THE PHARMACOLOGY AND PHYTOCHEMISTRY STUDY OF MAHOGANY SEEDS

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UNIVERSITI SAINS MALAYSIA

2012

THE PHARMACOLOGY AND PHYTOCHEMISTRY STUDY OF MAHOGANY SEEDS

by

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Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science (Pharmacy)

AUGUST 2011

ACKNOWLEDGEMENT

First and foremost I would like to extend my heartfelt gratitude to the Supreme Lord, without whose mercy, I wouldn't be able to complete my research successfully. I would like to acknowledge herein my supervisor, Dr. Surash Ramanathan, and co-supervisors Prof Sharif Mahsufi Mansor, Dr. Mohd Nizam Mordi and Dr. Sasidharan for their expert guidance for me to finish my project. Besides them, I would like to thank Dr. Vickneswaran Murugaiyah from School of Pharmacy who helped me a lot to accomplish my research work. My supervisors and co-supervisors were always there for me to clear my doubts immediately. Without their guidance and support I would not have finished my project successfully on the given time. I would also like to especially thank my parents, family members and Mr. Jeevandran who are always there for my success and for their moral supports. Here I take an opportunity to thank Research University Grant for funding my project and USM Fellowship Scheme from Institute for Postgraduate Studies (IPS) of University Sains Malaysia. Also here, I would like to take this opportunity to thank other lecturers and lab staffs including Miss. Vickneswarinee, En. Hilman, En. Zulkiflli, Pn. Juwita, En. Zamri, En. Rahim, En. Ashok, En. Salam, En. Amir, Cik. Aida and En. Khairil, who always gave me their full support and helped me to complete my project. In addition, I would like to thank my seniors, Dr. Lai, Mr. Gowda, and Dr. Venkatash, who had always cleared my doubts regarding my project. Last but not least, I would also like to thank my friends who had helped in my research project.

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$(C_2H_5)_2O$	Diethyl ether
$^{\bullet}OH$	Hydroxyl radical
µl	Micro litter
µM	Micro molar
$^1O_2$	Singlet oxygen
5-FC	5-fluorocytosine
AAI	Antioxidant activity index
ACN	Acetonitrile
AH	Antioxidant
AIDS	Acquired immunodeficiency syndrome
AL	Alveolar lining cells
$AlCl_3$	Aluminum chloride
AmB	Amphotericin B
ANOVA	Analysis of variance
AOT	Acute Oral Toxicity
AS	Alveolar air space
ATP	Adenosine triphosphate
BC	Bowman's space
BHA	Butylhydroxyanisole
BHT	Butylated hydroxytoluene
BSLT	Brine shrimp lethality test
C	Alveolar capillary
C. albicans	*Candida albicans*
CA	Central Artery

CE mg/g	Catechin equivalent (mg) per gram
CFU	Colony forming unit
CH_2Cl_2	Dichloromethane
$CH_3COOCH_2CH_3$	Ethyl acetate
$CHCl_3$	Chloroform
cm	Centimeter
CNS	Central nerve system
COX-1	Cyclo-oxygenase 1
COX-2	Cyclo-oxygenase 2
COX-3	Cyclo-oygenase 3
DCA	Drug Control Authority
DNA	Deoxyribonucleic acid
DPPH	Diphenyl-1-picrylhydrazyl
E	Endocardium
ESI-MS	Electrospray ionization mass spectrometric technique
ET	Single electron transfer
EtOH	Ethanol
Fe (II)-TPTZ	Ferrous tripyridyltriazine
Fe (III)-TPTZ	Ferric tripyridyltriazine
$Fe(SO_4)_2$	Ferric sulphate
$FeCl_2$	Iron (II) chloride
$FeCl_3$	Ferric chloride
FRAP	Ferric ion reducing antioxidant parameter
FTIR	Fourier transforms IR spectrophotometer
g	Gram

g/L	Gram per liter
H	Hepatocytes
h	Hour
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
HAT	Hydrogen atom transfer
HCl	Hydrochloric acid
HCOOH	Formic acid
HIV	Human immunodeficiency virus
HPLC	High Performance Liquid Chromatography
i.p	Intraperitoneal injection
i.v	Intravenous injection
IAEC	Institutional Animal Ethics Committee
IC_{50}	Concentration providing 50% inhibition
INT	Iodonitrotetrazolium violet
IR	Infrared
KBr	Potassium bromide
L	Liver lobules
L/min	Liter per minute
LC_{50}	Lethality Concentration at 50 %
LD_{50}	Median lethal dose
LDL	Low-density lipoproteins
M	Molar
M	Myocardium
m/z	Mass-to-charge ratio

MBC	Maximum Inhibitory Concentration
MeOH	Methanol
mg GAE/g	Milligrams of Gallic acid equivalent per gram
mg/kg	Milligrams per kilogram
mg/mL	Milligram per milliliter
MH broth	Mueller Hinton Broth
MHA	Mueller Hinton agar
MIC	Minimum inhibitory Concentration
MID	Minimum inhibitory dose
min	Minutes
mL	Milliliter
mL/kg	Milliliter per kilogram
mL/min	Milliliter per minutes
mM	MilliMolar
mm	Millimeter
mol/L	Mol per liter
mRNA	Messenger RNA
MS spectrum	Mass spectrum
NA	No activity
Na_2CO_3	Sodium carbonate
NaCl	Sodium chloride
$NaNO_2$	Sodium nitrate
NaOH	Sodium hydroxide
NCCLS	National Committee for Clinical Laboratory Standards
NM	Nucleus of Myocytes

nm	Nanometer
NMR	Nuclear magnetic resonance
NSAIDs	Nonsteroidal anti-inflammatory drugs
O_2^-	Superoxide anion
OD	Optical density
OECD	Organization of Economic and Cooperative Development
OsO_4	Osmium tetroxide
PAF	Platelet-activating factor
PAS reagent	Periodic Acid Schiff reagent
PBS	Phosphate buffer solution
PG	Propyl gallate
PPARγ	Peroxisome proliferator-activated receptor γ
RC	Renal corpuscles
R_f	Relationship to the Front
RI	Radical species
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RP	Red pulp
Rt	Retention time
S	Sinusoids
S. mahagoni	*Swietenia mahogani*
S.E.M	Standard error of the mean
SD	Standard deviation
SD broth	Sabouraud dextrose broth

