SCREENING OF PHYTOCHEMICALS AND BIOLOGICAL ACTIVITY (ANTIFUNGAL, ANTIBACTERIAL, AND ANTICANCER) OF MEDICINAL PLANTS

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THIS DISERTATION IS PRESENTED TO FULFILL PART OF THE REQUIREMENT TO OBTAIN BACHELOR OF SCIENCE WITH HONOURS

BIOTECHNOLOGY
SCHOOL OF SCIENCE & TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

2007

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

UDUL: Screening of Phytochemicals and Biological Activity (Antifungal, Antibacterial, and Anticancer) of Medicinal

azah: Sarjana Muda Kepujian Bioteknologi

SESI PENGAJIAN: 2004/2005

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CONFESSION

I hereby confess this project report is based on my laboratory work except for adaptations from other sources as stated.

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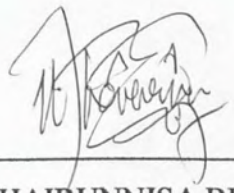

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ACKNOWLEDGEMENT

Firstly, I would like to forward my gratefulness to Allah for guiding me throughout the completion of my final year project. Thanks to Dr. Jualang Azlan Gansau, my supervisor, for helping me all along the process. Thank you for the support and ideas you've shared. Not forgetting my co-supervisor, Prof. Ho Coy Choke, thank you for the support and advices given.

To Mr. Frank and Patrick from Orchid De Villa in Inanam, thank you for helping me selecting samples and sponsoring samples for me. I would like to express my deep appreciation to my beloved Father, Ibrahim bin Gusti, and Mother, Farhana Loo Abdullah, my siblings, and my dearest, Syed Abdul Hadi bin Syed Hamzah, thanks for the motivations and love. I personally thank Kumuda Suppayah and Chee Jo Anne who are always there when it rains to motivate me, support me, and making my life so cheerful and fun. Not forgetting Farrah Mohd. Hatta, Siti Aishah binti Rafee, and Aizati Azlin Khairul Anwar for being there whenever I am down and thank all of you for motivating me and supporting me.

Thanks to Miss Radizah, Madam Rokiah, Mr. Musbah, and Mr. Rizal for making things a lot easier in laboratory. To Miss Aina and Mr. Ho, thank you for the guidance given. Thanks to my course mates for giving me ideas and helping in finishing this project.

ABSTRACT

This project was conducted to study the presence of phytochemicals (alkaloid, saponin, and anthraquinone) and the biological activity (antifungal, anticabterial, and PP1 inhibitor) of plant samples. A total of nine samples were collected from Orchid De Villa located in Inanam, and one sample was collected around University Malaysia Sabah Campus. All 10 samples were extracted using acetone, methanol and chloroform solvent. Dragendroff's reagent and Wagner's reagent were the two indicator used to test the presence of alkaloid. The result obtained showed that all samples have alkaloid constituents. Foam test and Liebermann-Burchard (LB) test were conducted to testify presence of saponin in all of the samples. The results obtained showed that all samples have negative result for foam test and only *Terminalia catappa* showed positive result for LB test. For anthraquinone test, no sample showed positive result for both free anthraquinone and C-glycoside bounded anthraquinone. Antifungal screening system involves only *Candida albicans*. Samples that showed inhibition zone were *Mimosa pudica* (2.0 cm), *Elephantopus tomentosus* (2.4 cm), *Dendrobium crumenatum* (1.7 cm), *Opuntia dillenii* (1.5 cm), *Terminalia catappa* (1.6 cm), and *Curcuma longa* (2.0 cm). Antibacterial screening involves screening against *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhi*. Extract of *Opuntia dillenii* (1.3 cm), *Terminalia catappa* (1.4 cm), and *Phyllanthus urinaria* (1.3 cm) were testified to have anti-*Escherichia coli* activity. As for *Bacillus subtilis* screening, all 10 samples showed positive result. In *Salmonella typhi* test, all samples showed inhibition except for *Elephantopus tomentosus* and *Curcuma longa* extracts. For PP1 inhibitor screening, *Terminalia catappa*, *Polygenum chinense,* and *Phyllanthus urinaria* inhibit both wild (PAY 704-1/ H10.018) and mutant (PAY 700-1/ H10.017) strain of yeast cancer cell. *Orthosiphon aristatus*, *Curcuma longa* and *Phyllanthus urinaria* are also testified to have inhibiiton. However, samples that showed inhibition were testified to be toxic towards both wild and mutant strains. Therefore, none of the samples are potential PP1 inhibitor.

