_{22}H_{46}N_4O$), and one quaternary alkaloid C ($C_{22}H_{48}N_4O_2$). Alkaloid C was reported here for the first time as a probably novel compound and thus was tentatively given a new trivial name, samosine. These three alkaloids were identified on the basis of spectroscopic methods and by a comparison with those reported in the literatures. Alkaloids A and C exhibited a significant *in vitro* antimicrobial activity against both bacteria and fungi. The MIC values against *B. subtilis* and *P. oxalicum* were in the range of 0.019 - 0.625 mg/ml, whereas alkaloid B had an inhibitory effect only against *B. subtilis* with MIC value of 0.312 mg/ml. Results of this research would hopefully be useful in preparing a more environment and occupant-friendly plant-based compounds to control microbial bioaerosols causing sick building syndromes and building deterioration.

CHAPTER 1

INTRODUCTION

Contamination of indoor air by microbial pollutants had been increasingly recognized as a public health problem, and may be responsible for building-related illness (BRI) and sick-building syndrome (SBS) (Green and Scarpino, 2002). Numerous investigations have shown the association of various species of bacteria and fungi with indoor air quality problems and sick building syndromes (Shahamat *et a*l., 1997; Cooley *et al.,* 1998; Obbard and Fang, 2003; Nielsen, 2003; Chapman, 2006; Straus, 2009).

Deterioration of materials, offensive odour and adverse health effects are associated with microbial contamination of indoor environments (Srikanth *et al*., 2008). Bioaerosols such as indoor fungi, bacteria and viruses can cause allergic and irritant responses, infectious diseases, respiratory problems, hypersensitivity reactions, kidney failure and cancer (Sorenson, 1999; Green and Scarpino, 2002).

Control of microorganisms in the indoor environments has traditionally focused on source control, ventilation, and air cleaning. Although commercially available disinfecting compounds have long been used as active agents, however they are costly, and have potentially harmful effects on the environment. These disinfecting compounds are toxic and they interfere with the vital body processes by destroying enzymes, blocking oxidation-reduction processes, restricting the functions of various organs and initiating cellular changes and mutations (American Air and Mold Specialists, 2010).

Verma *et al*. (2008) reported that treatment of decay in buildings by synthetic fungicides such as pentachlorophenol, tributyltin oxide, zinc carboxylate and boron esters has now been restricted due to their residual toxicity and carcinogenic nature. Moreover, the constant use of chemicals may induce resistance in a target organism (Okigbo and Ogbonnaya, 2006).

1.1 Problem statement

The search for ways of preventing and curing the harmful effects of fungal and bacterial bioaerosols in enclosed buildings has created a high demand for providing an efficient method to control them in eco-friendly manner. Biodegradable and environment friendly natural products may offer a safer alternative for microbial management without negative impacts of chemical biocides control (Okigbo and Ogbonnaya, 2006).

Plants represent a reservoir of antimicrobial agents from nature and many of which remain highly effective in fighting microbial infections (Doughari and Obidah, 2008). Several studies reported the antimicrobial activity of various herbs and spices in plant leaves, flowers, stems, seeds, roots and fruits (Obafemi *et al*., 2006; Rani and Murty, 2006; Guneshwor *et al*., 2007; Moorthy *et al*., 2007; Okowri *et al*., 2008; Lachumy *et al*., 2010; Radhika and Lakshmi, 2010). There are more than 35,000 plant species being used in various human cultures around the world for medicinal purposes (Philip *et al*., 2009).

Malaysia is blessed with an abundant and diverse flora, much of which is believed to possess medicinal values. It is among the world's top 12 biodiversity rich countries (Handa *et al*., 2006). In peninsular Malaysia, 12,000 species of higher plants and 2,000 species in Sabah and Sarawak are reported to have medicinal values

and have been used for generating various traditional health care systems (Jantan, 1998). Due to the vast potentiality of plants as a source of antimicrobial agents, this research was undertaken to screen the local flora for antibacterial and antifungal activities to develop new herbal biocides.

Generally,The present study is designed to obtain preliminary information on the antimicrobial activity of 104 crude extracts of plant parts of 75 plant species belong to 35 families in Malaysia against one species of bacteria (*Bacillus subtilis)* and four species of fungi (*Aspergillus niger*, *Aspergillus flavus*, *Cladosporium oxysporum* and *Penicillium oxalicum*). The fungal and bacterial isolates were collected to represent indoor air sampling in enclosed air-conditioned buildings in Penang state of Malaysia.

1.2 Research objectives

This research aimed to achieve the following objectives:

i. To screen the local flora for antimicrobial activity against bacteria and fungi isolated from enclosed air-conditioned buildings in Penang state of Malaysia.

ii. To purify the compounds from the crude 80% MeOH leaves extract of *Samanea saman* based on the bioactivity guided fractionation, and

iv. To characterize the compounds from*Samanea saman* leaves that showed antimicrobial activity.

CHAPTER 2

LITERATURE REVIEW

2.1 Sick–Building Syndrome

2.1.1 Definition and History

Buildings have been suffering from air related problems for centuries (Spengler and Sexton, 1983; Hodgson, 1992). World Health Organization (WHO) has estimated that 1/3 of buildings worldwide are suffering from indoor air pollution. According to WHO, indoor air quality related issues rank among the top five global health concerns (www.air-conditioning-syndrome.com/pages/science .phd).

