ISOLATION OF BIOACTIVE COMPOUNDS EXTRACTED FROM *Eurycoma longifolia* Jack OLD LEAVES, SCREENING FOR ANTI-BACTERIAL, ANTI-FUNGAL AND GSK-3BETA INHIBITION

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THIS DISSERATATION IS PRESENTED TO FULFILL THE PARTIAL REQUIREMENT TO OBTAIN A BACHELOR DEGREE OF SCIENCE WITH HONOURS

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ABSTRACT

The plant *Eurycoma longifolia* Jack or known more commonly as Tongkat Ali has long been a source of traditional remedy curing infertility and also to treat mild illnesses like dysentery and rashes. In this study, the screening and isolation of compounds from *Eurycoma longifolia* Jack old leaves, by extraction using solvent-solvent method and chromatography methods, have been tested for anti-bacterial, anti-fungal and Glycogen Synthase Kinase-3beta inhibition activity. The separations of the phytochemicals were performed by liquid-liquid extraction to allow separation according to its classes and specific characteristics. It was determined from the test that the leaf extracts showed activity against *Escherichia coli, Staphylococcus aureus* and *Candida krusei,* but had no activity against *Bacillus subtilis, Candida albicans* or GSK-3beta. The study includes isolation via Column Chromatography and verification by Thin Layer Chromatography. It was shown that the leaf extract possesses activity at possibly a single compound against *E.coli,* two possible compounds showing activity against *C.krusei* and 7 possible compounds against *S.aureus.* It was assumed that during isolation a single compound was collected at a specific time in these compound and further study in the future. The comparison of the leaf activity to the test performed using the root and the bark of the plant had also been performed and it was proved that the plant has no activity against GSK-3beta, but the leaf may have precursors in producing even more effective anti-microbial drugs. The experiment had proven that chemicals exhibiting anti-bacterial and anti-fungal exist in the plants and had been purified to a certain extent.

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PENULENAN KOMPAUN BIOAKTIF YANG DIEKSTRAK DARI DAUN TUA *Eurycoma longifolia* Jack, MENYARING AKTIVITI ANTI-BAKTERIA, ANTI-FUNGI DAN PERENCATAN GSK-3BETA

ABSTRAK

Eurycoma longifolia Jack atau lebih dikenali sebagai Tongkat Ali telah lama digunskan sebagai perubatan tradisional untuk mengubati masalah kelelakian dan sakit seperti sakit perut dan gatal kulit. Dalam kajian ini, penyaringan dan pemisahan kompaun-kompaun dari daun tua *Eurycoma longifolia* Jack, menggunakan kaedah pengekstrakan secara sistem pelarut-pelarut and kaedah kromatografi, untuk diuji aktiviti perencatan anti-bakteria, anti-fungi dan Glycogen Synthase Kinase-3beta. Pemisahan fitokimia dilakukan secara pengekstrakan cecair-cecair untuk pemisahan yang berasaskan pada kelas-kelas dan ciri-ciri spesifik fitokimia tersebut. Telah dibuktikan melalui ujian bahawa ekstrak daun telah menunjukan aktiviti yang merencat *Escherichia coli, Staphylococcus aureus* dan *Candida krusei,* tetapi tidak menunjukkan aktiviti penyaringan terhadap *Bacillus subtilis, Candida albicans* atau GSK-3beta. Penerusan kajian meliputi pemisahan menggunaan Kromatografi Berturus dan pengesahan menggunakan Kromatografi Berlapis Nipis. Ini telah menunjukkan bahawa ekstrak daun berkemungkinan mempunyai satu kompaun yang bertindak ke atas *E.coli,* dua kompaun yang bertindak ke atas *C.krusei* dan mungkin tujuh kompaun yang mempunyai aktiviti ke atas *S.aureus.* Ini adalah menganggapkan bahawa satu kompaun telah berjaya diekstrakkan dalam satu jangka masa yang berturutan dan berpotensi untuk diteruskan kajian. Perbandingan antara aktiviti pada daun terhadap aktiviti pada akar dan kulit kayu tumbuhan tersebut juga dikaji dan ini membuktikan bahawa tumbuhan ini sahnya tiada aktiviti menentang GSK-3beta, tetapi daun berkemungkinan untuk mempunyai prekursor untuk menghasilkan ubat yang lebih berkesan ke atas mikrob. Eksperimen ini telah menunjukan bahawa eksrak daun ada aktiviti ke atas bakteria dan fungi wujud dan telah disepara-tulenkan.