SDA	Sabouraud dextrose agar
SEM	Scanning Electron Microscopy
SM	*Swietenia mahogani*
SM Sp1	*Swietenia mahagoni* crude methanolic Spot 1
SM Sp2	*Swietenia mahagoni* crude methanolic Spot 2
SM Sp3	*Swietenia mahagoni* crude methanolic Spot 3
SMCM	*Swietenia mahagoni* crude methanolic
SMSA	*Swietenia mahagoni* seed alkaloid
T	Renal tubules
TBHQ	Tert-butylhydroquinone
TCA	Trichloroacetic acid
TCM	Traditional Chinese Medicine
TEAC	Trolox equivalent antioxidant capacity
TEM	Transmission Electron Microscopy
TLC	Thin Layer Chromatography
TPTZ	Tripyridyltriazine
TRAP	Total radical trapping antioxidant parameter
tRNA	Transfer RNA
U.K	United Kingdom
USA	United States of America
USD	United States Dollar
UV	Ultra violet
V	Central vein of lobule
v/v	Volume/volume
WHO	World Health Organization

WP	White Pulp
XO	Xanthine oxidase

KAJIAN FARMAKOLOGI DAN FITOKIMIA TERHADAP BIJI MAHOGANY

ABSTRAK

Swietenia mahagoni yang berasal daripada famili Meliaceae digunakan sebagai ubat-ubatan tradisional. Setakat ini, tiada laporan atau artikel yang telah diterbitkan tentang biji benih *S. mahagoni* yang berasal dari Malaysia. Oleh kerana itu, kajian ini direka bentuk untuk menentukan sifat-sifat farmakologi biji benih *S. mahagoni* untuk analisis agen antimikrob, antioksidan, analgesik dan untuk pemeriksaan tahap ketoksikan. Analisis fitokimia menunjukan kandungan alkaloid, terpenoids, antrakuinon, cardiac glikosida, saponin, dan minyak-minyak essential sebagai unsur-unsur aktif utama. Penilaian ketoksikan akut mencit menunjukan bacaan LD_{50} yang lebih dari 5000 mg/kg. Ini bermakna, ekstrak biji benih SMCM berpotensi untuk mengandungi bahan terapeutik yang selamat dari sebarang kesan toksik. Kajian telah dijalankan untuk mengetahui aktiviti antimikrob ekstrak biji benih SMCM ke atas bakteria Gram positif, Gram negative, yis and kulat. Ekstrak ini didapati aktif terhadap yis *Candida albicans.* SMCM biji benih ekstrak mengandungi aktiviti-aktiviti yang berkesan untuk *in vitro* dan *in vivo* aktiviti anti-Candida terhadap *C. albicans.* Seterusnya aktiviti antioksidan dan analgesik telah dikaji dan mendapati ekstrak biji benih SMCM mempunyai aktiviti antioksidan yang sederhana. Ekstrak biji benih SMCM menunjukan analgesik aktiviti pada dos yang lebih tinggi. SMCM ekstrak menunjukan aktiviti anti-Candida yang tinggi. Oleh kerana itu, kajian telah dilanjutkan untuk mengenal pasti bahan bioaktif yang mungkin memberi kesan aktiviti anti-Candida. Kumpulan bioaktif terpenoid (SM Sp1) didapati berkesan terhadap *C. albicans* dalam kajian bioautografi dengan nilai MIC 150 μg/mL. FTIR

analisis menyokong bahawa SM Sp 1 mempunyai ciri-ciri regangan seperti kompound Swietmanin J. Penemuan menunjukkan ekstrak biji benih SMCM mempunyai aktiviti anti-Candida yang ketara. Penemuan ini boleh menyumbang ke arah pembangunan bidang penggunaan ubat-ubat tradisional berasaskan tumbuhan dalam sektor bioteknologi dan industri farmasi di Malaysia. Oleh yang demikian, kajian lanjutan dalam pengasingan dan pencirian bahan bioaktif anti-Candida adalah disarankan pada masa hadapan.

THE PHARMACOLOGY AND PHYTOCHEMISTRY STUDY OF MAHOGANY SEEDS

ABSTRACT

Swietenia mahagoni which belongs to the Meliaceae family has been applied in folk medicine. To date there are no report or articles that published on the Malaysian origin *S. mahagoni* seeds. Therefore, this research was designed to investigate the pharmacological efficacy of *S.mahagoni* seeds for phytochemical analysis, antimicrobial, antioxidant, analgesic activities and for toxicity screening. The phytochemical analysis exhibited the presence of terpenoids, saponins, volatile oils, alkaloids, antraquinones, and cardiac glycosides as major active constituents. Acute oral toxicity evaluation exhibited the LD_{50} was relatively greater than 5000 mg/kg. This shows that, SMCM seed extract may contain potential therapeutic bioactive which is free from toxic effects. Antimicrobial activity of SMCM seed extract against Gram positive and Gram-negative bacteria and fungus strains was undertaken. The extract was found to be more active against *Candida albicans*. SMCM seed extract is effectively active for *in vitro* and *in vivo* anti-Candida activity against *C. albicans*. The SMCM seed extract was evaluated for antioxidant and analgesic activities and found to possess moderate antioxidant activity and analgesic activity at tested higher dose. SMCM seed extract shows good anti-Candida activity. Therefore, further work was undertaken to identify the possible bioactives for the anti-Candida activity. A terpenoid bioactive group was found to be active against *C. albicans* in bioautography assay with MIC value of 150 µg/mL. The FTIR analysis of SM Sp1 showed almost comparable stretching groups with an identified compound Swietmanin J. Our findings showed that, SMCM seed extract possess

therapeutic property for anti-Candida activity. These findings could assist the herbal drug development in Malaysian biotechnology and pharmaceutical industry. Therefore, in future, further works on isolation and characterization of the pure anti-Candida bioactive are recommended. From the overall findings, the most promising activity of the SMCM seed extract was observed in its anti-Candida activity.

CHAPTER 1

INTRODUCION

Swietenia mahagoni (Linn.) Jacq. (Meliaceae) is a large, deciduous, and economically important timber tree, which is native to the West Indies. *S. mahagoni* is one of the most popular traditional medicines in Africa (Abdelgaleil and Aswad, 2005). Currently large numbers of pharmacological studies have being carried out on this plant parts. The chestnut brown colour seeds have also been reported to have medicinal value for treatment of cancer, amoebiasis, coughs, chest pains and intestinal parasitism (Alrdahe *et al.,* 2010). However, there is lack of scientific investigation on *S. mahagoni* seed extract from Malaysia. Therefore, to explore the pharmacology effects of *S. mahagoni* seeds, a few groups of studies were focused in this project.