The term sick building syndrome (SBS), which generally applies to symptoms resulting from problems with indoor air quality (IAQ), was first known in 1982 as a serious problem affecting people in certain buildings (Cooley *et al*., 1998). The first official study of SBS that examined more than one building was published in 1981 (Finnegan *et al*., 1984). Poor IAQ can have a negative impact on health and can be classified as an acute or chronic disease such as asthma, respiratory infections, allergic rhinoconjunctivities, lung cancer and pulmonary tuberculosis. Such health impacts are referred to as Sick Building Syndrome (SBS) and Building-Related Illnesses (BRI) (Ambu *et al.,* 2008).

The Environmental Protection Agency (EPA) defined SBS as a "situation in which building occupants experience discomfort and acute health effects that appear to be linked to time spent in building". SBS symptoms are complaints of discomfort, e.g. headache, eye, nose or throat irritation; dry cough; dry or itchy skin; dizziness and nausea; fatigue; and sensitivity to odors. Most of the affected people get relieved after leaving the polluted building. Less common than SBS is

BRI. This term is used to describe illness that can be identified and attributed directly to building air contaminants. Complaints common to BRI include cough, chest tightness, fever; chill and muscle aches. Occupants may need prolonged recovery times after leaving the building (http://www.epa.gov/iaq/pubs/sbs.html).

It was estimated that 10 - 25 million workers in 200,000 to 1.2 million commercial buildings in the United States demonstrated symptoms associated with SBS annually (Woods, 1989; Burrell, 1991). The resultant impact on health can have significant influence on the economic life of a country and thus increasing health care costs because productivity will be less and absenteeism will increase (Shahamat *et al*., 1997). It has been estimated that in the United States, BRI symptoms have a relationship with work performance which decreased (3 - 5%) in an affected population resulting in an annual loss of US$ 60 billion in revenue (Ambu *et al*., 2008).

2.1.2 Causes of Sick–Building Syndrome

The Environmental Protection Agency (EPA) listed the following key contributing factors to sick building syndrome:

a - Inadequate ventilation

b - Pollutants emitted inside of building

c - Chemical contaminants from outside sources

d - Biological contaminants

These factors interact with other environmental considerations such as temperature, humidity or poor lighting (Baechler, 1991). The discussion below will concentrate on the biological contaminants and their impact on IAQ.

2.1.2.1 Biological contaminants

Microbial contamination of indoor environment is associated with adverse health effects in human and may be responsible for SBS and BRI (Molina *et al.*, 1989; Hiipakka and Buffington, 2000; Laumbach and Kipen, 2005; Srikanth *et al.*, 2008; Aibinu *et al.*, 2009; Straus, 2009). In the 1970′s and 1980′s, microbial contamination was identified as the primary cause for poor air quality in 5% of more than 500 indoor air quality (IAQ) investigations conducted by the National Institute for Occupational Safety and Health (http://www.osha.gov/dts /osta/otm/otm_iii/otm_iii_2.html). In the last decades, the microorganisms were found to be the primary source of indoor air contamination in as many as 35 - 50% of IAQ cases (Bas, 2004).

Microorganisms or their products implicated in IAQ problems include bacteria, Gram-negative bacterial endotoxins, fungi, fungal volatile organic compounds (VOCs), mycotoxins, viruses, protozoans, algae, actinomycetes, and parasites such as dust mites. Exposure to microbial bioaerosol such as fungi, bacteria, and viruses in indoor environment can cause allergic and irritant responses, infectious diseases, respiratory problems, and hypersensitivity reactions (Green and Scarpino, 2002). Air contains a significant number of microorganisms which act as a medium for their transmission or dispersal. Inhalation, which is predominant, ingestion and dermal contact are the routes of human exposure to air borne microorganisms (Srikanth *et al*., 2008).

A part of poor IAQ effects on human, a large number of microbial aerosols has been found to be responsible for the offensive odor, defacement and deterioration of organic material in indoor environment, especially when a microclimatic condition favors their development (Aira *et al.*, 2007; Alwakeel,

2008). Many bacterial and fungal species are isolated from indoor air with the species found dependent on nutrient source, water availability and temperature (http://www.Ie.ac.uk/ieh/). Sources of microbial content of indoor air includes furnishings and building materials, fungal contamination within walls, ceilings and floor cavities by the movement of the cells, spores and cell fragments via wall openings and gaps in structural joints (Srikanth *et al.,* 2008). The discussion below will focus on the effects of fungi and bacteria as these tend to be implicated in major health concerns more readily than other microbes.

2.1.2.2 Fungi and sick building syndrome

Fungi are eukaryotic, heterotrophic, unicellular yeast or multicellular filamentous microorganisms classified in their own kingdom. They reproduce sexually and asexually, producing spores. Fungal spores vary in shape, size and other properties related to their various roles in dispersal or dormant survival. In contrast to plants, fungi are characterized by the absence of chlorophyll. They use their enzymes to absorb simpler soluble nutrients from preformed organic compounds through the wall and cell membrane (Deacon, 1997). They are highly diverse and versatile organisms that can adapt to all kinds of environment.