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LIST OF SYMBOLS

^{o}C	Degree Celcius
mgl^{-1}	milligram per liter
nm	*nanometer*
cm	centimeter
g	grams
ml	*milliliter*
mins	minutes
X	times
v/v	volume over volume
w/v	weight over volume
%	percent
E.longifolia	*Eurycoma longifolia* Jack
E.coli	*Escherichia coli*
B.subtilis	*Bacillus subtilis*
S.aureus	*Staphylococcus aureus*
C.albicans	*Candida albicans*
C.krusei	*Candida krusei*
P.P	Pour Plate Method
S.P	Spread Plate Method
PE	Polar Extract
MPE	Moderately Polar Extract
BE	Basic Extract
NE	Neutral Extract
CE	Crude Extract
PD	Paper Disk (Control)
MeOH	Methanol
$CHCl_3$	Chloroform
EtOAc	Ethyl Acetate
R_f	Retention factor
SC-Ura	SC minus uracil media
GSK-3beta	Glycogen Synthase Kinase 3 beta

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CHAPTER 1

INTRODUCTION

The plant *Eurycoma longifolia* Jack, has long been a medicinal plant, which has been used across the South East Asian region by the natives as herbs and medical ailments. In these cultures, the herb has been used to treat Malaria, Tuberculosis, fever and as well as serve as an antihistamine and also a source of aphrodisiac.

The countries that use such a produce are mostly found in the countries that have a rather diverse rainforest such as those in Malaysia, Indonesia, Vietnam, Brunei, Thailand and a few other equatorial climate countries in South East Asia. The plant itself has been given many traditional names that have been made famous over the last decade, because the plant has proven to have a certain economic value. In Malaysia, the plant has been called commonly as *Tongkat Ali* and *penawar pahit.* In Indonesia, it is known as *Pasak Bumi, Bidara Pahit* and *Bidara Laut* (Goh *et al.,* 1995). In Vietnam, the plant is known as the herb with 101 remedies or in their own language is known as *Cai Ba Binh.* According to statistics acquired from the ASEAN forum, the usage of traditional means of herbal remedies have been given the most attention in Indonesia, followed by Vietnam. The part of the plant that is of great interest is the root and the bark of the tree as it has the most powerful effect on the health of men when applied. This is proven from the inventory of the local forest in Malaysia done by the Forest Research Institute of Malaysia (FRIM), which has shown a great

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tests used in this study are the anti-bacterial in which screening has been performed using bacterium *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* and anti-fungal which screening has been performed using *Candida albicans* and *Candida krusei.*

It is hoped that the quantification of phytochemicals from the plant leaflets will be able to be used as a herbal cure for diseases and reducing the reliance on the extract from the root and the bark of the plant.

This is also to see if by getting the desire result, the possibility of paving the road to genetic manipulation of the plant, to which in the near future, it is possible to mass-produce the phytochemical product by using bioreactors, as well as transgenic plants.

The objectives of the project:

1. To isolate compounds that exhibit anti-fungal, anti-bacterial and GSK-3b from extracts of *Eurycoma longifolia* Jack old leaves.
2. To fractionate the major classes of the chemical compounds via bioactivity screening methods and chromatographic methods.

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decrease of the plants population in the late 20th century (Noraini and Azmi; 2001). As a matter of protection to the forest, our government has categorized the plant as a protected species. In Indonesia, President Susilo Bambang, has announced that the forest harvesting of natural sources are only allowed to be done by the local natives of the jungle (Kreutz, 2004).

During the past years; this herb has been used exclusively as a remedy that can cure large number ailments, from infection to incapability of men libido. However, these are just practices that have been performed by local and traditional witchdoctors in the countries in South East Asia. The potential of this plant has not fully been studied for any other properties other than as an aphrodisiac, in which has been given most attention of the industrial sector. It is however crucial to be able to understand the full potential of the plant, in which has so much hope in the industrial sector. In this, the study to see if the consumption of the plant will allow the reduction of microbial growth, as a cure for surface infection and the capability of prolonging cell life.

Thus, this study is to isolate the chemical compounds that shows bioactive activities like anti-bacterial, anti-fungal and shows inhibition of Glycogen Synthase Kinase 3β or GSK-3β. This is because the compounds that show these bioactive activities may be found in other plants or may be synthetically produced in the laboratory; this will no doubt serve as a possibility of solving the problem that is the continual reduction of the *Eurycoma longifolia* populations in the jungle. The other factor that prompted this study is that the plant precursors to the bioactive compounds that can be found in the leaves. Thus, this is the best place to test for the compounds that can determine the chemicals that has the most value in the plant. The bioassay

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CHAPTER 2

LITERATURE REVIEW

2.1 *Eurycoma longifolia* Jack

This is a plant, which is in the group under the family of Simabarouceae and has been given a lot of scientific names and traditional names. It wasn't until the recent 20th century that the names have been reviewed and later synchronized to the standard *Eurycoma longifolia* Jack. The plant have been given a lot of traditional names like *Tongkat Ali* and *penawar pahit* in Malaysia; *Pasak Bumi, Bidara Pahit* and *Bidara Laut* in Indonesia (Goh *et al.*, 1995) and *Cai Ba Binh* in Vietnam. It is also known in scientific journals before this as *Eurycoma merguensis* Planch; *Eurycoma eglandulosa* Merv and *Picraxylon siamensis* (Goh *et al.*, 1995). The identification via the phylogeny scheme has been shown below (Sabarina, 2001).

