

## CHAPTER 1

### INTRODUCTION

*Eurycoma longifolia* is one of the small Asian trees in the genus *Eurycoma*, commonly called as ‘Tongkat Ali’. It is a flowering plant in the family Simaroubaceae, which is native to Malaysia and Indonesia, and to a lesser extent in Vietnam, Thailand and Laos. There are four species which are known to be ‘TongkatAli’, *Eurycoma longifolia*, *Eurycoma apiculata*, *Polyathiabulata* and *Gholiothalamus* sp. Among them *Eurycoma longifolia* is the most popular species with high usage(Gimlette and Thomson, 1977).

Tongkatali extracts has been used as a natural treatmentfor a host of medical conditions. There are more than 200 Tongkat Ali products in the market, with most emphasizing the aphrodisiac properties (McKerry, 2010). Studies of *E.longifolia* on male rats showed high sexual stimulation towards receptive females(Bhat and Karim, 2010). These studies support the aphrodisiac properties of Tongkat Ali. Experiments have demonstrated that consumption of this herb can raise sperm counts and increase both testosterone levels as well as sexual behavior (Anget *al.*, 2003).

Extracts of this herb have often been incorporated in some natural body building supplements due to the positive effect of Tongkat Ali on testosterone production. Recently, it has been effectively marketed as an aphrodisiac and a natural libido enhancer that can help many middle aged men to retrieve their youthful appetite(Jarvie, 2011).

The amount of extract used in each tablet is vital as low concentrations are ineffective. Tongkat Ali tablets are commonly made with ratios of between 20:1 (i.e. 20kg of plant used to produce 1kg of extract) and 100:1. Higher concentrations are not recommended as the true nature of its efficacy and safety are not known, while some unpleasant effects in male adolescents due to excess of testosterone has been demonstrated (McKerry, 2011).

### 1.1 SIGNIFICANCE OF THE STUDY

Most publications on aphrodisiac properties were focused more towards identification of the chemical constituents of Tongkat Ali and screening for other potential activities. Previous studies have reported the isolation of quassinoids such as eurycomanone, 13 $\alpha$  (21)-epoxyeurycomanone, eurycomanol, eurycomanol-2-*O*- $\beta$ -D-glucopyranoside, and 13,21-dihydroeurycomanone from *Eurycoma longifolia* using High Performance Liquid Chromatography (HPLC) (Chin *et al.*, 2011). The quassinoid extracts from *Eurycoma longifolia* have been examined for cytotoxic properties and potential uses as anti-malaria, anti-cancer and anti-ulcer agents. However, the popularity of Tongkat Ali for its aphrodisiac activity and its potential as a remedy for sexual dysfunction and male infertility are not related to quassinoids. Certain components of *Eurycoma longifolia* aqueous extracts demonstrated bioactivity by increasing synthesis of testosterone and sperm activity and number. These components were peptides having molecular weights of about 4300 daltons and these peptides were known as Eurypeptides (glycopeptides) (Sambandan *et al.*, 2004). Analysis of purified bioactive fractions isolated using reverse HPLC indicated the presence of bioactive peptide which were associated with several types

of sugar residues. The molecular weights of these proteins were about 4300 daltons (Perry *et al.*, 1980). However, little is known on the plants genomics and proteomics. There is also the need to develop methods to authenticate claims made by manufacturers of the various products.

## **1.2 JUSTIFICATION**

The results of earlier research based on collaboration between MIT-FRIM linked the active components of *Eurycoma longifolia* to be proteins (Sambandan *et al.*, 2004). In this current study proteomics technology (conventional method for protein characterization) was used to investigate potential biomarkers and the authenticity of some commercial Tongkat Ali products in the market. The products tested include those approved by Ministry Of Health (MOH approved) and products which have not been approved (Non-MOH approved).

The objectives of this research were to:

- Evaluate potential protein biomarkers in various commercial Tongkat Ali products.
- Compare presence of the biomarkers in Ministry of Health (MOH) approved and non approved products.
- Characterize the protein biomarkers utilizing Mass Spectrophotometry.