ABSTRAK

Projek ini dijalankan untuk mengkaji kehadiran sebatian fitokima (alkaloid, saponin, dan antrakuinon) dan aktiviti biologi (antifungi, antibakteria, dan antikanser) sampel tumbuhan. Sembilan sampel diambil di Orchid De Villa, Inanam, dan satu sampel diambil di sekitar Kampus Universiti Malaysia Sabah. Kesemua sampel diekstrak menggunakan aceton, metanol, dan klorofom. Reagen Dragendroff dan Wagner adalah penunjuk digunakan untuk mengkaji kehadiran sebatian alkaloid. Keputusan menunjukkan semua sampel mempunyai alkaloid. Ujian Biuh serta ujian Liebermann-Burchard (LB) diadakan untuk mengkaji kehadiran saponin dalam sampel. Keputusan ujian Biuh menunjukkan semua sampel tidak mengandungi saponin. Bagi ujian LB, hanya *Terminalia catappa* menunjukkan kehadiran saponin. Semua sampel memberikan keputusan negatif untuk ujian kehadiran antrakuinon bebas dan antrakuinon terikat pada C-glikosidik. Ujian antifungi melibatkan *Candida albicans* sahaja. Sampel yang didapati berpotensi merencat pertumbuhan *C. albicans* ialah *Mimosa pudica* (2.0 cm), *Elephantopus tomentosus* (2.4 cm), *Dendrobium crumenatum* (1.7 cm), *Opuntia dillenii* (1.5 cm), *Terminalia catappa* (1.6 cm), dan *Curcuma longa* (2.0 cm). Ujian antibakteria melibatkan *Escherichia coli*, *Bacillus subtilis*, dan *Salmonella typhi*. Ekstrak *Opuntia dillenii* (1.3 cm), *Terminalia catappa* (1.4 cm), dan *Phyllanthus urinaria* (1.3 cm) mempunyai potensi merencat pertumbuhan *Escherichia coli*. Dalam ujian anti-*Bacillus subtilis*, semua sampel mempunyai potensi sebagai perencat. Untuk ujian perencatan *Salmonella typhi* pula, hanya *Elephantopus tomentosus* dan *Curcuma longa* tidak menunjukkan keputusan positif. Ujian antikanser yang dijalankan menunjukkan *Terminalia catappa*, *Polygonum chinense*, dan *Phyllanthus urinaria* mempunyai aktiviti perencatan. Sampel-sampel tersebut merencat pertumbuhan kedua-dua yis iaitu jenis liar (PAY 704-1/H10.018) dan jenis mutan (PAY 700-1/H10.017). *Orthosiphon aristatus*, *Curcuma longa*, dan *Phyllanthus urinaria* juga menunjukkan perencatan. Walau bagaimanapun, sampel-sampel tersebut adalah toksik terhadap yis yang digunakan.

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Lists of Symbol

°C	Celsius
β	Beta
α	Alpha
γ	Gamma
µl	microliter
ml	mililiter
pH	Hydrogen concentration
mm	millimeter
cm	centimeter
g	gram
%	percent
g/ml	gram per milliliter
(v/v)	volume over volume
(w/v)	weight over volume
mg	miligram
M	Molar
m	meter
C	Carbon
O	Oxygen
Mg	Magnesium
Ca	Calcium
kDa	kiloDalton
$Bi(NO_3)_2$	Bismuth (III) Nitrate
$HgCl_2$	Mercury Chloride
KI	Potassium Iodide
H_2SO_4	Sulfuric Acid
$NaSO_4$	Sodium Sulphate
$CHCl_3$	Chloroform
NH_4OH	Ammonium Hydroxide

NaOH	Sodium Hydroxide
$FeCl_2$	Ferum (II) Chloride
HCl	Hydrochloric Acid
Rpm	Rotation per minute
PP1	Protein Phosphatase Type 1
CGA	C-Glycoside bounded anthraquinone.