Kingdom	:Plante
Phylum	:Spermatophyta
Sub-phylum	:Angiospermae
Class	:Dycotyledon
Order	:Rutales
Family	:Simabarouceae
Genus	:*Eurycoma*
Species	:*Eurycoma longifolia* Jack

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2.1.1 Characteristics

The plant is very unique for many reasons, as it is a rather queer plant that thrives in the every competitive rain forest around the South East Asia region. Due to the rather equatorial climate, the plant has learned to adapt to it's surroundings and thus allowing the general understanding of it's basic character. The plant (Plate 2.1) is generally a short tree and usually grows up to about the height of about 10 meters in height with a diameter of 10cm and takes about 5 years to mature (Chang *et al.*, 2000). The plant achieves it's highest commercial value at the age of 30 (Shaari *et al.*, 1992). Its leaves grow up to about 1 meter in length and are known to have leaflets which spans from 5cm to 20 cm in length and 1.5cm to 6cm wide. The leaflets are being arranged in a manner that are opposite of sub opposite with the shape of ovate-lanceolate; ovate-oblong. It is known to bear flowers at it's season, to which the flowers are reddish; unisexual, which could be on the same plant or on different plants at a time, arranged in a dense formation, much-branched, which is axillary symetry and is also known to have stiff but pendent panicles (Perry and Metzger, 1980). The flowers are also known to emit strong sweet aroma similar to the aroma that can be found on the trees of guava plants. It also bears fruits that can be identified to have the characteristics that are known for its bitterness. The fruit, which can be from 5 to 12mm when matured has the dark reddish brown colour and is ovoid and found at the distinct ridge of the tree. The plant too, if up root, displays a whitish yellow root which has a tap root system *(sistem akar tunjang)* and can be distinctively differentiated from each other by its taste. The locals test the purity of the plant and it's age by the length of the root and also by scratching the root and tasting the scratched area, as it will give a bitter taste. The more bitter the plant, the more value the root has (Goh *et al.*, 1993).

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As mentioned above, the plant lives in the equatorial climate or in the diverse rainforest in South East Asian Region. It is a small tree and lives in shaded conditions to which the plant is known to have poor mortality rate when placed in the open grounds with direct sunlight. Due to the fact that the plant synthesizes large amounts of secondary metabolites in the metabolic pathway, it is known to have a great accumulation of these chemicals at the rooting area. It thus has adapted itself to grow around the hilly areas to which there are constant rainfall to wash off the build-up of these metabolic chemicals. It is also known to grow around 500m above sea level and is widely distributed around Burma, Southern Vietnam, Southern Cambodia, Indonesia, Thailand, Malaysia, Sumatra, Borneo, and Philippines (Goh *et al.*, 1995).

The plant has a close relative to which the plant is indistinguishable if observed by an untrained eye, can thus confuse the person which harvest the plant itself. *Eurycoma harmandiana* is known to grow at 700m above sea level. It is also different from *E.longifolia* as it's roots are red and is known to be more bitter in taste and has more commercial value in the market (Kanchanapaam *et al.*, 2001).