### **1.3 HYPOTHESIS**

- Particular protein spots are useful for biomarkers in authentication of Tongkat Ali herbal products.
- All of the MOH approved products must have both active biomarkers.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 THE PLANT *EURYCOMA LONGIFOLIA* (TONGKAT ALI)

##### 2.1.1 General description and taxonomy of Tongkat Ali

*Eurycoma longifolia* Jack from the Genus *Eurycoma* and family Simaroubaceae is one of the popular tropical medicinal plants (Fig 2.1). The plant is indigenous to South East Asia, mainly Indonesia, Malaysia and Vietnam (Goreja, 2004). *Eurycoma longifolia* Jack is also known as long jack and “Tongkat Ali”. Other vernacular names include “penawar pahit”, “penawar bias”, “bedara merah”, “bedara putih”, “lempedu pahit”, “payong ali”, “tongkat baginda”, “muntah bumi”, “petala bumi” (Malay), “bidara laut” (Indonesian), “babi kurus” (Javanese), “cay ba binh” (Vietnamese) and “tho nan” (Laotian) (Zhari *et al.*, 1999; Goreja, 2004). The common names of the plant usually refer to the plant's medicinal use and extreme bitterness for example "penawar pahit" in the Malay language translates simply as "bitter medicine" (Zhari *et al.*, 1999; Goreja, 2004; Wyatt-Smith, 1953).



Figure 2.1 *Eurycoma longifolia*

(Source: University Science Malaysia; Bhat and Karim, 2010).

### **2.1.2 Distribution and cultivation of *Eurycoma longifolia***

There are four different species of Tongkat Ali, which includes *Eurycoma longifolia*, *Entomophthora apiculata*, *Polyathia bullata* and *Goniothalamus* sp. However, *E. longifolia* is used more routinely for traditional medicinal purposes (Kamarudin and Latiff, 2002; Aziz *et al.*, 2003).

Indonesia is the only country in the world with considerable resources of wild Tongkat Ali. The plant was once very common in Malaysia, but the search for Tongkat Ali has taken on a similar dimension as the search for truffles in France or Italy. Thus, Tongkat Ali has since been declared a protected plant and is guarded by forest rangers in Malaysia (Kreutz, 2010b).

Tongkat Ali is native to Southeast Asia. As one of the flowering shrubs, it is a small tree that grows slowly and reaches a height of about 12 m with one or two branches. The plant has pinnate compound leaves of about 1 m in length, which tended to be crowded towards the end of branches. The lower leaves fall off leaving large scar on the stem. The leaflets are in nearly opposite pairs, dark green and ovate-lanceolate. The flowers are borne on densely branched panicles, about 60 to 70 cm in length from the leaf axils. *Eurycoma longifolia* plants are dioecious, with either male or female flowers. The male flower has a sterile pistil, while the female has sterile stamens. The flowers have green calyx lobes with five red corolla lobes. The fruit is a yellowish-brown ovoid drupe (Zanoli *et al.*, 2009).

## **2.2 TONGKAT ALI AS ALTERNATIVE MEDICINE**

### **2.2.1 Traditional use of Tongkat Ali**

Extracts of Tongkat Ali have been applied as natural treatment for a host of medical disorders. The roots are the most valuable component and are consumed for treating diarrhea, fever, malaria, and swollen glands or used topically to heal sores and ulcers. Tongkat Ali is most commonly used for treatment of erectile dysfunctions (Gimlette and Thomson, 1977; Perry, 1980; Goreja, 2004). The root extract of the

plant has also been consumed for restoring energy and vitality, enhancing blood flow and as a herbal ingredient for women after child birth (Ismail *et al.*, 1999). The treatment follows the traditional way of preparing most remedies, where the roots are cut into smaller portions, boiled to blood-warm temperature, and consumed as a tea. Intense boiling in fresh water for several times was also suggested to remove the bitterness in the root's decoction. Nevertheless, the product in the form of chipped roots can also be mixed with honey, sugar syrup or dates to reduce the bitterness; or in certain cases the root powder is mixed with alcohol and incubated for 6 to 8 weeks and consumed. However, according to experienced traditional healers, the bitterness of the plant is essential for efficacy of the treatment. As such, bitterness is associated with higher efficacy (Bhat and Karim, 2010).

