Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 15 (6): 831-844, 2015
ISSN 1812-5654

ansinet
Asian Network for Scientific Information

REVIEW ARTICLE OPEN ACCESS

DOI: 10.3923/jas.2015.831.844

Review on *Eurycoma longifolia* Pharmacological and Phytochemical Properties

Aini Norhidayah Mohamed, Jaya Vejayan and Mashitah Mohd Yusoff
Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Malaysia

ARTICLE INFO
Article History:
Received: January 14, 2015
Accepted: March 04, 2015

Corresponding Author:
Aini Norhidayah Mohamed,
Faculty of Industrial Sciences and Technology,
Universiti Malaysia Pahang,
Lebuhraya Tun Razak, 26300,
Gambang, Pahang, Malaysia
Tel: +60122476193

ABSTRACT
Eurycoma longifolia or Tongkat Ali is famous for its aphrodisiac property and the traditional uses range from tonic after childbirth to treating malaria. Phytochemical studies revealed the presence of bioactive compounds such as quassinoids, alkaloids, squalene derivatives, tirucallane-type triterpenes and biphenylneolignans. Existing research revealed that plant has potential to treat various diseases and to replace the current treatment. Purpose of this article is to evaluate and summarize the existing literatures concerning phytochemical, biological and toxicological studies of *E. longifolia*. It is expected that critical evaluation will be useful for researchers working on the potential role of *E. longifolia* in treating diseases or for product development.

Key words: *Eurycoma longifolia*, Tongkat Ali, Longjack, aphrodisiac properties, anti cancer, anti malaria

INTRODUCTION

In Malaysia, it is considered as a national treasure while other countries refer it as Malaysian Ginseng. Tongkat Ali Long Jack is the common name while *Eurycoma longifolia* is the Latin name. A well-known folklore herbal medication in Southeast Asia, the water decoction of this plant parts have been used to treat ailment for centuries. The decoction is bitter and it is assumed that the more the bitterness, the better the efficacy. Based on personal experiences and cultural beliefs, the traditional uses have been passed from generation to generations. This plant has been used to increase energy and vitality, as health tonic, relieve fatigue, anti-malaria treatment, tonic for woman after childbirth, diarrhea, dysentery, glandular swelling, bleeding, dropsy, cough, fever, ulcer and high blood pressure (Bhat and Karim, 2010). The aborigines are known to drink the decoction of this plant before entering deeper into mosquito infested jungle. This plant is investigated to contain canthin-6-one and β-carboline alkaloids which are naturally occurring amine compounds formed as metabolic by-products in order to repel insects and herbivores (Chua *et al*., 2011).

The traditional uses are well documented in literature. Hout *et al*. (2006) conducted an ethnobotanical survey of application of *E. longifolia* as medication among traditional healers in regions of Cambodia. The survey revealed that *E. longifolia* has been used for fever, rheumatism, dysentery, diuretic and tonic. Al-Adhroey *et al*. (2010) investigated plants that are traditionally used in the treatment of malaria, among 233 aboriginal as well as rural households and traditional healers in malaria endemic areas in Malaysia. The studies revealed that among the many other medicinal plants used, *E. longifolia* is the only one mentioned most by the studied groups. Adnan and Othman (2012) found that *E. longifolia* is a common plant species that have important value in Malay community and it is used commonly for healing and consumption.

There are a total four species being referred by locals with the name Tongkat Ali, Eurycoma longifolia, Entomophthora apiculata, Polyalthia bullata, *Goniothalamus* sp. Nevertheless only *E. longifolia* has been the most widely used species among them (Athimulam *et al*., 2006). Additionally, scientific research has been found to be numerous on *E. longifolia* (Hassan *et al*., 2012). It is known as Tongkat Ali in Malaysia and Singapore, Pasak Bumi in Indonesia, Cay Ba Binh in Vietnam and Tung Saw in Thailand. Tongkat Ali name or Ali's walking stick, may come from the morphology of the plant, where its long, straight, woody roots looks like walking stick. In Malaysia, it is also known as Payung Ali, Penawar Pahit, Setunjang Bumi, Bedara Pahit, Tongkat Baginda, Pokok Syurga, Tongkat Ali Hitam, Pokok Jelas and Jelaih

(Athimulam *et al*., 2006). Among these common names, Tongkat Ali is the most famous of all in Malaysia as well as internationally. The latter is indicative based on the many products sold in western world stating "Tongkat Ali" at their labels or packaging.