At present, there is a dilemma of microbial drug resistance and there is a raise of opportunistic infections especially with AIDS patients and individuals on immunosuppressive chemotherapy (Stark *et al.,* 2009). Moreover, there are several antifungal and antiviral prescriptions, which have limited use due to their toxicity effect, while we have yet to find a cure for viral diseases (Pauli, 2006 and Scorzoni *et al.,* 2007). Presently human fungal contagion has increased at an alarming rate in the last 20 years, mainly among immunocompromised individuals (Perea *et al.,* 2002; Scorzoni *et al.,* 2007). Oral candidiasis is the most widespread opportunistic infection associated with 90% of HIV-infected individuals (Torssander *et al.,* 1987; Feigal *et al.,* 1991; Patel *et al.,* 2008). *Candida albicans* is the most recurrent etiological agent associated with candidiasis infection. Furthermore, isolates of this species from HIV-positive patients are more virulent and genotypically altered than

strains from HIV-negative patients (Challacombe *et al.,* 1995; Sweet *et al.,* 1995; Patel *et al.,* 2008). With the mounting recognition of herbal remedy as an alternative form of health care, the screening of therapeutic plants for bioactive is becoming increasingly important (Rabe and Van Staden, 1997; Shai *et al.,* 2008). Therefore, anti-Candida activity against *Swietenia mahagoni* seed extract was mainly given importance in this study.

In addition to emerging problems of drug resistance in infectious diseases, multi-factorial diseases due to imbalance of antioxidant system in human body is also becoming another major existing crisis (Tiwari, 2004). Reactive oxygen species (ROS) formed *in vivo* are highly reactive and potentially damaging the transient chemical species in our body system (Ali *et al.,* 2008). Over-production of this reactive species, induced failure in the defense mechanisms and damage to cell structures, DNA, lipids and proteins (Valko *et al.,* 2006) and increases the risk of more than 30 different disease processes in human body (Aruoma, 1998). Currently the presences of enzymatic and non-enzymatic antioxidants are possible to reduce the risks of chronic diseases and prevent disease progression (Stanner *et al.,* 2004; Ali *et al.,* 2008). Many studies have proven that, either by enhancing the body's natural antioxidant defenses or by supplementing with dietary antioxidants, we can overcome the effects of ROS. Therefore, major efforts are concentrated towards discovering and synthesizing novel antioxidants. Due to this reasons, *S. mahagoni* seed extract was also used to evaluate the property of the antioxidant activity.

Another foremost cause that leads for the evaluation of analgesic effects on *S. mahagoni* seed extract is to discover an alternative medication for pain emancipation. Pain is the most apparent reason for an individual to search for therapeutic attention.

Presently the treatment for chronic pain has turned into a major problem due to the use of available medications and their undesirable side effects. The side effects of currently used pain medications are addiction, tolerance, gastrointestinal effects, and abuse (Elisabetsky *et al.*, 1995). There are a lot of up coming literature reports regarding the analgesic activity in medicinal plants. Therefore, *S. mahagoni* seed extract was also exploited to discover new analgesic drugs as alternatives to synthetic pain relief drugs.

This search on *S. mahagoni* seed methanolic extract could also reveal more about the pharmacological effects and phytochemistry cores of the seeds and simultaneously it would assist for the growth of new herbal drug in the Malaysian biotechnology industry.

1.1 OBJECTIVES

The objectives of this the present study is as follows:

- To conduct phytochemical analysis for major constituents of *Swietenia mahagoni* methanolic crude extract seed extract.
- To conduct acute oral toxicity study on *Swietenia mahagoni* methanolic crude extract.
- To evaluate antimicrobial activity on the test samples (methanolic crude extract) of *Swietenia mahagoni* seed
- To carry out various antioxidant tests on *Swietenia mahagoni* seeds test samples.
- To investigate the analgesic property of *Swietenia mahagoni* crude methanolic seed extract.

1.2 RESEARCH FLOW

Swietenia mahagoni seeds were macerated using methanol solvent to obtain *Swietenia mahagoni* crude methanolic (SMCM) extract. This crude extract was subjected to a few pharmacological screening using various standard methods (as described in Chapter 2: Materials and Methods) for analgesic, antioxidant, and antimicrobial activity evaluation. Based on the evaluation, SMCM seed extract retained good anti-Candida activity against *Candida albicans*. Therefore, further study was mainly focused on the identification and characterization of the bioactive for anti-Candida activity. The research flow for this study is clearly explained in Figure 1.1.