Some traditional healers in Malaysia advanced the use of Tongkat Ali as a sound treatment for a variety of ailments and conditions. Applications of the herbal medicine include the treatment of high blood pressure, improve blood circulation, and the treatment of fatigue and impotency. In 2005, ZRT Laboratories released test results which indicated that the continued use of the Tongkat Ali greatly increased the human testosterone levels (McKerry, 2010).



### **2.2.2 Combinations of Tongkat Ali with other herbs**

Combinations of Tongkat ali with other substances is not new, as it has been done by traditional healers long ago by mixing the chipped root extract with honey, sugar syrup or dates to reduce the bitterness of the root. In certain cases the root powder is mixed and incubated in alcohol for a specified period of time (Packer *et al.*, 2004).

Tongkat Ali currently is hardly sold as a separate entity. In fact it has been mixed with many other herbs. Tongkat Ali in combination with Arginine is reported to solve male problems such as weak erection, low testosterone, penile fatigue, erection dysfunction or disorders, impotency, sexual dysfunction, and other penis erection problems (Goreja, 2004 and McKerry, 2010). Horny Goat Weed is another good addition to Tongkat ali for those who somehow do not get the full effect of pure Tongkat Ali.

One complication in using supplements with Tongkat Ali is that though the mixture was indeed proven to be efficient and extensive research, support the extract's effect, on a larger scale however the amount or content of Tongkat Ali extract in the supplements were found to be insufficient to exert its beneficial effects on the consumers.

## **2.3 FUNCTIONS OF TONGKAT ALI**

The root of Tongkat Ali has a long history of use as an indigenous natural remedy and has become globally popular as a remedy for male sexual dysfunction and infertility after the joint patent filing by the Massachusetts Institute of Technology and the Malaysian Government in 2001 (Burkill, 1966; Perry, 1980; Soepadmo 1996). The root's aphrodisiac and tonic properties were also supported by many preliminary scientific reports (Ang and Ngai, 2001; Ang *et al.*, 2002, 2003).

### **2.3.1 Bioactive constituents and components of Tongkat Ali**

The part used is the root which is dried and ground into a powder or a liquid that resembles coffee. Tongkat ali extract includes isolated active components from the bark or root of the shrub. The crude root extract has a higher concentration of active components than in a similar amount of powdered root. It is usually used at concentrations of between 1:20 and 1:200, extraction is stronger at higher ratios, due to it having less cellulose. Tongkat Ali extract is a famous aphrodisiac and sexual performance enhancer. It is imported by a number of supplement manufacturing companies and has become a popular component in natural testosterone boosting formulations marketed to bodybuilders and fitness enthusiasts (Occhiogrosso, 2007).

Scientific research on *Eurycoma longifolia* (Tongkat Ali) was carried since the year 1968. Other than antioxidants and phytochemicals, previous chemical studies had revealed the presence of canthin-6-one alkaloids,  $\beta$ - carboline alkaloids, quassinoids, quassinoid diterpenoids, eurycomaoside, tirucallane-type triterpenes,

squalene derivatives, biphenylneolignans, eurycolactone, laurycolactone, eurycomanone, eurycomanol, eurycomalactone D,E and F; laurycolactone, 21-dihydroeurycomanone, 13-alpha(21)-epoxyeurycomanone, beta-carboline alkaloids, 9-methoxycanthin-6-1 and new compounds are still being characterized (Darise *et al.*, 1982; Chan *et al.*, 1989; Kardono *et al.*, 1991; Itokawa *et al.*, 1993; Morita *et al.*, 1993a; Morita *et al.*, 1993b; Ang *et al.*, 2002; Bedir *et al.*, 2003; Kuo *et al.*, 2004; Goreja, 2004). However, not every compound has been screened to prove its traditional claims as many are present in very low amounts (<0.05% dry weight of raw material).