Fungi are ubiquitous, found on earth, in water, in moist soil, in compost or in decomposing organic matter as saprophytes. They are also present in plants and animals as parasites or symbionts and play an essential part in the economy of nature (Mehrotra and Aneja, 1990). The fungi together with the bacteria are responsible for decay of organic matter. Fungi, as a group, contain species of mushrooms, toadstools, yeasts, molds, mildews, smuts and rusts. Molds are the most common fungi found in indoor environment (McNeel and Kreutzer, 1996).

Fungal growth in buildings has been a problem since the time of Moses (Straus, 2009). It was also described in detail in the Bible (Leviticus) over 3300 years ago (Schwab and Straus, 2004). Fungi usually enter a building through outdoor air intakes of the heating, ventilation, and air conditioning system, through doors and windows, and as contaminants on building materials and contents (Shelton *et al.,* 2002). In a pilot study, Gravesen *et al.* (1999) reported that molds can grow on cloths, carpets, leather, wood, sheet rock, insulation (and on human foods) when moisture conditions exist. Due to the ability of molds to grow in moist or wet indoor environments, it is possible for people to become exposed to molds and their products, either by direct contact on surfaces or through the air if mold spores, fragments or mold products are aerosolized (Aleruchi, 2010). Their spore-bearing structures facilitate aerosolization without help from human activity (Burrell, 1991).

Several studies have shown the correlation between various species of fungi with SBS and air quality problems (Finnegan *et al.,* 1984; Harrison *et al.,* 1992; Page and Trout, 2001; Shelton *et al.,* 2002; Kuhn and Ghannoum, 2003; Laumbach and Kipen, 2005; Srikanth, 2008). Most fungi are not pathogenic to human health, but molds and other fungi may cause adverse effects to human health through three ways: allergy, infection and toxicity. They do so through the production of spores, mycotoxins and volatile organic compounds (VOCs) emissions (Singh, 2005).

Several studies have indicated that repeated exposure to particular fungal propagules present in indoor environment develops of allergic disorders in humans such as asthma, sinus rhinitis and pneumonia (Flannigan, 1997; Lugauskas *et al.,* 2000). Singh (2005) reported that out of 100,000 species of fungi, only a limited number of these are pathogenic to human. People with weak immune system such as cancer patients undergoing chemotherapy, organ transplant patients receiving

immunosuppressive drugs, AIDS patients with uncontrolled diabetes are at significant risk for more opportunistic fungal infection. In addition, fungi can release volatile organic compounds (VOCs) such as alcohols and ketones (Ezeonu *et al.,* 1994). These compounds, which are responsible for the musty or moldy odours, are associated with mould growth in damp buildings. Korpi *et al.* (1999) proved the potency of VOCs to induce sensory irritation to eyes and upper respiratory tract.

Many fungi, including some molds, also produce mycotoxins which are non-volatile secondary metabolites with the potential to cause deleterious health effects known as myotoxicosis (Sorenson, 1999; Page and Trout, 2001; Abbott, 2002). Mycotoxins are carcinogenic, teratogenic, tremorgenic, haemorrhagic, and dermatitic to a wide range of organisms and they cause hepatic carcinoma in man (Wary, 1981; Soliman, 2003).

Some of the most common fungi and molds isolated from contaminated buildings belong to the genus *Penicillium, Aspergillus, Cladosporium, Alternaria, Aureobasidium* and *Stachybotrys* (Hiipaka and Buffington, 2000; Shelton *et al.*, 2002; Schwab and Straus, 2004; Zeng *et al.*, 2006). The importance of the most common genera and species found in indoor environment in Malaysia will be highlighted below:

A. The importance of *Aspergillus* spp. in sick building syndrome

Aspergillus species belongs to the class Deuteromycetes, which is ubiquitous molds widely distributed in the environment. Most other molds isolated from sick buildings fall into this class for which a sexually reproducing stage is unknown (Kurup *et al.,* 2000). Although about 185 different species of *Aspergillus* are known, only around 20 of them have been reported to cause human diseases

(Krishnan *et al*., 2009). The spore sizes of *Aspergillus* spp. are about 2 – 5 μm aerodynamic diameter (Schwab and Straus, 2004); therefore, they easily enter the lungs, developing serious respiratory symptoms, including allergic disorders and invasive infection. Invasive aspergillosis is the second most common invasive fungal infection in humans in the United States which comes after candidiasis (Krishnan *et al*., 2009).

Several studies done by numerous investigators have shown the association of various species of *Aspergillus* with poor air quality and SBS (Karol., 1991; Kiertiburanakul *et al*., 2007). *Aspergillus* species that can grow indoors include *A. fumigatus, A. flavus*, *A. niger*, *A. tereus, A. versicolor*, *A. sydowii*, *A. ustus* and *A. flavipes* (Verma *et al*., 2003; Schwab and Straus, 2004; Alwakeel, 2008). Species of *Aspergillus* have been isolated from damp walls, wall paper, polyvinyl chloride covering, gypsum board, floor, carpet and mattress dust, upholstered-furniture dust, acrylic paint, filters and fans, humidifier water, shoes, bird droppings and potted plant soil, plastic and decomposing wood (http://www.moldbacteria.com /learnmore /aspergillus.html).