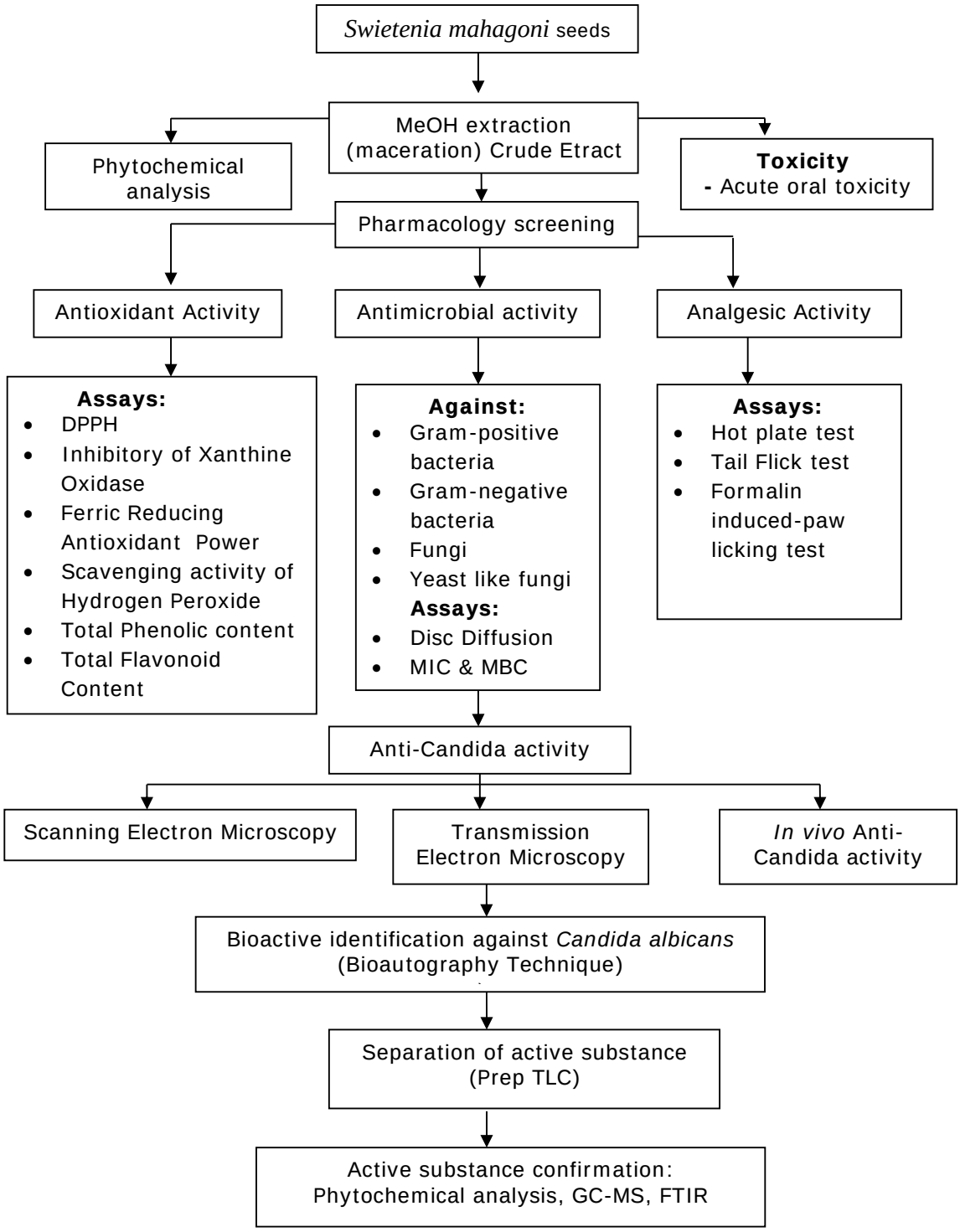

Figure 1.1: Research flow for the pharmacology and phytochemistry studies of *Swietenia mahagoni* seed extract.

CHAPTER TWO

LITERATURE REVIEW

2.1 Herbal drug

Therapeutic plants have been natural resources for the treatment of various diseases since ancient times. Medicinal plants would be the paramount resource to obtain a variety of drugs according to the World Health Organization (WHO) survey **(**Santos *et al.,* 1995 and Sukanya *et al.,* 2009); reports show that about 80% of individuals from developed countries are still accustomed to traditional medicine practice. WHO statistic testify that about 20,000 plant species are used as curative targets (Gullece *et al.,* 2006; Maregesi *et al.,* 2008) for hundreds of diseases.

Current surveys estimate that globally 25 % of prescribed drugs are derived from plants products and around 121 active compounds are presently in use. Indeed 11 % of the total 252 drugs in WHO's essential medicines list are exclusively from plant based origin (Rates, 2001). Almost 80 % of the African and Asian populace depends on traditional medicine for their primary healthcare (WHO, 2008). Mukherjee reported that the indigenous systems of medicine have dominated around 80% of the rural areas in India (Mukherjee and Wahile, 2006). In general, the information above indicates that herbal medicine has been widely used by people from all cultures throughout history. Examples of modern clinical medications derived from natural products are salicylic acid, a precursor of aspirin is originally derived from the white willow bark and meadowsweet plant; Quinine derived from cinchona bark actively use in treatment of malaria and vincristine obtained from periwinkle is used to treat certain types of cancer (Mukherjee and Wahile, 2006).

Searches reveal that, 74 % of pharmacologically active plant derived components were discovered after following up on the ethnomedicinal use of the plants (Ncube *et al.,* 2007). The growth of pharmaceuticals involves identification of active principles, its pharmacological effects, dosage formulations and clinical studies to establish safety, efficacy and pharmacokinetic profile of the novel drug (Iwu *et al.,* 1999; Ncube *et al.,* 2007). Hence, for the development of herbal drugs from natural sources, ethnobotanical and ethnopharmacological research is very essential.

In ethnobotanical approaches, there is three generation of plant drugs according to Iwu *et al.* (1999) classification. The first generation of herbal drugs is in the crude form of plant extract. Several first generation effective medicines used in their natural state are such as cinchona, opium, belladonna and aloe (Iwu *et al.,* 1999). The second generation of plant-based drug emerged after scientific processing of the plant extracts to isolate their active constituents. This generation of herbal drugs is pure molecules and the notable examples are quinine from *Cinchona*, reserpine from *Rauvolfia*, and taxol from *Taxus* species (Iwu *et al.,* 1999). The third generation of phytotherapeutic agent is usually developed based on top-bottom approach. It consists of clinical evaluation of the particular drug and followed by cytotoxicity, acute and chronic toxicity screening. An acceptable safety index substance will only be subjected to detail pharmacological/biochemical studies (Iwu *et al.,* 1999).

Ethnopharmacology is defined as the scientific study on medicinal plants which is correlated with ethnic groups, their health and how it is related to their physical condition. The ethnopharmacological approach is used to study the pharmacopoeias of Traditional Chinese Medicine (TCM), the European

pharmacopoeias, or the medicinal plants from traditional ethnic groups. Various pharmacological activities including analgesic, antimicrobial, antioxidant, anti-inflammatory, antidiabetic and other activities were undertaken in laboratory conditions. In a review by Maganha *et al.* (2010) it is stated that, *Hibiscus* genus has potential bioactive molecules, such as phenolic compounds, triterpene derivatives, phytosteroids, with antioxidant, cardioprotective, antihypertensive and antiproliferative effects (Maganha *et al.,* 2010). *Lycium barbarum* polysaccharide, showed antioxidative properties and some interesting pharmacological activities in the context of age related diseases such as atherosclerosis and diabetes (Potterat*,* 2010). This shows that the development of plant based therapeutic is growing quite remarkably. The main core for the usage of herbal medicine is that, it causes fewer side effects than pharmaceuticals. Common herbs function physiologically to restore balance in the body rather than just to target the symptoms. For this reason, herbs often tend to show effects more gradually but the effects derived are more efficient than the modern medicines.

Major pharmaceutical companies and academic researchers are undertaking extensive studies on all parts of plant materials. Ordinarily, leaves, fruits, bark, flowers, seeds, and roots are essentially targeted for their therapeutic effects. Sufficient understandings of the herbal system including biological, chemical, genetic and agronomic aspects are required for the formulation of an herbal medicine. Bioactive element reliability throughout the development process such as extraction, bio-guided assay, purification and shelf life is of utmost importance to certify medicinal effectiveness and consumer safety. Generally, various steps are involved in the development of an herbal medicine (Figure 2.1) starting from raw material collection to the isolation of bioactive compounds (Sahoo *et al.,* 2010).