### **2.3.2 Mechanism of action of Tongkat Ali**

Tongkat Ali works by increasing levels of the hormone testosterone. Testosterone is an important male hormone associated with sexual performance produced in the gonads. The hormone functions to stimulate the differentiation of the male reproductive tract in the embryo, the descent of the testes into scrotum, further development of the reproductive tract and penis during puberty and maturation of the sperm. Testosterone is also responsible for the development of male secondary sex characteristics such as moustache, beard, enlargement of the larynx (deep voice) and increases production of the sebaceous glands, all of which are associated with masculine features. The hormone plays a major role in the maintenance and development of male libido and sexual behavior. Tongkat Ali acts through an increase in testosterone and also c-GMP (cyclic guanosine monophosphate) production. The plant extract takes a longer time period to exert its effects as it

enhances the testosterone synthesis. Thus, the benefits are felt gradually and the optimal effectiveness should be felt within a week of continuous uninterrupted use. Testosterone also takes care of bone strength in males as the hormone helps to overcome osteoporosis. Tongkat Ali has an impact on the male's testosterone levels directly. Actually the herb is a testosterone booster. The herb helps the body to produce more testosterone and increase strength by increasing ATP products (Chye, 2006). Indirectly, this testosterone-enhancing herb improves the libido, energy as well as muscle mass. These result in increased sex drive, thickening of semen with increased sperm count and reduced incidence of premature ejaculation, as well as maintain erections.

As it is well known, the most important part of the semen is the spermatozoa, the male generative cells which fertilize the female ovum. Tongkat Ali tones and strengthens the spermatozoa. The plant acts by assisting in carrying a normal amount of blood to all parts of the body, equalizing circulation and restoring imbalances in circulation. Imbalance in blood circulation normally begins to occur in men from the age of 40 and onwards, that is when androgen, a steroid hormone becomes deficient. Androgen has specific effects on tissue growth and brain function. Possible mechanism of action of Tongkat Ali is to block the negative feedback to the pituitary gland and hypothalamus that initiates this deficiency, and lets the body produce sufficient amounts of testosterone to counter the deficiency (Stoxen, 2003).

Experiments using rodents revealed many interesting observations. Typically, the Tongkat Ali has a direct effect on the levels of testosterone in the rodent's body. When the dose was high, the rodents showed high levels of sexual activities due to

high levels of testosterone in the blood. When the user stops taking Tongkat Ali, the level of testosterone goes down due to the normal body functions. Thus, Tongkat Ali does not have a long term effect in boosting testosterone levels in the male's body. Whenever the person needs to boost his testosterone level, he can take Tongkat Ali and experience the effect within a couple of hours' time. Tongkat Ali testosterone effects are considered healthy as there has not been any negative or strong side effects reported.

The Malaysian government has been testing the Tongkat Ali on animals. These tests inevitably demonstrated enhancement of sex drive and rise in energy in the animals. Most experiences of the Tongkat Ali were performed on mice. To-date Tongkat Ali's extract has been tested successfully by three independent laboratories outside of Malaysia. The results of the tests showed Tongkat Ali extract can increase testosterone levels in both males and females (McKerry , 2010).

#### **2.4 SCIENTIFIC FINDINGS ON TONGKAT ALI**

Scientific investigation on Tongkat Ali in 1995 showed that the plant's quassinoid content has potential as an anti-fever agent. The quassinoids were shown to be two times more effective than aspirin (Goreja, 2004).