Aspergillus flavus

Aspergillus flavus has a worldwide distribution in the environment. It normally occurs as saprophyte on many organic nutrient sources like plant debris, tree leaves, decaying wood, animal fodder, cotton, dead insect and animal carcasses, outdoor and indoor environment (Klich, 1998). *Aspergillus flavus*, which comes after *A. fumigatus*, is the second most common cause of invasive and non-invasive aspergillosis in humans, animals, and insects (Yu *et al.,* 2005). It also causes allergic reactions, sinusitis, keratitis and cutaneous infections in humans (Kaushik

et al., 2001; Qiang-qaing *et al*., 2005; Freda, 2006; Jahromi and Khaksar, 2006; Krishnan *et al*., 2009; Leema *et al*., 2010; Liela *et al*., 2010).

Moreover, *A. flavus* is known to be responsible for the production of the most potent cancer-causing groups of mycotoxins, the aflatoxins. Aflatoxins are known to cause liver cancer in humans (Krishnan *et al.,* 2009). Several studies reported the association of lung cancer in humans with inhalation of aflatoxin-contaminated dust, (Dvorackova, 1976; Olsen *et al.,* 1988). Additional health effects of this toxin include: mutagen, teratogen, hepatotoxin, nephrotoxin, immunosuppressant and hemorrhage of intestinal tract and kidneys (Klich, 2009).

Aspergillus niger

Aspergillus niger is ubiquitous in the environment, growing upon a wide variety of substrates and is regarded as common food spoilage fungi (Abarca *et al.,* 2004). Among *Aspergillus* spp., *A. niger* has been considered as a significant causative agent of allergic diseases and invasive aspergillosis in humans (Krishnan *et al.,* 2009; Liela *et al.,* 2010). Moreover, ochratoxins produced by *A. niger* have been shown to have nephrotoxic, immunotoxic, teratogenic, gemotoxic properties and have been classified as a possible human carcinogen (Kuiper-Goodman and Scott, 1989; Accensi *et al.,* 2001). In addition, *A. niger* has been reported to cause fungal otomycosis (ear infection), keratitis, skin and pulmonary diseases (Grecovich *et al*., 1975; Mishra *et al*., 2004; Shah *et al*., 2004; Fasunla *et al.,* 2008; Kredics *et al.*, 2008; Person *et al*., 2010).

B. The importance of *Penicillium* spp. in sick building syndrome

Penicillium species belongs to the class Deuteromycetes, which is the same class as *Aspergillus* spp. (Kurup *et al*., 2000). There are over 200 species, widely distributed in all environments. Species belongs to this genus are some of the most commonly isolated from contaminated buildings (Cooley *et al.,* 1998; Hiipakka and Buffington, 2000; Lugauskas *et al.,* 2000; Hyvarinen *et al.,* 2002; Shelton *et al.*, 2002; Alwakeel, 2008). The species prefer damp and dark places and may be found in house dust, books, wallpaper, stuffed furniture, foam rubber mattresses, refrigerator doors and rubber tubing. *Penicillium* can cause the black spots on window sills (http://www.phadia.com>...Mold and other Microorganisms-Cached). Numerous species of this genus were isolated from sick buildings e.g. *P. chrysogenum* (formerly *P. notatum*), *P. citrinum, P. brevicompactum, P. oxalicum, P. commune, P. corylophilum* and *P. palitans* (Shen *et al*., 1999a; Nielsen, 2003). *P. chrysogenum* was one of the predominant organisms isolated from multiple buildings (Cooley *et al.,* 1998). The spore size of this mold ranges from 1 – 5 μm in diameter with an average size of 3.5 μm (Schwab and Straus, 2004).

Recent studies have shown the association of *Penicillium* spp. with poor indoor air and SBS (Flannigan *et al.,* 1997; Cooley *et al.,* 1998; McGrath *et al*., 1999). Repeated exposure to propagules of *Penicillium* spp. was found to cause respiratory and allergic disorders such as asthma, rhinitis and alveolitis (Karol, 1991; Flannigan, 1997; Lugauskas *et al.,* 2004). Some *Penicillium* species are known to produce mycotoxins such as patulin, citrinin, ochratoxin A, penicillic acid, cyclopiazonic acid and roquefortine. Citrinin and ochratoxin A may generate nephrotoxic effects. Patulin and penicillic acid mainly target gastrointestinal tract and liver, respectively (Keblys *et al.,* 2004).

Penicillium oxalicum

This species is widespread in tropical agricultural commodities such as maize, rice, peanut, kemiri nuts, soybeans, cowpeas and sorghum in Southeast Asia. This species can rapidly grow at 37ºC (Pitt, 2002). *P. oxalicum* was found to be one of the most dominant species of *Penicillium* in indoor environment (Sawane and Saoji, 2004). Shen *et al.* (1999b) reported that multiple allergens have been characterized from *P. oxalicum* that could induce the symptoms associated with exposure to this mold in sick buildings. The toxic teratogenic, hepatotoxic and slightly mutagenic secalonic acid D metabolite of *P. oxalicum* has been identified as a natural contaminant of grain dust (Fleischhacker *et al*., 1986).