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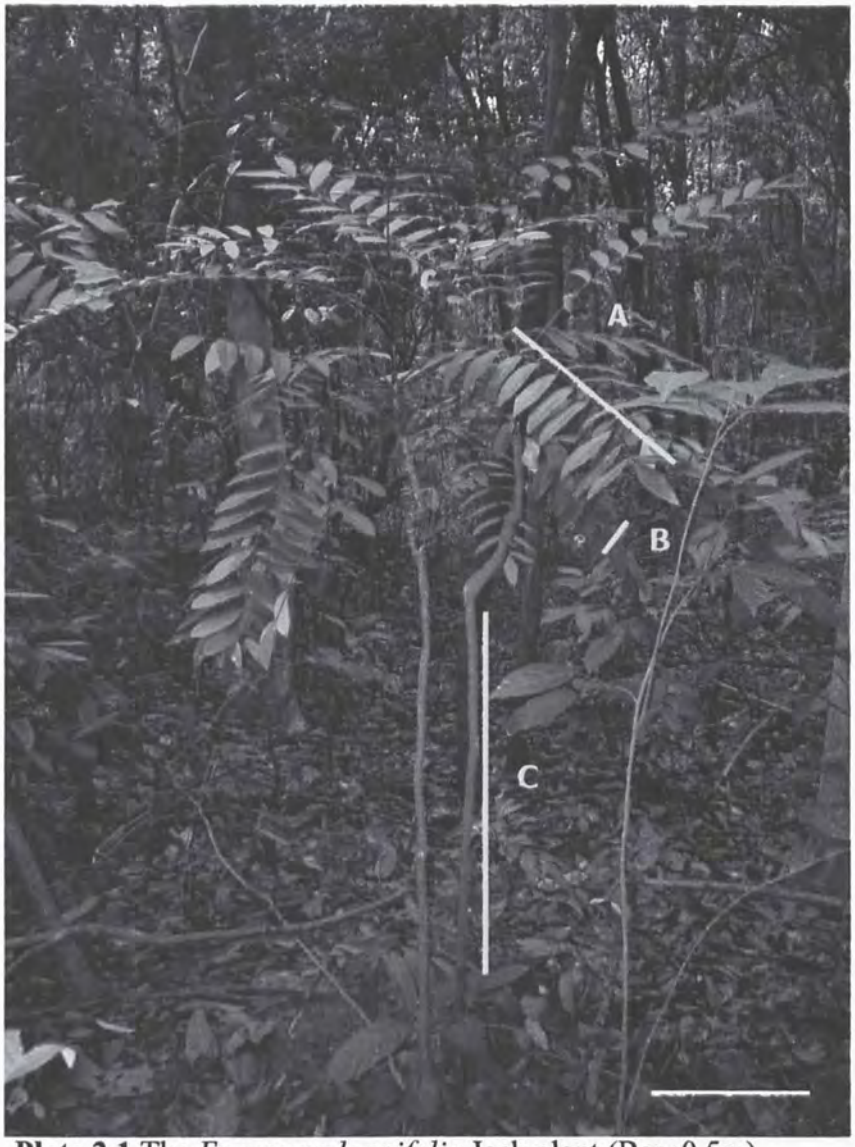

Legend
A : Leaf

B :Leaflet

C :Stem

Plate 2.1 The *Eurycoma longifolia* Jack plant (Bar :0.5m)

(The plant picture was taken from the Cu Chi forest in Vietnam)

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2.1.2 Uses and medical practices using the plant

A local survey of the herbal stall vendors in the market around Penampang, Kota Kinabalu and Kota Belud, in Sabah has been done. From the interview, the plant is known to have properties that is curative to the internal health of the body. It is also known that the concoction if applied to the skin will allow the skin to heal and make it look younger. The plant is also known to cure tumor and cancer, as well as relive hypertension. It is also known to give a boost in energy and can slow the aging of men and women as well. It is also known stabilize the pH level in the human body.

Interviews with the local herbal practitioners too have been done in Vietnam, to which the plant is known to have flourish at the areas around the Ho Chi Minh City. The plant which is known to us as the as the plant of 101 cures, or in their local tongue is known as *Cai Ba Binh,* is used exclusively during the Vietnam War and has been used by the Communist Soldiers as local traditional medical remedies, to which it is known to have boost energy level of soldiers as well as allow the soldiers to be more resilient to attacks and injury. The juice of the plant is also known to be used as antiseptic to the soldiers to which has been inflicted with injuries. It is clear that from here, that the plant has potential to be an anti-bacterial agent, as well as an anti-fungal agent too. Nowadays, the plant is used as a concoction to cure flu and as well ease minor pain and strain by the locals and has been known to be sold in the market (Plate 2.2).

From past research, it is discovered that the plant has the properties of having bioactivity for anti-histaminic, anti-malarial, anti-tumor, anti-ulcer, antiseptic and also known to be cytotoxic at high concentrations (Goh *et al.*, 1995).

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Plate 2.2 The author holding on to the root of a *Eurycoma longifolia* Jack plant at the bazaar in Ho Chi Minh city, Vietnam

It also known to be an antidotal, antipyretic, anti-tuberculosis, anti-viral, aid in indigestion, lumbago, vermifuge, ferbifuge, relieve back aches, relieve jaundice, dropsy, cachexia, cure for fever, cure for diarrhea, to wash and relieve itches, curing dysentery and serve as the source of aphrodisiac, which give the honorary name of "Tongkat Ali Jantan", which means male Tongkat Ali (Chang *et al.,* 2000). It is in turn a balance in which the name of Tongkat Ali Betina, or female Tongkat Ali is being aptly given to the relative of the plant, which is *E. harmandiana* in which has been known to relieve the pangs of menstrual pain, as well as a tonic used for mothers which has given birth. It is being believed by the locals that the reason as to why the plant has been called the female plant is that the red roots, and that symbolizes the menstrual periods to that of a women, and this belief that "the cure of the disease can be cured by spiritual manifestation in plants and animals", which has pushed the locals to believe the power of the plants to serve it's purpose (Goh *et al.,* 1995).

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