Researchers in Japan and America reported that some chemicals in Tongkat Ali such as quassinoids and alkaloids have an inhibitory effect on the growth of cancer cells in laboratory experiments, which included colon cancer, breast cancer and leukemia. Beta-carboline alkaloids isolated from Tongkat Ali have demonstrated

cytotoxicity against human lung cancer (A-549) and human breast cancer (MCF-7) cell lines (Kuo *et al.*, 2004). As such some compounds in Tongkat Ali are able to kill cancer cells (Kuo *et al.*, 2003). In another independent study on the effects of extracts and their chromatographic fractions from the root of Tongkat Ali on the growth of human breast cancer cell lines, MCF-7, Tongkat ali extract was proven to possess cytotoxic, antimalarial, anti-ulcer, antipyretic, and plant growth inhibiting activities. These research findings led to a joint patent by the government of Malaysia and the Massachusetts Institute of Technology (MIT) to further study and improve the extract's potential as a successful remedy for breast cancer (Itokawa *et al.*, 1993; Ang *et al.*, 2000 and Jiwajinda *et al.*, 2002).

Besides, Tongkat ali extract was also proven to have antioxidant activity based on studies conducted by the Forest Research Institute Malaysia (FRIM) and the Department of Science, University Kebangsaan Malaysia (UKM). It was discovered that Tongkat Ali contains an anti-oxidant enzyme and SOD (superoxide dismutase) which could inhibit the chain reaction of radicals harmful to the body system. These finding correlates well with the findings of several similar studies on Tongkat Ali conducted in Malaysia (Tambi and Imran, 2010).

In addition, Tongkat Ali at the right dose is also known to be thermogenic or heat-generating, and increases metabolic rate of the body and enhances blood circulation. However, very high doses lead to insomnia and fatigue (Sehelian, 2004). It was suggested that Tongkat Ali's thermogenic effect was due to its action on ATP.

In another study, Tongkat Ali's potential as an anti-anxiety treatment showed that test mice treated with Tongkat Ali's extract had a greater decrease in episodes of

fighting and were able to complete more squares on a maze. Interestingly, these results were found to be consistent with anxiolytic effects produced by diazepam, a well known drug to treat anxiety disorders. These studies support the medicinal use of Tongkat Ali as an anxiety disorder remedy (Goreja, 2004)

In the 1980's research on the biological effects of Tongkat Ali showed that the roots of Tongkat Ali had the property to kill malarial parasites (Miller *et al.*, 1986). In another separate study, sixty-five compounds were isolated from the roots of Tongkat Ali and among them; four quassinoid diterpenoids were reported to be novel compounds from natural sources. The isolated compounds were also screened for anti-HIV, cytotoxicity and antimalarial activity by *in vitro* assays (Kuo *et al.*, 2004).

Beside all the above uses of Tongkat Ali in the medicinal field, the most important one is the ability of this plant to increase virility and sexual desire, libido, sexual performance and to treat erectile dysfunction. The plant has thus gained a reputation as an aphrodisiac (Gimlette and Thomson, 1977). The word aphrodisiac comes from 'Aphrodite', the Greek goddess of beauty, love and sexuality, and aphrodisiacs are substances which stimulate sexual desire (Choudhary and Rahman, 1997). Studies by the School of Pharmaceutical Science in University Science Malaysia reported that Tongkat Ali extracts had aphrodisiac effects on animals which mean that the extract could increase the number and length of time of the sexual performance in test animals (Ang and Sim, 1998; Ang and Cheang, 1999)

Treatment with *Eurycoma longifolia* (Tongkat Ali) increased the sexual motivation in sexually naive male rats. The aphrodisiac property of Tongkat Ali was

evident in this study as it was observed that Tongkat Ali continued to enhance and maintain a high level of total number of successful crossovers, mountings, intromissions and ejaculations during the 9-12th week observation period (Ang and Sim, 1998). A study by Ang and Cheah (2001) showed the effects of *Eurycoma longifolia* Jack on the levator ani muscle in both un-castrated and testosterone-stimulated castrated intact male rats. Tongkat Ali significantly increased the levator ani muscle activity compared to the control (untreated). The pro-androgenic effect demonstrated in this study further supported the traditional use of *Eurycoma longifolia* jack as an aphrodisiac (Ang and Cheah, 2001). This was further supported by results of the study by Ang and Ngai (2001) where the aphrodisiac effect of Tongkat Ali was evaluated on non-copulator male rats in electrical cages. A transient increase in the percentage of the male rats responding to the right choice was observed after 3 weeks post Tongkat Ali treatment and the effect became more prominent after 8 weeks post-treatment (Ang and Ngai, 2001).

