

**SCREENING FOR INHIBITORS FROM MEDICINAL PLANTS EXTRACTED
AGAINST BACTERIA, FUNGI, GSK-3 β AND MKK1**

KOAY KEAN HUANG

**THIS DISSERTATION IS SUBMITTED TO FULFILL THE PARTIAL
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extracted against bacteria, fungi, GSK-3 β dan MEK1

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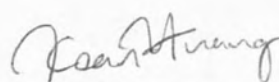
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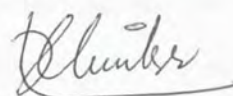
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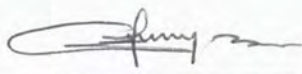
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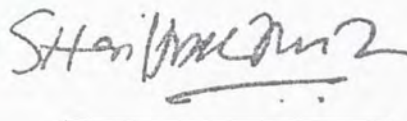
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4. DEAN

(SUPT/KS PROF MADYA

DR. SHARIFF A. KADIR S. OMANG)





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ABSTRACT

The objective of this project was to extract secondary metabolites from medicinal plants and to screen for phytochemicals; alkaloids, saponin, cardiac glycoside, tannin and polyphenol in the samples. It was also in determining the inhibition potential of plants extraction towards serine/threonine protein kinases, especially GSK-3 β and MKK1, bacteria and fungal. Total of 10 medicinal plants which are *Nephrolepis auriculata*, *Selaginella doederleinii*, *Dicranopteris linearis*, *Bauhinia purpurea*, *Lonicera japonica*, *Synediella nodiflora*, *Cycas revoluta*, *Strobilanthus crispus*, *Opuntia dillenii* and *Kyllinga brevifolia*, have been collected from Orchid De Villa, Inanam, Sabah. Only methanol extract has been tested for phytochemical analysis. In alkaloid test, *Lonicera japonica* showed precipitation in Mayer, Wagner and Dragendroff test while *Cycas revoluta* showed slight cloudy only in Dragendroff test. Gelatin test and FeCl₃ test was performed in tannin and polyphenol test. *Nephrolepis auriculata* and *Opuntia dillenii* extracts showed presence of tannin while *Dicranopteris linearis*, *Cycas revoluta* and *Kyllinga brevifolia* extracts showed presence of polyphenol. *Selaginella doederleinii*, *Bauhinia purpurea*, *Lonicera japonica*, *Synediella nodiflora*, *Cycas revoluta*, *Strobilanthus crispus*, *Opuntia dillenii* and *Kyllinga brevifolia* showed the presence of cardiac glycoside. In saponin test, foam test was carried out, *Nephrolepis auriculata*, *Selaginella doederleinii*, *Dicranopteris linearis*, *Bauhinia purpurea*, *Lonicera japonica*, *Synediella nodiflora* and *Kyllinga brevifolia* extracts showed positive result; meanwhile *Strobilanthus crispus* and *Opuntia dillenii* showed negative result mean the presence of free acid after added Na₂SO₄. In bacteria screening system, only *E. coli*, *Staphylococcus aureus* and *Bacillus subtilis* showed inhibition activity; meanwhile there is no activity against *Samonella typhi* and *Enterobacter aerogenes* and also *Candida krusei* and *Candida albicans*. Yeast strains H10075 transformed with pKT10-gsk-3 β and latter transformed with YEP24-MCK1 was used n GSK-3 β screening system. This system is based on temperature sensitivity (25 °C and 37 °C), in which this transformed yeast strain are able to suppress temperature sensitivity phenotype. Results found that no GSK-3 β potential inhibitors found from the samples. In MKK1 screening system, all methanol extracts showed potential inhibitory activity. This system utilizes mutant yeast MKK1^{P386} which is under control of the strong GAL1p. Overexpression of the mutant gene will cause cell growth arrest with the addition of galactose.