Despite wide range of traditional uses known, *E. longifolia* is most famous solely for its aphrodisiac property. It is highly sorted out in the western world for its potential in enhancing sexual performance. Ab Rahman *et al*. (2011) found out, among 1331 men aged 40 years and above who were se eking treatments in sexual problem, *E. longifolia* was used besides Viagra, genital massage and ginseng. Ethnobotanical survey by Ong and Nordiana (1999) in the states of Kelantan and by Samuel *et al*. (2010) in Perak, Malaysia discovered the roots were used as aphrodisiac by men. Besides the Malay community, *E. longifolia* also has been used by the Chinese and Indian ethnics in Malaysia (Low and Tan, 2007).

Aphrodisiac property of *E. longifolia* is contributed by its testosterone enhancing effect. Instead of containing testosterone itself, *E. longifolia* works by promoting the tests to increase the production of testosterone naturally. Current androgen replacement therapy applies supplementation of testosterone gel, injection or pellet. The continuous supplementation can result in the stopping of body natural production system thus, the natural testosterone promoting effect of *E. longifolia* is much favored treatment.

Due to its energy enhancing property and aphrodisiac potential, lots of products with Tongkat Ali formulations have been sold in the market. Usually from the raw crude powder or after the freeze- or spray drying process of the water extracts, the products come in the form of capsule, pills, liquid formulation, pre-mixed coffee and canned processed drinks (Effendy *et al*., 2012). The standardized water soluble extract has been patented in 2004 (US2004/0087493 A1) after having gone extensive animal and human clinical evaluation (Tambi, 2009). Usually in the market, the concentration of Tongkat Ali in a product is declared as x:y which means y gram of Tongkat Ali is used to produced x gram of extract.

Wide spectrum of pharmacological activities of *E. longifolia* is attributed by the presence of several classes of metabolites. The major metabolites in *E. longifolia* are quassinoids, squalene derivatives, biphenylneolignans, tirucallane-type triterpenes, canthin-6-one and beta-carboline alkaloids. The bitter taste of this plant is contributed by quassinoids. The water soluble extracts contains tannins, high molecular weight polysaccharides, glycoproteins and mucopolysaccharides. Compounds such as eurycomanone, 9-methoxycanthin 6-one, 14, 15-β-dihydroxyklaineanone and 13, 21-epoxyeurycomanone usually are used as standard markers for the standardization of *E. longifolia* products (Chan *et al*., 2004). The presence of these compounds has generates dresearch interest in variety of other ailments and some of these compounds were shown to possess antimalarial, antiulcer and anticancer in publications.

This review paper discusses the studies that have been undertaken on the pharmacological effect of *E. longifolia*. Safety, efficacy, quality control aspects as well as ecological concern such as steps that have been undertaken to tackle depleting natural resource are also discussed. To the author's knowledge, there has been only one review pertaining *E. longifolia*, written by Bhat and Karim (2010).

Pharmacological effect: The traditional uses of *E. longifolia* have been cultivated for generations. However, scientific validation is needed to verify the effectiveness in treating ailments. Mechanism of action is also needed to be understood for better understanding of the safety and efficacy. In the next sections the pharmacological effects of *E. longifolia* are discussed.

Aphrodisiac effect: *Eurycoma longifolia* has many traditional uses but it has gained its notoriety as sexual enhance (Ang and Sim, 1998). However, the efficacy are based on personal experience, testimony and more of a subjective opinion rather than verified scientifically. Ang and Sim (1998) started the research pertaining to the aphrodisiac property of this plant. They investigated the potential of this plant in increasing sexual motivation of sexually naive male rats where the rats were administered with different fractions (chloroform, water, butanol) of *E. longifolia* extracts. The successful crossover through electric grid in electrical copulation cage was used to measure the motivation of the male rats to find the receptive female. The result reported that repeated and chronic dosing of EL fractions enabled the rats to cross the electrical grid. The number of mounting, intromission and ejaculation also improved. They also discovered that the results did not show much different between fractions and they claims that it might be due to the presence of active compounds in more than one fraction.

Ang *et al*. (2001) in their investigation on castrated rats discovered that feeding castrated rats with *E. longifolia* fractions (butanol, methanol, water, chloroform) significantly increased the weight of the rat's levator ani muscle. However, which fraction gave the best stimulation was failed to be determined.

Ang *et al*. (2001) noticed that *E. longifolia* extracts (butanol, methanol, water, chloroform), in dose dependent manner gave significant increase in the potency activity which were measured by penile reflexes of treated male rats; quick flips, long flips and erections.