A team of scientists at University of Malaya demonstrated that water-soluble extracts of Tongkat Ali increased testosterone formation 4.4 fold (male sex hormones). Studies showed significant changes in the male to female ratio of the offspring in the treated group compared to 1 to 1 ratio in the untreated group. The water-soluble extract was able to increase the concentration of sperm, mobility rate and percentage of progressive sperm. The results showed that the water-soluble extract could increase the quantity and quality of the sperm, and increase the fertility rate. Furthermore, it had the potential to increase the litter size (Wahab *et al.*, 2010).  
Effect of *Eurycoma longifolia* Jack (Tongkat Ali) on libido in middle-aged male rats



was studied by (Ang and Lee, 2002). The results of their study show that Tongkat Ali extracts can increase libido in middle- aged male rats.

In another separate study, the effect of *Eurycoma longifolia* Jack (Tongkat Ali) on orientation activities in middle-aged male rats by Ang and Lee (2002) revealed that mice treated with 800 mg/kg of Tongkat Ali increased orientation activities towards the receptive females (anogenital, licking, licking and mounting), increased genital grooming towards themselves and restricted movements to a particular area of the cage, but decreased interest in the external environment (raring, climbing, exploration) as compared with the controls (Ang and Lee, 2002).

Influence of *Eurycoma longifolia* on the copulatory activity of sexually sluggish and impotent male rats was studied by Zanolli *et al* (2009). Treatments with Tongkat Ali root powder significantly reduced ejaculation latencies and increased the percentage of mounting and ejaculation in animals as well as reduction in post-ejaculatory interval. Testosterone serum levels also increased in treated rats compared to the controls.

A preliminary interview conducted with 30 married men (aged 31-52 years old), with regular consumption of Tongkat Ali for three consecutive weeks indicated that 48% of the subjects felt healthy, active and energized, and 40% felt increased sexual desire. Biochemical study of the subjects too showed an increased free testosterone index and levels of DHEA (Chua, 2002). James Stoxen in his review stated that the production of testosterone falls progressively from age 40 where experts call this condition as "partial androgen deficiency of the aging male."

Androgens are steroid hormones with specific effects on tissue growth (muscle, fat, skin, hair and others) and brain function. They play important roles in men and women, but are produced in much larger quantities in men. In men after puberty, the testicles produce most androgens, mainly as testosterone. It has been suggested that Tongkat Ali may block negative feedback to the hypo-thalamus and pituitary glands, so the body continues to produce testosterone at elevated levels (Stoxen, 2003). Kuo *et al.*, (2003) reported the isolation and identification of three new  $\beta$ -carboline from *E. longifolia*, together with cytotoxic, anti-HIV, and antimalarial activity of selected alkaloids from this plant. Ang *et al.*, studied the butanol, methanol, water and chloroform extracts of the roots of *Eurycoma longifolia* Jack on the potency of treated male rats. It was observed that *Eurycoma longifolia* produced a dose-dependent, recurrent and significant increase in the episodes of penile reflexes as evidenced by increases in quick flips, long flips and erections of the treated male rats during the 30 min observation period. These results provide further evidence that *Eurycoma longifolia* increases the aphrodisiac potency activity in treated animals (Ang *et al.*, 2001).

## **2.5 DRUGS AND PHARMACEUTICAL PRODUCTS FROM TONGKAT ALI**

### **2.5.1 Herbal products of Tongkat Ali**

There are different forms of Tongkat Ali products. Firstly, chipped roots are the most basic form of Tongkat Ali available. Consumers rarely use chipped forms because it is quite inconvenient to process and extract. The chipped form is used largely by the manufacturers.