C. The importance of *Cladosporium* spp. in sick building syndrome

Species of *Cladosporium* are not characterized by sexual phase of reproduction and it also belongs to the class Deuteromycetes. Species occur both as saprophytes and as plant pathogen. Members of this genus are widely distributed in the air and dead organic matter, and they are known as food contaminants (Tasic and Tasic, 2007). They are ubiquitous in nature with wide distribution in the tropics and subtropics (Dixon and Polak-Wyss, 1991; de Hoog *et al*., 2000). Zeng *et al*. (2006) reported that *Cladosporium* is one of the most common airborne molds found in indoor and outdoor environments. The most isolated species are *C. elatum*, *C. herbarum, C. sphaerospermum* and *C. cladosporioides* (de Hoog *et al.,* 1995; Masclaux *et al.,* 1995).

Cladosporium spp. are very common on wet building materials such as gypsum board, acrylic painted walls, wood, wall papers, carpet and mattress dust, fans, and wet insulation in mechanical cooling units (www.moldbacteria. com/learnmore/cladosporium.html.). Several species of *Cladosporium* can cause

cerebral phaeohyphomycosis, cutaneous infections, onychomycosis, sinusitis, allergic diseases as well as intrabronchial lesions (Hironaga and Watanabe, 1980; Aldape *et al.*, 1991; Annessi *et al*., 1992; Romano *et al*., 1999; de Hoog *et al.*, 2000; Yano *et al*., 2003; Hilmioglu-Polat *et al*., 2005; Tasic and Tasic, 2007). It has also been reported that some species are able to produce cytotoxic and mutagenic toxins such as cladosporin, emodin as well as other less toxic compounds (Tasic and Tasic, 2007).

Cladosporium oxysporum

Cladosporium oxysporum is widespread in the tropics, mainly on the dead parts of leaves and stems of herbaceous and woody plants (Holliday, 1980). This species was also reported to cause a leaf spot on plants (Hammouda, 1992; Lamboy and Dillard, 1997). The spore size of this species is 2.5~10.0 X 2.0~4.5 μm (Paul and Yu, 2008); therefore they are easily carried through the air. *C. oxysporum* has been reported as causative agents of cutaneous phaeohyphomycosis and bronchopulmonary disorder in humans (Singh *et al.*, 1992; Romano *et al.*, 1999).

2.1.2.3 Bacteria and sick building syndrome

Bacteria are prokaryotic single-celled organisms that lack nuclei. They live on and in the human body and the bodies of other living things, in dirt, in the air, and in water. They are the most numerous organisms on Earth (Favor, 2004). Bacteria are autotrophs or heterotrophs, and that feed on dead and rotting plants and animals are called saprophytes. Some bacteria are parasites and those bacteria that live with other organisms in mutualism relationships may harm the others. Bacteria that cause diseases are parasites. All bacteria have simple cell structures and most of them are microscopic. On average, the cell size is about 2 μm in length or diameter. Bacteria are of different shapes, some are rod, round, spiral, or curved

rods. Based on their Gram's staining ability, these microorganisms are classified into two distinct groups: Gram-positive bacteria and Gram-negative bacteria (Wearing, 2010).

Bacteria are important biological components of bioaerosols (Fang *et al*., 2007). It has been estimated to account for 60% of Earth's biomass (Pevsner, 2009). They are able to grow and propagate on a variety of building materials and indoor surfaces, causing indoor air pollution (Zhu *et al.,* 2003). Although bacteria cause health hazards, they have not received as much interest by researchers as in molds when it comes to indoor quality. Goh *et al.* (2000) reported that the indoor concentrations of bacteria in air were approximately ten times higher than those measured outdoors, indicating a significant internal source of bacteria. Indoor bacterial population primarily comes from human and animal activities (Peterson and Talcott, 2006).

Pathogenic bacteria can cause severe diseases in humans if inhaled, ingested or if they come into contact with the skin (www.moldbacteriaconsulting. com/tag/acinetobacter). Bacteria were found to be the cause of many nosocomial and community-acquired infections in humans. In the United States, 2 million hospital-acquired infections occur each year. Moreover, it was estimated that 20,000 deaths are directly attributed to nosocomial pneumonias each year. These infections are caused by *Staphylococcus aureus, S. pneumoniae, S. pyogenes*, coagulase-negative *Staphylococci*, *Enterococcus faecium*, and *E. faecalis*. Bacteria can cause many types of respiratory, skin, hair, and other infections. They also cause bacteremia, endocarditis, meningitis, osteomyelitis and postoperative wound infections (Utrup *et al.,* 2003). On the other hand, some Gram-negative bacteria are able to produce toxic compounds (endotoxins). These toxins have been shown to provoke symptoms of humidifier fever, cough and chill (Karol, 1991).

The predominant common bacteria found in indoor environments include *Bacillus* and *Staphylococcus* (Alwakeel, 2008; Van Houdt *et al*., 2009). Other species have been reported, especially from genus *Micrococcus, Flavobacterium, Acinetobacter, Alcaligenes, Epiococcus, Corynebacterium, Pseudomonas, Bacterioides* and *Clostridium* (Hung *et al*., 2005; Alwakeel, 2008).