CHAPTER 1

INTRODUCTION

Plant is the most important organism in today's world as it is the biggest and the first organism in food chain which contributes to conversion of energy from one organism to another. However, plants do not only work as energy supplier but also as medicine. These types of plants are better known as medicinal plant. This project main scope is to screen phytochemicals from these medicinal plants and also to screen its biological activity. Plants in today revolutionized world are used for screening of pharmaceuticals property. Plant produces secondary metabolites that are testified to fight some diseases by interacting with the sources. It is very important to find out the precious treasure hidden in plants to improve health.

Malaysia is a well known country with its green scenery where variety of plants can be obtained. Compounds produced by plants are further tested for its potential in medicinal property. This leads to new drug discovery in the whole world. Phytochemicals research in Malaysia started as early as 1950s and centered in University Malaya Singapore. January 19979, research on medicinal plants started with

the establishment of Traditional medicine Research team. (KUBATRA). This team consists of chemists, botanists, microbiologists and zoologists which approach multidiscipline (Ikram, 1995). Research on biological activity of medicinal plants is getting more important as the increase of population leads to high deman of food, land, and infrastructures (Clegate & Molyneux, 1993).

Phytochemicals screening is a method of testing plant extracts for presence of phtochemicals like saponin, alkaloid, antraquinone, tannin and other natural compound as desired. Plant extracts are also used in biological activity screening. This is done by conducting assays for plant extracts against microorganism to find out whether the plant has medicinal property.

There are many commercialized plants that are known to cure and prevent diseases but this does not mean that research on other plants for new drug discovery should stop. Research should be done continuously to discover the most efficient drugs to treat certain diseases. Besides that, certain microorganisms that cause diseases in human have the ability to be resistance towards certain drugs.

The objectives of this project is to prepare plant extracts using various solvents, screen sample for the presence of phytochemicals (saponin, alkaloid, and anthraquinone), and to test the biological activity of plant samples (antibacterial, antifungal and PP1 inhibitor).

CHAPTER 2

LITERATURE REVIEW

2.1 Medicinal Plants

Medicinal plants are plants that have medicinal property. These plants are used for curing infections and diseases. Ancestors attempted thousands of time in using plant as medicine as they believe that plants are gifts from God (Kapoor, 1990). With latest technology, we can discover the miracle of these medicinal plants by researching the plant chemical substances that work actively against infections and diseases. Plants are known to have active compounds that can be use for therapeutic purpose. Some of the diseases that is usually cured using medicinal plants includes cough, flu, diarrhea, headache, malaria, high blood pressure, diabetes mellitus, gastric, and many more (Fasihuddin & Hasmah, 1993).

2.2 Phytochemical Screenings

Phytochemical is chemical substances derived from plant for example alkaloid, saponin, tannin, and phenolic compound (Padua *et al.*, 1999). Phytochemicals is secondary metabolite produce by plants. It is not a necessity for a plant to use it for metabolite activity. Infact, the presence of phytochemical did not result in any deficiency for the plant (V&HSG, 2006). However, it is important in protecting the plants from environmental pressures or in controlling the growth of plant. Plant has pharmaceutical property and this gives it a very high value when it comes to chemical substances it contains. There are two product produce by plants: Primary metabolite and secondary metabolite.

Primary metabolites have cell function that works as building unit for all components of cell. This includes amino acids, nucleic acid, lipid and carbohydrate. Secondary metabolite has no function for cell. However, it might function as antibacterial agent that makes the plant resistance towards certain bacteria for example alkaloid.

2.2.1 Alkaloid

The word alkaloid is derived from the word alkaline which is derived from Arabic word al-qali. Alkaloid has been used in extracted form for a very long time as poison; for example narcotic and medicine (Kutchan, 1995). Alkaloid has structure of 1 or more nitrogen and it is basic due to the presence of nitrogen. It is toxic to

human and has physiological activity. It is generally colourless but a few with colour like berberine and betaine; bitter and it is an optically active compound. Alkaloid is in liquid form in room temperature (Harbone, 1984). Alkaloid is soluble in non-polar solvents like chloroform or ether (Ali, 2002).