The powdered root is another raw form of Tongkat Ali that is widely used and also available. Most people use two tablespoons of Tongkat Ali powder a day to experience the enhancements in testosterone. The conventional way of using Tongkat Ali is in its powder form. Tongkat Ali in the liquid form (extract) is one of the most powerful and easy to use forms available in the market. The easiest way for consuming Tongkat Ali is as pills and tablets. Tongkat Ali tablets and pills are easy to consume and carry and unlike powder and liquid forms, people can easily take the tablets or pills anywhere and use them when required. This is the reason why tablets or pills have become popular with most consumers (Kreuts, 2010).

### **2.5.2 Production of Tongkat Ali tablets and capsules**

Capsules are made using the Tongkat Ali root extracts. Different doses of extracts are available (ratios of 1:50, 1:100, and 1:200). A 1:200 Tongkat Ali Extract dosage, implies purchasing a capsule, tablet, or liquid where 200 grams of raw root was used to produce 1 gram of the final product. This is far the most popular mixture of the extract available. Basically, Tongkat Ali tablets and pills contain the root powder. However, manufactures may add other components to the pure root powder, to give added value to the consumers.

Tongkat Ali products are available either in the form of raw crude powder or as an additive mixed with coffee (and ginseng) or in certain health products as a replacement for ginseng. Malaysian manufacturers usually provide the Tongkat Ali Extract products in tightly sealed containers for easy shipping of the products (Jaganath and Ng, 2000; McKerry, 2010).

The demand in the international market is for the water extracts which has a market value of 26 US dollars per bottle of 60 capsules. This is followed by dried Tongkat Ali roots which fetch somewhere between 20 and 25 US dollars/kg (Kaur *et al.*, 2002). Tongkat Ali products are available in the form of raw crude root powder or in mixtures with other aphrodisiac herbs, coffee and ginseng; or with certain health products (Jaganath and Ng, 2000).

### **2.5.3 Tongkat Ali Products in Industry**

Currently Tongkat Ali is a protected plant in Malaysia. At the same time, Malaysian herbal suppliers have been engaged in aggressive marketing in the US (backed by scientific studies that confirmed Tongkat Ali's testosterone-boosting powers). Increasing demand coupled with decreasing supply has several effects, including rising prices and the emergence of counterfeits or imitation products. Malaysia is one of the most important exporters of Tongkat Ali in the world. Hence, the local authority has pressed manufacturers of Tongkat Ali products to standardize the industry. Most manufacturers do not have the basic equipment or technology to keep pace with the new demand. There are so many factories which provide fake extracts. This placebo can arise from immature trees to defective sterilization products. The extent of deviations in the business is so bad that in Thailand, Malaysia, and Vietnam certain manufactures have turned to using branches and stems of the shrub instead of the roots. Some Malaysian producers of Tongkat Ali minimized the dosage of their products and sold root powder instead of extract, and some even turned to mis-labeling. In fact most of the Tongkat Ali products in the US are under dosed by a factor of up to 1000 (McKerry, 2011).

Recently, Tongkat Ali has begun to emerge in European and western markets due to its reputation and high regards in the eastern cultures as an alternative treatment. On-line providers have reported an increase in consumer interest and sales across Europe and throughout the U.S. Furthermore, a television program by Dr. Mehmet Oz on the positive effects of Malaysian Tongkat Ali roots had increased the herbs popularity across the United States. The tree must be 20 – 25 years old otherwise the product will not be reliable in terms of its activity. The Malaysian government has spent millions of dollars in updating the clean and sterilized manufacturing centers for this plant. Malaysian manufactured Tongkat Ali extracts must assure the buyer of its safety and reliability in increasing testosterone. Dr. Oz's speech had added pressure on the manufacturers in Malaysia to keep pace with safety and quality (Gimlette and Thomson, 1977).

Tongkat Ali products in Malaysia can be divided into two groups, the first is Ministry of Health (MOH) approved and second is non-MOH approved products, which are cottage products and not regulated. All products which are exported legally to other countries are generally regulated and MOH-approved. While the Malaysian government has invested millions of dollars in perfecting the manufacturing process, the industry needs standardization and there must be guarantees on product quality.