A. The importance of *Bacillus* spp. in sick building syndrome

The genus *Bacillus* comprises 51 validly described species as well as many species of uncertain taxonomic standing. This genus is part of the family Bacillaceae. The distinguishing feature of this family is the production of endospores. Members of this genus are widely distributed in soil, air, fresh water, foods, animals, sugar, milk, cocoa, spices, and frozen foods. This genus contains the rod-shaped aerobic or facultatively anaerobic spore formers. With exception of anthrax *Bacillus,* bacilli are not highly pathogenic to mammals (Harwood, 1989). *B. cereus, B. licheniformis* and *B. subtilis* are examples of this genus. *B. cereus* has been associated with a range of opportunistic infections such as bovine mastitis, abscess formation, septicemia, endocarditis and ear, eye, and wound infections (Logan and Rodrigues-Diaz, 2006).

B. *Bacillus subtilis*

Bacillus subtilis is a Gram-positive, facultative, aerobic, sporulating bacillus normally found in soil. Although it is considered as a non-pathogenic bacterium, it is linked to food-borne illnesses, causing diarrhea, nausea and vomiting (Logan, 1988). *B. subtilis* is also a potential occupational allergen (Karol, 1991). Moreover, this species has been described in about 15 systemic infections such as bacteremia, meningitis, pneumonia, endocarditis in drug abuse, and in 160 ophthalmic infections (Cox *et al.*, 1959; Tuazon *et al.*, 1979; Richard *et al.*, 1988; Patrick *et al.*,

1989). *B. subtilis* has also been identified in a case of cholangitis in a patient receiving immunosuppression after kidney transplantation (Wallet *et al.,* 1996).

2.1.2.4 Deterioration of building materials

Different species of fungi and bacteria have been isolated from all building materials and surfaces (both organic and inorganic) such as wood, papers, carpets, gypsum board, ceramic products, plastics, mineral insulation, paints and glues (Anderson *et al.,* 1997; Hyvarinen *et al.,* 2002; Aira *et al*., 2007; Alwakeel, 2008). These organisms cause corrosion and degradation of materials and components of building materials and thereby load the indoor air environment with harmful substances, thus leading to poor indoor air quality, SBS and BRI (Anderson *et al*., 1997; Nielsen, 2003; Gorny, 2004; Aibinu *et al*., 2009).

2.1.3 Bioaerosol remediation

The growing concern about problems resulting from exposure to indoor air contaminants is based on evidence that urban residents spend 80 - 90% of their time indoors whether they are at home or work (Green and Scarpino, 2002). The easiest way to deal with bioaerosol problem is to prevent it. A variety of methods have been used. The most common control methods are ultraviolet (UV) irradiation, electric ion emission and an application of disinfectants.

The UV irradiation is known to affect the viability of bioaerosols because of its germicidal effect. Although UV can be easily applied by installing and turning on a UV lamp, the 254 nm wavelength UV light has harmful effects to humans. Electric ion emission has also been used to control bioaerosol. When the efficiency of the filter is increased, the efficacy of respiratory protection devices against bioaerosols can be enhanced. However, the generation of ions produces ozone, a pollutant, and also causes electric charges to accumulate on surrounding surfaces

(Jung *et al.,* 2009). Disinfectants (biocides) have long been used as active agents. These compounds are pollutants and most of them contain toxic chemicals which may have carcinogenic nature, residual problems and harmful effects on the environment (Verma *et al*., 2008).

In case of fungi, when mold infects a surface, it is impossible to eradicate them completely because of the protective layer formed by the molds itself. When molds are exposed to ultraviolet light or toxic chemicals such as bleach, only the top layer of the molds is usually killed. If the original spore is unaffected, it will continue to exist on the surface in a dormant state; once additional moisture is present, the molds will again reproduce and develop (Chen *et al.,* 2004; Aibinu *et al*., 2009). On the other hand, research has shown that *B. subtilis* form tough protecting endospores allowing it to endure extreme environmental conditions thus, contaminating foods and building surfaces (Awodele *et al*., 2007).

Moreover, the major problem with excessive and indiscriminate use of these chemicals is that resistance can be induced in the target organism. There have been several reports of the reduced microbial susceptibility to these agents (Bundgaard-Nielsen and Nielsen, 1996; McDonnell and Russell, 1999; Russell, 1999; Russell, 2004). The emergence of the bacterial and fungal resistant has encouraged researchers to develop new alternatives to control microbial infection which may be harmless to humans and may have wide-ranged antimicrobial activity. Biological methods of control have been preferred because they are selective with no side effect.

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals, and plants (Menghani *et al.,* 2011). Plants may offer a new source of antimicrobial agents with a significant activity against infective microorganisms (Cowan, 1999). An antimicrobial agent is

a substance that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells (Sahgal *et al.*, 2009).

2.2 Plants as a source of antimicrobial compounds

Plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008). A considerable number of literature deals with the action of plant extracts on microorganisms pathogenic to plants or animals (Solomon-Wisdom and Shittu, 2010). These observations have helped in identifying the active principles responsible for such properties.