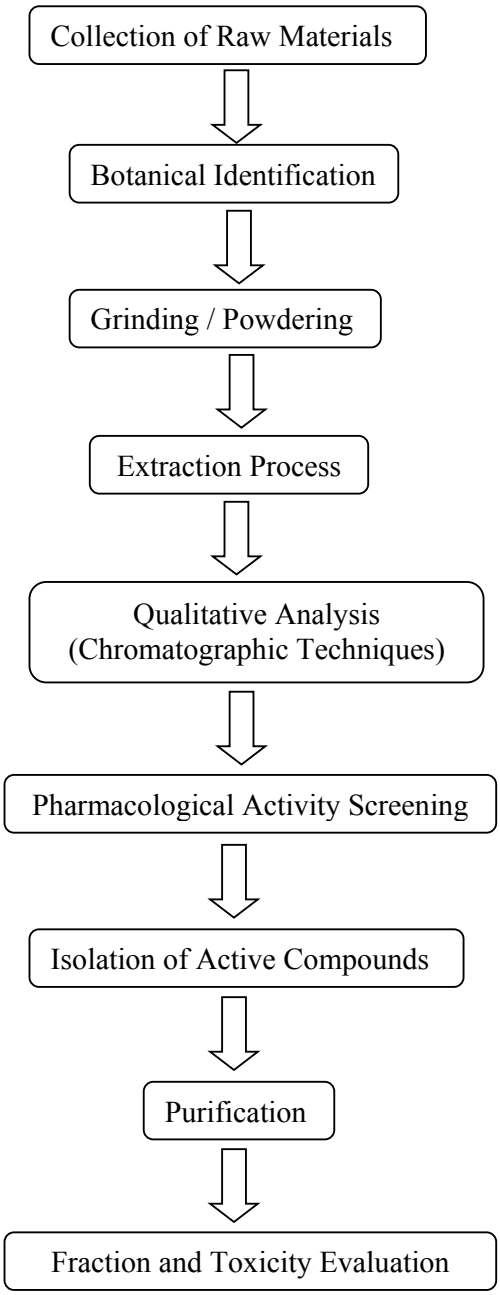

Figure 2.1: Steps involvend in the developmet of a phytomedicine in market (Sahoo *et al.*, 2010).

2.2 Herbal medicine in Malaysia

Nature has blessed Malaysia with a great quantity and quality of varied medicinal plants. At present, Malaysia has been rated the world's 12th mega biodiversity rich with its flora and fauna (Institute for Medical Research, 2002 and Ang, 2004). Various medicinal plants have been used for years in the daily life of Malaysians. Numerous studies have been carried out to survey the practices of traditional medicines in villages such as Gemenceh, Negeri Sembilan and Machang, Kelantan (Ong and Nordiana, 1999).

In Malaysia, commercial traditional herbal medicines, popularly known as "jamu" and "makjun", are readily available and consumed regularly (1–3 times daily) to promote health (Ali *et al.,* 2005). In a survey, Aziz and Tey (2009) state that, one in three Malaysians adults in urban areas use herbal medicines daily. A few major factors which influence the use of herbal medicines are gender, ethnicity, age, and perceived health status (Aziz and Tey, 2009). Aziz and Tey (2009) found that, Malays rank first in the use of traditional herbal medicines as compared to Chinese and Indians. Malays are 6 times more likely to use herbal medicines than Indian and 1.5 times higher than the Chinese (Aziz and Tey, 2009).

As such, the Drug Control Authority (DCA) of Malaysia implemented the Phase Three registration of traditional medicines on 1 January 1992. This Act necessitates for special emphasis on the quality, efficacy and safety in all pharmaceutical dosage in any form of traditional medicine preparations (National Pharmaceutical Control Bureau, 1993 and 1999; Ang, 2004).

A survey shows that, in 1997, Malaysian consumers spent about six times more on herbal product than the United States consumers per capita, with their respective populations of 22 and 273 milions (Malaysian National News Agency, 2006; Aziz and Tey, 2009). According to Frost and Sullivan market research report, the Malaysian pharmaceutical industry was valued at approximately USD 1.03 billion in 2007 and is estimated to reach USD 1.8 billion by 2013 (Tham and Yahya, 2009; Chua *et al.,* 2010). As to date, there are 234 pharmaceutical companies that are registered with the DCA with 67 companies involved in the production of modern medicines while the remaining 167 companies are local traditional and herbal medicine manufacturers (Malaysian Industrial Development Authority, 2009; Chua *et al.,* 2010).

The current situation of herbal medicine practices and development of the pharmaceutical industry in Malaysia highlights the importance of herbal drug discovery and development. With this in view, a traditionally used plant (*Swietenia mahagoni*) was selected to scientifically investigate and to determine the pharmacological effects of the plant in this thesis. The review of the plant species is described below.

2.3 Swietenia mahagoni

2.3.1 General view of plant

Swietenia mahagoni Jacq. is clustered under the Meliaceae family. It is a large, deciduous, and economically important timber tree which is native to the West Indies (Mulholland *et al.,* 1992; Chen *et al.,* 1997; Chen *et al.,* 2007). The common names for *S. mahagoni* (SM) are small leaved, West Indian, Spanish or Cuban mahogany, caoba, Madeira, coabilla, caoba dominicana, and acajou. The *S. mahagoni* Jacq. synonyms are *Swietenia mahogoni* (L.) Lam., *Swietenia fabrilis* Salisbury, *Cedrus mahogany* (L.) Miller.

S. mahagoni is a humid zone species, with natural distribution in the Caribbean region (South Florida, Bahamas, Antilles, Haiti and Jamaica) (Schmidt and Joker, 2000). The species is over exploited in much of its natural area of distribution and has been registered on CITES Appendix II (1992) as an endangered species. It has been extensively planted in southern Asia (India, Sri Lanka, Bangladesh) and in the Pacific (Malaysia, Philippines, Indonesia and Fiji), and has been introduced for cultivation in West Africa (Schmidt and Joker, 2000).

2.3.2 Botanical description of the plant

S. mahagoni is an evergreen to semi-evergreen tree and can grow up to 30-35 m. The bark of this tree is grey in colour and smooth when it is young and turns to dark brown, ridged and flaky when mature. The leaves are clustered, glabrous, 12-15 cm long and paripinnately compound with 2-4 pairs of leaflets. The leaflets are ovate-lanceolate shape, 5 to 6 cm long, 2 to 3 cm wide, dark green and glabrous,

while the flowers are unisexual, small and white to greenish in colour and in the form of 8 to 15 cm long slender panicles (Schmidt and Joker, 2000).