ABSTRAK

Objektif kajian ini adalah untuk mengekstrak metabolik sekunder daripada tumbuhan ubat-ubatan dan penyaringan fitokimia; alkaloid, saponin, glikosida kardium, tanin dan polifenol daripada sampel-sampel. Ia juga bertujuan untuk mengkaji sample yang berpotensi untuk merencatkan serine/threonine protein kinases, terutamanya GSK-3 β and MKK1, antibakteria dan antifungi. Sebanyak 10 sampel yang digunakan dalam kajian ini, iaitu *Nephrolepis auriculata*, *Selaginella doederleinii*, *Dicranopteris linearis*, *Bauhinia purpurea*, *Lonicera japonica*, *Synediella nodiflora*, *Cycas revoluta*, *Strobilanthus crispus*, *Opuntia dillenii* and *Kyllinga brevifolia*. Ekstrak metanol telah digunakan untuk kajian fitokimia. Untuk alkaloid, *Lonicera japonica* menunjukkan mendakan setelah ditambah reagen Mayer, Wagner dan Dragendroff, *Cycas revoluta* pula menunjukkan kekeruhan yang tidak jelas hanya pada ujian Dragendroff. Ujian gelatin dan FeCl₃ digunakan dalam penyaringan tanin dan poliphenol, ekstrak *Nephrolepis auriculata* dan *Opuntia dillenii* telah menunjukkan kehadiran tanin manakala ekstrak *Dicranopteris linearis*, *Cycas revoluta* dan *Kyllinga brevifolia* telah menunjukkan kehadiran polifenol. Ekstrak *Selaginella doederleinii*, *Bauhinia purpurea*, *Lonicera japonica*, *Synediella nodiflora*, *Cycas revoluta*, *Strobilanthus crispus*, *Opuntia dillenii* dan *Kyllinga brevifolia* menunjukkan kehadiran glikosida kardium. Untuk saponin, ujian buih digunakan, ekstrak *Nephrolepis auriculata*, *Selaginella doederleinii*, *Dicranopteris linearis*, *Bauhinia purpurea*, *Lonicera japonica*, *Synediella nodiflora* dan *Kyllinga brevifolia* menunjukkan keputusan positif; manakala *Strobilanthus crispus* dan *Opuntia dillenii* yang keputusan negatif menunjukkan kehadiran asid bebas selepas dikaji dengan Na₂CO₃. Untuk bakteria, hanya *E. coli*, *Staphylococcus aureus* dan *Bacillus subtilis* menunjukkan perencatan aktiviti, manakala tiada aktiviti terhadap *Samonella typhi* dan *Enterobacter aerogenes* dan juga fungi, *Candida krusei* dan *Candida albicans*. Strain yis H10075 yang telah ditransformasi dengan pKT10-gsk3 β dan YEp24-MCK1 digunakan dalam penyaringan sistem GSK-3 β . Sistem ini adalah berdasarkan sensitiviti yis terhadap suhu (25 °C dan 37 °C), strain yis yang telah



ditransformasi ini boleh mengatasi fenotip sensitif terhadap suhu. Tiada perencat GSK-3 β ditemui daripada sampel-sampel. Untuk MKK1 penyaringan sistem, semua ekstrak metanol berpotensi dalam perencatan aktiviti yis. Sistem ini menggunakan strain yis MKK1^{p386} dimana dipengaruhi oleh GAL1p. Ekspresi melampau gen mutan ini akan menyebabkan pertumbuhan sel terhalang dengan penambahan galaktosa.



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LIST OF UNITS

cm	Centimeter
ft	Feet
g	Gram
L	Liter
M	Molar
mg	Miligram
ml	Mililiter
mm	Milimeter
rpm	Rotation per minute
v/v	Volume per volume
w/v	Weight per volume
%	Percentage
μl	Microliter or 10^{-6} liter
$^{\circ}\text{C}$	Degree Celsius



CHAPTER 1

INTRODUCTION

Medicinal plants contain medicinal properties and they are widely used to treat various diseases today. Thus, it is very important for human. Today, approximately 80 % population of worldwide are depend on medicinal plants and herbs for their treatment of diseases. Data from World Health Organization (WHO) indicates that by today, around 21,000 of plant species have been used as medicine. Many plants extract have been analyzed by scientists through the processes of fractionation, isolation, purification and identification of their compounds for further study and analysis (Fasihuddin and Hasmah, 1993). Therefore, many drugs have been produced by medicinal plants as treatment for different kinds of diseases.