Wiart *et al.* (2004), for instance, have reported a broad spectrum of antibacterial activities of extracts of *Polyalthia laterifolia, Peristrophe tinctoria*, *Knema malanyana, Solanum torvum, Celosia argentea, Eclipta prostrata, Dillenia suffruticosa, Piper stylosum* and *Rafflesia hasseltii*. Antimicrobial activities of *Senna alata* against *A. niger, Mucor* sp., *Rhizopus* sp., *C. albicans, Saccharomyces* sp., *E. coli, B. subtilis, P. aeruginosa* and *Saphylococcus* sp. were reported by Owoyale *et al.* (2005). Twelve extracts obtained from nine plants belong to six different genera of Clusiaceae were analyzed against Gram-negative (*E. coli* and *P. aeruginosa)* and Gram-positive (*S. aureus* and *E. faecalis*) bacteria. *Tovomita longifolia, T. brasiliensis, Clusia columnaris, Haploclathra paniculata* and *Caraipa grandifolia* showed significant results against the bacteria (Suffredini *et al.*, 2006). Vlietinck *et al.* (1995) have screened 100 Rwandese plant species. Of these, 45% were active against *S. aureus*, 2% against *E. coli*, 16% against *P. aeruginosa*, 7% against *C. albicans*, 80% against *Microsporum canis* and 60% against *T. mentagrophytes*.

In Turkey, Kordali *et al*. (2003) reported the antifungal activity of crude extracts obtained from *Pistacia vera, Pistacia terebinthus* and *Pistacia lentiscus*

against three pathogenic agricultural fungi, *Phythium ultimum*, *Rhizoctonia solani*. Kianbakht and Jahaniani (2003) have studied the antibacterial activity of methanolic extracts of different parts of *Tribulus terrestris* (fruits, stems plus leaves and roots) against *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*. The MIC value of fruits extracts and stem plus leaves against all bacteria was 2 mg/ml and the MIC value of roots against *S. aureus*, *E. faecalis* and *E. coli* was 4 mg/ml and the MIC value of roots against *P. aeruginosa* was 2 mg/ml.

Table 2.1 summarizes various research reports published on different plant species having antimicrobial properties against a variety of species of bacteria and fungi. This table includes the plant botanical name, family, bacteria and/or fungi tested, plant part used, tested extract, antimicrobial activity and references.

.

Table 2.1: Several plants with reported antimicrobial activity in the literatures

Samples (Family)	Plant part used	Tested extract	Tested bacteria and /or fungi	Antimicrobial potency	Reference
Acacia nilotica (Mimosaceae)	Leaf	Aqueous	*A. columnaris, A. flavus, A. fumigatus, A. niger, A. ochraceus* and *A. tamarii*	80.00±1.82, 75.25±1.31, 82.00± 1.29, 91.00±1.29, 85.00±1.29 and 84.25±1.79 %[a]	Satish *et al*. (2007)
Acalypha indica (Euphorbiaceae)	Leaf	Chloroform	*Proteus mirabilis*	18 mm[b]	Devi *et al.* (2009)
Andrographis paniculata (Acanthaceae)	Stem	Chloroform	*S. aureus, B. subtilis, E. coli, P. vulgaris, A. niger* and *P. chrysogenum*	11, 11, 11, 12, 12 and 11 µg/ml[c]	Radhika and Lakshmi (2010)
Acorus calamus (Acoraceae)	Rhizome	80% ethanol	*Alternaria alternata, Curvularia lunata, Fusarium equiseti, Macrphomina phaseolina, Botryodiplodia theobromae* and *Colletotrichum corchori*	100, 100, 100, 100, 100 and 100 %[a]	Begum *et al*. (2007)
Balanites aegyptiaca (Balanitaceae)	Leaf	Ethanol	*Salmonella typhi*	6.5 mg/ml[c]	Doughari *et al*. (2007)
Bryonopsis laciniosa (Cucurbitaceae)	Leaf	80% ethanol	*S. aureus, Micrococcus luteus, B. cereus* and *P. aeruginosa*	0.625, 0.625, 1.25 and 10 mg/ml[c]	Ehsan *et al*. (2009)
Conyza scabrida (Asteraceae)	Leaf and stem	Ethyl acetate	*S. aureus, C. albicans* and *Mycobacterium smegmatis*	0.625, 2.5 and 5 mg/ml[c]	Thring *et al*. (2007)