Figure 2.2: *Swietenia mahagoni* leaves and fruits.

2.3.3 Flowering and fruiting habit of *Swietenia mahagoni*

Mahoganies usually have ordinary annual flowering and begin bearing fruits 10 to 15 years of age. The *S. mahagoni* flowers are unisexual and the trees are monoecious. In general, the pollination occurs by insects. Usually only one flower of the inflorescence develops into a fruit with the other flowers being aborted, even though fertilization has taken place in the particular flowers. Generally, development of the flower into a mature fruit will take from 8 to 10 months. The flowering sessions vary according to the climate of the growth environment i.e. in a geographical site flowering usually takes place shortly before the rainy season. Based

on the observation, *S. mahagoni* flowering in Malaysia (Penang) occurs between April and July and the fruits mature between December and February.

Figure 2.3: *Swietenia mahagoni* flower.

2.3.4 Fruit and seed description of *Swietenia mahagoni*

The fruits are erect, 5 to 10 cm length, 3 to 6 cm in diameter, oblong, and usually are in 5-celled dehiscent capsules. The valves are thick and woody with a coriaceous surface when mature. Normally, the outer valves are 4 to 5 cm thick and the inner valves will be very thin (Schmidt and Joker, 2000). The fruit splits open from the base or simultaneously from the base and the apex when it is mature. The centre of the fruit has a thick, woody, and 5-angled columella part which extends to the apex. The seeds hang pendulous by their wing in the woody part. There are usually about 35 to 45 seeds per fruit (Schmidt and Joker, 2000).

The seeds are chestnut brown in colour, 4 to 5 cm long, compressed, crested and extended into a wing at the attachment end. The cotyledons are fused in the upper two thirds along the axial surface. In general, the seeds are dispersed by wind (Schmidt and Joker, 2000).

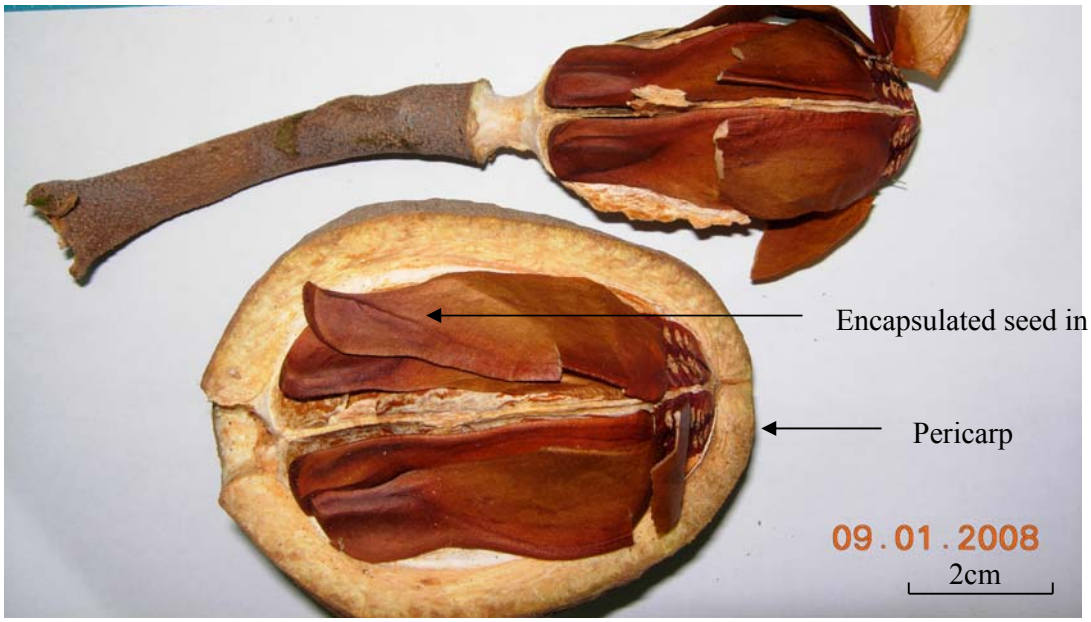

Figure 2.4: Fruit and seed within the wing.

Figure 2.5: Seed of *Swietenia mahagoni*.

2.3.5 General usage of *Swietenia mahagoni*

S. mahagoni has the potential for huge scale timber production plantations, especially in dry areas, due to its excellent timber quality. It is also used in agroforestry, for soil improvement and as an ornamental plant (Schmidt and Joker, 2000). *S. mahagoni* is closely related to the African genus Khaya and it is one of the most popular traditional medicinal plants in Africa.

2.3.6 Pharmacology effects of *Swietenia mahagoni*

S. mahagoni seeds have been applied as a folk medicine for the treatment of hypertension, diabetes, and malaria, while the decocted bark has been used as a febrifuge (Darzeil, 1937; Mulholland *et al.,* 2000; Chen *et al.,* 2007). The decocted bark of *S. mahagoni* is used as febrifuge which can be associated with its use as an antimalarial drug (Darzeil, 1937). *S. mahagoni* has also been used as an antipyretic, tonic, and astringent (Anon, 1986; Gautam *et al.,* 2007) and was used as folklore medicine in Puerto Rico (Antoun *et al.,* 2001; Gautam *et al.,* 2007). *S. mahagoni* has been reported to have medicinal uses, such as for the treatment of cancer, amoebiasis, chest pains and intestinal parasitism (Bascal *et al.,* 1997). The biologically active ingredients, tetranortriterpenoids and fatty acids are considered to be responsible for these therapeutic effects (Bascal *et al.,* 1997). In addition, 6-acetylswietenine and 6-acetyl-3-tigloyl-swietenolide from *S. mahagoni* has been shown to effectively reduce the number of rust pustules on detached groundnut leaves (Govindachari *et al.,* 1999a). Ether extract from the stem bark of *S. mahagoni* (studies from Alexandria, Egypt) showed potent activity against Spodoptera insects (Saad *et al.,* 2003). Whereas, Swietenialides A, B, and C; swietenialides D and E and mexicanolide, 2-hydroxyswietemin showed antifeedant activity against *S. littoralis*