Phytochemicals are sometimes referred to as phytonutrients. Phytochemicals are to be any chemical or nutrient derived from a plant source. It is a chemical metabolites compound from plants which contains of primary and secondary metabolite. For primary metabolites, it is directly involved in the normal growth, development, and reproduction of organism which are able to produce a lot of



component such as amino acid, lipid, carbohydrate, and enzyme. Photosynthesis, glycolysis and krebs cycle are those process involved in primary metabolites. However, secondary metabolites are those chemical compounds in organisms that are not directly involved in those processes, but usually have important ecological function (Fasihuddin and Hasmah, 1993).

In this project, screening for glycogen synthase kinase 3 β (GSK-3 β) and mitogen-activated protein kinase kinase 1 (MKK1) inhibitors from protein serine/threonine kinases (MAPK) pathway of the yeast *Saccharomyces cerevisiae* were carried out. These enzymes in the signal transduction and cell cycle of *Saccharomyces cerevisiae* are homologous from yeast to human. Therefore, this is my purpose of choosing them for this project. Potential inhibitors of the GSK-3 β and MKK1 gene can be especially useful in cancer therapy and also in the study of cell signaling. Besides of these, bacteria and fungi inhibitors are also screened in this project. These screening systems are using secondary metabolites from medicinal plants and used as inhibitors for antibacterial, antifungal and anticancer.

The objectives for this project are to extract secondary metabolites compounds from medicinal plants. It is also aimed to screen for phytochemical compounds in medicinal plants. Besides that, it is also important in determining the inhibition potential of different solvent fraction of medicinal plants extraction towards bacteria, fungi, GSK-3 β and MKK1.



CHAPTER 2

LITERITURE REVIEW

2.1 Plant Samples

2.1.1 *Nephrolepis auriculata*



Photo 2.1 *Nephrolepis auriculata*, photo taken from Orchid De Villa

Kingdom	: Plantae
Phylum	: Pteridophyta
Class	: Filicopsida
Order	: Polyodiales
Family	: Dryopteridaceae/Oleandraceae
Genus	: <i>Nephrolepis</i>
Species	: <i>Nephrolepis auriculata</i>



Nephrolepis auriculata also known as Tuber sword fern. It is originated from tropical Asia. It distributes abundant in the warm habitats, disturbed area and wasteland (Kamarudin & Latiff, 2002). It consists of tufted stipes, 5-15 cm long, densely covered when young with yellowish-brown, entire, narrowly linear, hair-pointed scales; stolons long shining sometimes bearing scaly tubers with 1-3 cm long, fronds 30-60 cm long, pinnate; rachis grooved, scaly above, pinnae sessile, unequal at base, auricle acute, articulate to rachis, both surfaces glabrescent; lower pinnae obtuse, gradually shortened, upper soriferous pinnae to nearly 4 cm long, more or less acute; margins serrulate to crenate (Huang, 2003). It is believed that *N. auriculata* can be used to treat skin itch disease and hypertension (high blood pressure) (Kamarudin & Latiff, 2002).

2.1.2 *Selaginella doederleinii*



Photo 2.2 *Selaginella doederleinii*, photo taken after harvesting

Kingdom	: Plantae
Phylum	: Pteridophyta
Class	: Lycopsidea
Order	: Selaginellales
Family	: Selaginellaceae
Genus	: <i>Selaginella</i>
Species	: <i>Selaginella doederleinii</i>

Selaginella doederleinii is distributed in Asia such as Hong Kong, Japan, Taiwan, India, Malaysia or Vietnam. It is grown on the montane forest floors at low and medium altitude. It possesses of main stems erect with decumbent at base, 12-35 cm tall, bearing long rhizopores; branches alternate, 8-10 mm wide. Its leaves are dimorphic, lateral leaves are oblong, unequal-sided, pointing outward, the margins almost entire on abaxial side, ciliate on adaxial side; median leaves are inbricating, ovate, nearly equal-sided, aristate, pointing upwards; axillary leaves are equal-sided, ovate, ciliate. Strobili bear on tips and sides of the lateral branches, 5-12 cm long; sporophylls monomorphic, ovate, aristate, keeled, the margins ciliate (Huang, 2003). The leaf of *S. doederleinii* contains of alkaloid, sterol saponins and shikimic acid. The therapeutic uses of this plant are malignant hydatic mole and chorionic epithelioman (Kee, 1999)

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