#### **2.5.4 Safety and drug interaction**

Some side effects of Tongkat Ali products such as anxiety, insomnia, and restlessness have been reported. Tongkat Ali should not be used by nursing or pregnant women or children and also by men with prostate cancer, as Tongkat Ali increases testosterone levels. Persons with other diseases who should avoid Tongkat Ali are those with diabetes mellitus, kidney, heart, liver diseases, or sleep apnea. Furthermore Tongkat Ali should not be used by people with weak immune systems as evidence suggests that it may further weaken immune function. It is especially important to tell the doctor or pharmacist if the person is taking insulin. Tongkat Ali may decrease blood sugar levels (Wong, 2006) Several reported side effects of Tongkat Ali products include slightly increased level of aggression when exercising, slight restlessness and difficulty going to sleep while the body adjusts to the new testosterone level, raised libido and increased sexual performance and increased recovery rate and also upset stomach, blurred vision, headaches and constipation (McKerry, 2011).

## **2.6 PROTEIN BASED STUDY ON TONGKAT ALI**

### **2.6.1 Overview of 2-Dimensional Electrophoresis**

The term 2-dimensional electrophoresis (2-DE) means the technique in which first-dimension isoelectric focusing in a polyacrylamide gel with a pH gradient and a high concentration of urea is combined with a second-dimension separation on SDS polyacrylamide gels. In the first dimension, the proteins are separated according to their charges, and in the second dimension, according to their molecular masses. The

resulting spot patterns are usually oriented according to the Cartesian convention with the low, acidic isoelectric points to the left and the low molecular weights at the bottom. Depending on the 2-D application, different gel formats, reducing or non-reducing conditions, different pH ranges, and different detection methods can be used.

Gel electrophoresis has some advantages over other separation techniques. Starting materials, such as cell lysates or tissue extracts, can be applied to gels directly and fractionated with very high resolution. Electrophoretic techniques exhibit minimal loss of hydrophobic protein species. The separated proteins are embedded in the matrix, where they can be detected with very high sensitivity (essentially unlimited exposure time for fluorography, autoradiography, or storage phosphor imager). The isolated proteins can be readily extracted from the matrix for further characterization by sequence analysis or mass spectrometry. The two separation parameters of 2-D protein gel electrophoresis (isoelectric point and molecular weight) are essentially orthogonal and independent, even though they occur in the same matrix. Either dimension is capable of resolving 100–200 protein species, but the resolving power of the combined technique is approximately the product of the resolving power of the individual techniques. Up to 10,000 individual protein species have been resolved in a single gel, similar in magnitude to the estimated number of expressed proteins in a eukaryotic cell or bacterium. For studies of minute changes in protein expression or modification in a cell or tissue, it is crucial that the entire array of proteins can be displayed in one gel (Celis and Gromov, 1999).

### **2.6.2 Application of 2-DE in plant studies**

Though 2-DE is one of the most efficient and powerful methods to study complex patterns of gene expression at the level of proteins, electrophoretic separation of proteins from plant tissue extracts is often complicated by the plants' hard cell walls, high frequency of modified N-termini, and insolubility of membrane proteins as well as other non-protein contaminants indigenous to the plant, such as organic acids, lipids, polyphenols, pigments, and terpenes (Tsugita *et al.*, 1996; Wang *et al.*, 2003).

### **2.6.3 Proteomics as tool to detect biomarkers of *Eurycoma longifolia* in Tongkat Ali-based products**

Biomarkers are important molecular signposts of the biologic state of a cell at a specific condition. Active genes, their respective protein products, and other organic chemicals made by the cell create these signposts. Proteomic technologies provide the tools needed to discover and identify the biomarkers and this applies to biomarkers of plants as well. The conventional proteomics has been the high resolution 2-D gel electrophoresis followed by computational image analysis and protein identification using mass spectrometry. In this study, screening of a total of 23 MOH approved and 6 non-MOH approved samples were subjected to protein being quantified using Bradford analysis and followed by detection of 2-dimensional electrophoresis of developed markers for this plant's active extract from previous studies. (Hamid,2008; Ling 2010)