(Boisduval) (Saad *et al.,* 2003). The bark of *S. mahagoni* has been used as astringent for wounds due to the active tannin substances caused by the rich red colour (Falah *et al.,* 2008). The seeds of *S. mahagoni* have been used for leishmaniasis and as abortion medicine by an Amazonian Bolivian ethnic group (Bourdy *et al.,* 2000). The seed extract also can be used as a potential agent for diabetes therapy because it shows agonistic activity to peroxisome proliferator-activated receptor γ (PPARγ) and can ameliorate the blood glucose levels of diabetic db/db mice (Li *et al.,* 2005). To support this finding, De, and coworkers found an aqueous-methanolic extract of *S. mahagoni* seed is potential for the correction of diabetes and its related complications like oxidative stress and hyperlipidemia (De *et al.,* 2011). Other than these, swietemahonin A, D, E, and G and 3-O-acetylswietenol ide and 6-O-acetylswietenol ide, from the seeds showed a strong inhibition against platelet-activating factor (PAF)-induced aggregation *in vitro* and *in vivo* assays (Ekimoto *et al.,* 1991). Recent studies in Bangladesh showed that the chloroform extract of the seed and ethyl acetate extract of bark both demonstrated good cytotoxic activities. In addition, both chloroform and ethyl acetate extracts of the leaf and bark demonstrated a good activity against human pathogenic bacteria. The chloroform seed extract exhibited antimicrobial activity only against *Bacillus megaterium*, *Salmonella paratypi, Shigella dysenteriae, Pseudomonas aeroginosa* and *S. boydii* (Haque *et al.,* 2009). The isolated active principle 2-hydroxy-3-O-tigloylswietenolide from the *S. mahagoni* seed shows potent activity against tested multiple-drug-resistant bacterial strains (clinical isolates: *Streptococcus aureus, Staphylococcus aureus, Streptococcus pneumonia, Haemophilus influenzae, Escherichia coli, Klebsiella pneumonia, Salmonella typhi,* and *Salmonella paratyphi*) (Rahman *et al.,* 2009).

2.3.7 Chemical substances identified from *Swietenia mahagoni*

Previous chemical investigations on this West Indies mahogany species from a few countries such as India, Indonesia, and Egypt have led to the isolation of more than 40 limonoids belonging to the structural types of gendunin, mexicanolide, and phragmalin (Mulholland *et al.,* 1992; Saad *et al.,* 2003; Chen *et al.,* 2007). Currently two novel limonoids, swiemahogins A and B were isolated from the twigs and leaves of *S. mahagoni.* They are the first examples of andirobin and phragmalin types of limonoids from twigs and leaves reported by Chen *et al.,* (2007). Furthermore, a tetranortriterpenoid, 6-Desoxyswietenine was also identified by Govindachari *et al.,* (1999b). A number of limonoids have also been reported from the genus Swietenia (the true mahoganies) with structures assigned on the basis of spectral data (Kadota *et al.,* 1990a). Ever since two rings B,D-seco limonoids of methyl angolensate and its 6-hydroxy derivative were isolated by Taylor (1969) many mexicanolide-type compounds of rings B,D-seco limonoid having a bicyclo[3,3,1]-ring system such as swietenin (Connolly *et al.,* 1965) have been isolated from *S. mahagoni* (Kadota *et al.,* 1990a). A subsequent study of the leaves, indicates the presence of seven new phragmalins possessing an orthoester group at 8,9,30-position (swietephragmins A–F and G), together with two new different type rings B,D-seco limonoids (2-hydroxy-3-O-tigloylswietenolide and deacetylsecomahoganin). Three known limonoids [methyl 6-hydroxyangolensate (Adesogan and Taylor, 1968), swietemahonin G (Kadota *et al.,* 1990b) and 7-deacetoxy-7-oxogedunin (Kadota *et al.,* 1990a) were also isolated from the leaves of *S. mahagoni*. New compounds from the seeds of *S. mahagoni*, named swietemahonin A, D, E, and G and 3-O-acetylswietenol ide and 6-O-acetylswietenol ide were isolated by Ekimoto research team (Ekimoto *et al.,* 1991). From the seeds of *S. mahagoni*, 18 tetranortriterpenoids,

(five swietenins (B-F), three acylswietenolides, seven swietemahonins (A-G), swietemahonolide, mahonin and secomahoganin related to swietenine and swietenolide were isolated and identified (Kadota *et al.,* 1990c).

2.3.8 Conclusion

This review illustrates that the plant species is widely used traditionally for its pharmacological effectiveness. However the scientific investigation of S*wietenia mahagoni* seed in terms of its likely antimicrobial, antioxidant, analgesic activity are still lacking in literature. The toxicity aspects of *Swietenia mahagoni* seed extract yet to be reported. These pharmacological findings will assist the herbal drug discovery and development in Malaysian pharmaceutical and biotechnology industries.

2.4 Toxicity

Practices of traditional medicine vary greatly according to the place and influenced by culture, history, personal attitudes and philosophy factors. However, there is lack of scientific investigation in the safety and efficacy for the most of herbal medicines. Herbal medicines contain bulk of active organic compounds and are employed in the treatment of diseases of diverse origins. The practitioners most often prepare the medicines with a combination of two or more plant products for the treatment of more than one disease condition (Pieme *et al.,* 2006; Ogbonia *et al.,* 2009).

Herbal drugs usage are very vast, the doses are not quantified and most importantly toxicity of these plants' drugs are largely unknown (Saad *et al.,* 2006). The danger associated with the potential toxicity of such therapy and other herbal therapies used over a long period of time demand that the practitioners be kept

abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs (Tedong *et al.,* 2007; Ogbonia *et al.,* 2009). Although a number of scientific researchers have revealed activities of so many plants not many people venture into studying toxicity of these plant materials.

Currently a lot of reports are alarming side effects and toxicity resulting from the ingestion of herbal medicines. The standardized extract of *Ginkgo biloba* has an inhibitory action on blood pressure and it may influence cortisol release in response to some stress stimuli (Jezova *et al.,* 2002), may cause hemorrhage and hyphema (Schneider *et al.,* 2002) and may increase the risk of bleeding or potentiate the effects of warfarin therapy (Heck *et al.,* 2000). Plant flavonoids actually have the capacity to become carcinogenic at higher levels. High doses of these chemicals also carry other health risks including a small but documented risk for a rare form of leukemia in young children. Ginseng reinforces warfarin action by heterogeneous mechanisms. It should thus not be used in patients on oral anticoagulant and/or antiplatelet therapy (Argento *et al.,* 2000) and Ashwagandha is a potential source of hypoglycemic, diuretic and hypocholesterolemic agents (Andallu and Radhika, 2000). The current evidence suggests that oral evening primrose oil does not provide clinically significant improvement in persons with atopic dermatitis, and that it is also likely ineffective for the treatment of cyclical mastalgia and premenstrual syndrome (Bayles and Usatine, 2009) and hepatotoxic reactions have been observed after the ingestion of Senna (Stickel *et al.,* 2001) .