Alkaloid can be differentiated into a few groups based on its chemical structure; heterocyclic or non-heterocyclic which is divided into 12 major groups based on the ring structure (Padua *et al.*, 1999). Alkaloid is divided into five main groups: alkaloid with Nitrogen ecocyclic and amine aliphatic, alkaloid putresine, spermidine, and spermine, alkaloid peptide, alkaloid terpene and steroid, and alkaloid heterocyclic.

Alkaloid can normally be found in actively growing tissue, cell epidermis and hypodermis. It is waste derived from plant as nitrogen source. It plays an important role in supporting growth of plant. It is used as medication as analgesic or better known as pain relievers like narcotic and morphin; and as anesthetic. Most alkaloid dysfunction as they are degraded at temperature above 70°C (Fasihuddin & Hasmah, 1993). Nicotin is the first known alkaloid to be poisonous and has potential as pesticide (Kutchan, 1995).

Normally, to testify the presence of alkaloid, sample is tested with six different reagents; Dragendroff's, Mayer's, Wagner's, Hager's, Tannic acid test, and Ammonia Reineckate Test (Ali, 2002). All six tests must be performed in conformational of the alkaloid substance. However, due to limited chemical supply,

I will only conduct the Dragendroff's, Mayer's and Wagner's test. Dragendroff's reagent consists of potassium iodide and bismuth nitrate, the addition of this reagent will form orange coloured precipitation if there is alkaloid in sample tested. Mayer's reagent is potassium mercury-iodide solution, White or pale colour of precipitation will indicates presence of alkaloid except alkaloids with purine group and a few others. As of Wagner's, Iodide solution, brown or reddish colour precipitation indicates presence of alkaloid.

In plants, alkaloid acts as poisonous and stimulating agents (Ali, 2002). For example Tomatin, a major alkaloid in tomato that works as repellent. Alkaloids are capable to exhibit extensive and well-marked pharmacological activities like analgesic, antiamoebic and emetic, anticholinergic, antihypertensive, antimalarial, antitumor, antitussive, cardiac depressant, central nervous stimulant, diuretic, oxytocic, ophthalmic and cholinergic, skeletal muscle relaxant, and smooth muscle relaxant.

There are no systematic structural classification exists for alkaloids. However, there most widely accepted classification is true alkaloids, protoalkaloids, pseudoalkaloids. True alkaloids are toxic. It shows a wide range of physiological activity. This group's alkaloids contain nitrogen in a heterocyclic ring, derived from amino acids and normally occur in plants for example colchine, quinine, morphine, emetine etc.

Protoalkaloids are simpler amine in which the amino acid nitrogen is not in a heterocyclic ring for example mescaline and ephedrine. Pseudoalkaloids are not derived from amino acid precursor and are usually basic in nature for example steroidal alkaloid and purines.

2.2.2 Saponin

Saponins are an important group of glycosides which are widely distributed as plant constituents. It is the glycoside of steroid, steroid alkaloids or triterpenes which are found in plants especially plant with waxy protective coating. The most important saponin-containing drugs are Quillaia and Senega. Most of saponins are neutral and soluble in water. Saponins are hydrolyzed to form sugar, normally dextrose, and an aglycone, generally known as sapogenin (Ali, 2003). The sapogenins are soluble in water and in weak alcohol. Sapogenins may be steroid or triterpene and the sugar moiety maybe glucose, galactose, pentose or a methylpentose. Saponin with addition of water forms colloidal solution which forms froths on shaking and it produces stable emulsion on shaking with oil and fats.

Saponins are highly complex glycosides which are widely distributed in the higher plants. Saponin can cause haemolysis of red blood corpuscles, even at great dilution. Most of saponins are toxic to be injected to human body but it is much less toxic when consumed due to its haemolysis property mentioned earlier. Saponins are also very toxic to fish. Any markedly toxic saponins are known as sapotoxin.

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