Table 2.1: Continued

Samples (Family)	Plant part used	Tested extract	Tested bacteria and / or fungi	Antimicrobial potency	Reference
Cassia fistula (Fabaceae)	Seed	80% methanol	*S. aureus, B. thuringiensis, E. coli, Salmonella sp., Micrococcus sp., B. subtilis* and *C. albicans*	12.500, 12.500, 50.000, 3.125, 1.563, 3.125 and 6.25 mg/ml[c]	Lachumy *et al.* (2010)
Dodonaea viscosa (Sapindaceae)	Leaf and stem	Boiled aqueous	*S. aureus* and *M. smegmatis*	5 and 5 mg/ml[c]	Thring *et al.* (2007)
Distemonanthus benthamianus (Fabaceae)	Stem	Ethanol extract	*A. flavus, C. albicans, Microsporum gyseum* and *Trichophyton mentagrophytes*	13.40±0.14, 13.70±0.15, 11.40±0.18 and13.40±0.17 mm[b]	Adekunle and Odukoya (2006)
Eucalyptus camaldulensis (Myrtaceae)	Leaf	80% methanol	*Microsporum canis, M. gypseum, Trichophyton rubrum, T. schoenleini, T. mentagrophytes* and *Epidermophyton floccosum*	1.6, 1.6, 1.6, 0.4, 0.4 and 0.4 mg/ml[c]	Falahati *et al.* (2005)
Eugenia uniflora (Myrtaceae)	Leaf	96% ethanol	*Paracoccidioides brasiliensis*	750 mg/ml[c]	Santos *et al.* (2004)
Equisetum giganteum (Equisetaceae)	Aerial part	80% ethanol	*B. cereus, B. subtilis, Enterococcus faecalis, S. aureus, S. epidermidis, S. pyogenes* and *Bacterioides fragilis*	8, 16, 8, 8, 16, 4 and 8 mg/ml[c]	Kloucek *et al.* (2005)

Table 2.1: Continued

Samples (Family)	Plant part used	Tested extract	Tested bacteria and /or fungi	Antimicrobial potency	Reference
Erigeron floribundus (Asteraceae)	Leaf	Dichloromethane	*Epidermophyton floccosum, Microsporum canis, M. gypseum, M. langeroniise, T. mentagrophytes, T. rubrum, T. soudanense* and *Scopulariopsis brevicaulis*	0.25, 1, 0.25, 0.25, 0.50, 0.25, 0.25 and 0.25 mg/ml[c]	Tra Bi *et al.* (2008)
Ficus glomerata (Moraceae)	Bark	Ethanol	*S. aureus, B. subtilis, B. pumilus, E. coli, P. aerugenosa* and *C. albicans*	16±0.00, 12±0.00, 13±0.00, 12±0.00, 14±0.00 and 13±0.00 mm[b]	Mon *et al*. (2008)
Ficus benghalensis (Moraceae)	Branching root	Methanol	*B. cereus, S. aureus* and *K. pneumoniae*	16, 12 and 15 mm[b]	Parekh and Chanda (2007)
Jatropha curcas (Euphorbiaceae)	Leaf, seed, roots and barks	Methanol	*T. mentagrophytes, T. verrucosum, T. beigelii* and *C. albicans*	280, 400, 480 and 320 mg/ml[c]	Ayanbimpe and Fagbemi (2005)
Litsea glutinosa (Lauraceae)	Leaf	Ethanol	*S. aureus, E. faecalis, E. coli, P. aeruginosa* and *Proteus mirabilis*	15.1±0.6, 13.2±0.51, 12.1±0.23, 10.6, ±0.8 and 14.9±0.34 mm[b]	Prusti *et al.* (2008)
Leptadenia lancifolia (Asclepiadaceae)	Stem bark	Aqueous, acetone and methanol	*Cryptococcus neoformans*	15.0, 6.3 and 30.0 mm[b]	Doughari and Obidah (2008)
Lasianthera africana (Icacinaceae)	Leaf	95% Ethanol	*E. coli, S. typhi, S. aureus* and *C. albicans*	6.25, 12.50, 50 and 50 µg/ml[c]	Andy *et al.* (2008)

Table 2.1: Continued

Samples (Family)	Plant part used	Tested extract	Tested bacteria and /or fungi	Antimicrobial potency	Reference
Metasequoia glyptostroboides (Cupressaceae)	Floral cones	Methanol	*Phytophthora capsici*, *Colletotrichum capsici* and *Sclerotinia sclerotiorum*	1000, 500 and 500 µg /ml[c]	Bajpai *et al*. (2007)
Marrubium vulgare (Lamiaceae)	Whole plant	Methanol	*B. subtilis*, *S. epidermidis*, *S. aureus*, *E. coli* and *P. vulgaris*	100, 200, 100, 400 and 400 mg/ml[c]	Masoodi *et al.* (2008)
Moringa oleifera (Moringaceae)	Leaf	Ethanol	*S. typhi*	8 mg/ml[c]	Doughari *et al.* (2007)
Muntingia calabura (Elaeocarpaceae)	Leaf	Methanol	*S. aureus* ATCC25923, *S. aureus* ATCC33591, *C. coli*, *P. aeruginosa*, *C. albicans and M. canis*	1250, 2500, >5000, >5000, >5000 and >5000 µg/ml[c]	Zakaria *et al*. (2010)
Nigella sativa (Ranunculaceae)	Seed	Methanol	*S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *B. cereus*	25, 9, 13, 10 and 21 mm[b]	Zuridah *et al*. (2008)
Otostegia persica (Lamiaceae)	Aerial part	Chloroform	*S. aureus*, *S. epidermidis* and *E. faecalis*	0.62, 2.5 and 0.31 mg/ml[c]	Asghari *et al*. (2006)
Ocimum gratissimum (Lamiaceae)	Leaf	Ethanol	*Fusarium oxysporum* and *A. niger*	70.1 and 66.6 %[a]	Okigbo and Ogbonnaya (2006)