The *in vivo* toxicity study in animal is more indicative toxicity safety markers. Animals' toxicity tests are conducted prior to human clinical investigation as part of non-clinical laboratory tests of pharmaceuticals.

2.4.1 Acute oral toxicity in animal models

Acute toxicity is usually defined as unfavorable effects occurring within a short period of time with administration of a single dose or multiple doses substance(s) within 24 hours (Walum, 1998). Toxicity effects generally will be classified as any affect that results in functional destruction and/or biochemical lesions that may affect the performance of the whole organism or reduce the organ's ability (Rhodes *et al.,* 1993; Walum, 1998). The term acute toxicity is most often used in connection to lethality and median lethal dose (LD_{50}) determination.

The rationale behind of acute toxicity investigation is to collect information on the biologic activity of a tested material and gain insight into its mechanism of action. The dosed animal is closely observed during the first 24 hour and day by day for as long as two weeks and the behavioral changes are noted. To examine the toxic level of the herbal remedies, basic toxicity evaluation should be carried out according standard protocol.

There are a few numbers of toxicity evaluation guidelines such as Food and Drug Administration (FDA) guidelines for food and colour additives toxicity level testing (Rulis and Levitt, 2008); European Union (EU) guidelines to examine industrial chemical toxicity stage (Ambachtsheer, Kron, Liroff, Little, and Massey, 2007) and Organization for Economic Co-operation and Development (OECD) guidelines for evaluation of chemicals and plant extract toxicity intensities (Jaijoy, Soonthornchareon, Lertprasertsuke, Panthong, and Sireeatawong, 2010).

Here in, OECD guidelines 423 (adopted: 2001) was used for the evaluation of acute oral toxicity evaluation of SMCM seed extract. The OECD 423 guideline is

based on acute toxic class method where the toxicity evaluation is based on the stepwise dosing procedure using three animals of a single sex. This method is reproducible which uses a few animals only and able to rank toxicity of the test substance. In principle, the OECD guideline 423 is not intended to calculate precise LD_{50}, but does allow for the determination of defined exposure ranges where lethality is expected ("OECD guidelines for testing of chemicals 423," 2001).

2.5 Antimicrobial activity

Infectious diseases are the leading cause of deaths world-wide. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow *et al.,* 2003) and currently it has become a crucial global concern (Westh *et al.,* 2004; Ghalem *et al.,* 2009). Human fungal infections have increased at an alarming rate in the last 20 years, mainly among immune-compromised individuals (Perea and Patterson, 2002; Scorzoni *et al.,* 2007). New data indicate that the relative proportion of organisms causing nosocomial bloodstream infections has increased over the last decade (Scorzoni *et al.,* 2007). Now Candida species is being firmly established as one of the most frequent agents among nosocomial bloodstream infections. Candidemia normally will lead to high mortality and also extends the length of hospitalization of a person and increases the costs of medical care. *Candida* species usually spread widely as they reside on plants, alimentary tracts of mammals and as also on human mucocutaneous membranes (Goncalves *et al.,* 2006; Scorzoni *et al.,* 2007). In the human gastrointestinal tract, around 50-70% of total yeast isolates were identified as *Candida albicans*. A number of antifungal drugs, such as polyene macrolides (Amphotericin B) and azoles (Itraconazole and Fluconazole) are currently used in

antifungal therapies. However, these antifungal agents endure a number of restrictions that can render their use complicated. For an example, Amphotericin B is associated with dose-limiting nephrotoxicity, while flucytosine and azoles showed resistant due to the rapid development among pathogenic strains, drug-drug interactions among the antifungal agents and fungi-static mode of action. As such the search of an alternative antifungal with broad fungicidal spectrum of action, and with fewer dose-limiting side effects (Graybill, 1996; Maertens and Brogaerts, 2000; Scorzoni *et al.,* 2007) are of importance. Herbal medicines have been proven to be an excellent source of drugs, and this could serve in search of novel compounds with potential antifungal activity.

Antifungal and antibacterial properties have been reported more frequently in a wide range of plant extracts in an attempt to discover new bioactive drugs that could resolve the infectious disease problems (Ali *et al.,* 2001; Ho *et al.,* 2001; Osibote *et al.,* 2010). Countless infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. At present, a number of drugs to treat bacterial and other infections are being isolated from natural sources including ethnomedicinal plants (Coe and Anderson, 1996). In accordance to WHO report, 20 000 plant species are presently in use for medicinal purposes (Scorzoni *et al.,* 2007). Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug development. For example, the root crude extract of *Garcinia atroviridis* (asam gelugor) predominantly exhibited antibacterial activity with strongest inhibition against the test bacteria at a minimum inhibitory dose (MID) of 15.6 µg/disc (Mackeen *et al.,* 2000). The essential oil of *Satureja cuneifolia* (used to produce essential oil and aromatic water) exhibited antimicrobial activity against selected food borne and spoilage bacteria

(Oke *et al.,* 2009). Du *et al.* (2003) reported that, two novel antifungal activity triterpene saponins were isolated from aerial parts of a Tibetan herbal medicine *Clematis tangutica*. Recent finding stated that, orange essential oil was the most effective against *Aspergillus niger*, mandarin essential oil was most effective at reducing growth of *Aspergillus flavus* while grape fruit was the best inhibitor of *Penicilium chrysogenum* and *Penicillium cerrucosum* (Viuda-Martos *et al.,* 2008). There is a continuous and urgent need to discover new antifungal and antibacterial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.,* 2003; Kage *et al.,* 2009).

2.5.1 Antimicrobial sensitivity test

Antimicrobial activity of plants can be detected by observing the growth response of microorganisms towards the plants extracts/fractions/pure compound. This antimicrobial sensitivity test is used to establish the degree and spectrum of *in vitro* activity of a new antimicrobial drug. At present, the available methods for antimicrobial screening are such as agar diffusion, broth dilution and bioautographic techniques.

2.5.2 Agar-based disc diffusion Assay

Agar-based disc diffusion assay is effective because of its simplicity and low cost. In the diffusion technique, the organism under investigation is exposed to a reservoir-containing drug (plant extract) to be tested by an impregnated disc and the diameter (mm) of the zone around the reservoir (inhibition diameter) is measured (Figure 2.6). In order to lower the detection limit, the inoculated system is kept at low temperature during several hours before incubation, which favors the diffusion