The effect of *E. longifolia* on sexual qualities in middle age male rats was observed by feeding the rats with 0.5 g kg^{-1} of various fractions (chloroform, water, butanol, methanol) of *E. longifolia* for 12 weeks. After treatment, the hesitation time to meet receptive female in electrical copulation cage were measured (Ang *et al*., 2003). It was also observed that there was not much difference on the sexual qualities exhibited by the various fractions of *E. longifolia*.

The effects of *E. longifolia* on sexual arousal in sexually sluggish male rats were tested by Ang *et al*. (2004a). The act of yawning and stretching indicated sexual arousal. 200, 400 or 800 mg kg^{-1} of various fractions (methanol, butanol l, chloroform, water) of *E. longifolia* extracts were fed

on the rats. The concentration of 800 mg kg^{-1} of the extract gave the best sexual arousal, however, there was not much difference between fractions.

Zanoli *et al*. (2009) claimed most previous researches on the aphrodisiac effects of *E. longifolia* were carried out on normal rats, except work done by Ang *et al*. (2004b) on sexually sluggish male rats. Zanoli *et al*. (2009) also argued that studies on sexual disorder should be utilized on sluggish and impotent male rats to mimic human sexual problem. Instead of using *E. longifolia* solvent fractions, they used root powder to avoid any solvent interaction in pharmacological effect. In the test, rats were treated with acute, subacute and subchronic level of *E. longifolia* and copulatory performance during mating, sexual motivation in partner preference and testosterone serum levels were measured. Results showed that acute and subacute dose reduced ejaculation latencies, increased the mounting and ejaculating. However, the motivational behavior is not affected by the treatments.

Testosterone is important for male fertility such as normal spermatogenesis. Hormonal disturbances such as excess of estrogen can cause inhibitory effect on testosterone and disturb testicular function. Wahab *et al*. (2010) investigated the effect of *E. longifolia* on testicular histology and sperm count in estrogen-treated male rats. They discovered that *E. longifolia* could heightens testicular function and reverse the effect of excessive estrogen as rats treated with *E. longifolia* showed higher sperm count and mortality. Low *et al*. (2013) showed that quassinoids rich extract can increase sperm concentration and the amount of testicular testosterone.

The aphrodisiac property tests of *E. longifolia* mostly were carried out on rodents. Tambi and Imran (2010), conducted a clinical trial on the effect of standardized, water soluble extract in managing men with idiopathic infertility, namely men with low sperm concentration with or without low percentage motility and morphology of unknown causes. In the trial, several men were given 200 mg daily of the extract and follow up semen analysis were conducted. The supplementation resulted in increased sperm concentration and better sperm motility and morphology. There were also spontaneous pregnancies.

All of the researches above showed that *E. longifolia* has the potential to treat male sexual problems. Research conducted by Abdulghani *et al*. (2012) showed that *E. longifolia* extracts has a promising effect in treating female sexual disorders. They studied the ameliorative effects of *E. longifolia* extracts on reproductive disorders in female rats; an irregular estrous cycle and ovarian cystic follicles. The rats were treated with hormone to induce reproductive disorder. After treatment with the extract, the reversal effects were shown where fewer rats showed the disorder.

Generally, it can be said that *E. longifolia* can help in improving the sexual performance and treating sexual problems. All of the tests above utilized the plant extract instead of isolated bioactive compounds, so which bioactive compounds that contributed to the effect is not yet determined. The particular fraction of extract that gave the best stimulation is yet to be known. Synergistic effect of bioactive compounds that present in all fractions might contribute to the effect explaining why traditionally decoction of the whole extract is consumed for sexual effect. Although all the tests showed promising effect, still mode of action need to be evaluated and more clinical trials are warranted.

Anti-malarial effect: Malaria is a deadly disease, which is responsible for one million of deaths annually (Nguyen-Pouplin *et al*., 2007), caused by a parasite of *Plasmodium* genus. It is prevalent in tropical and subtropical regions such as South East Asia and Sub Saharan Africa. Current treatment for this disease is using drugs such as chloroquine, artemisinin and clindamycin. However, with the deadly situation, there is a need for the constant search for new anti-malarial compounds that is more effective, cheaper, easily accessible, exhibit better efficacy yet safe and ultimately without being resistant to the parasite.

Plants have been used in folklore to treat malaria and in respect, *E. longifolia* has also been commonly used as one of the treating remedy. Not surprisingly, the current drugs (chloroquine and artemisinin) for the malarial treatment are also plant derived. Al-Adhroey *et al*. (2010) conducted a survey on plants which have been traditionally used in Malaysia in the treatment of malaria and interestingly they discovered, among 233 aboriginal, rural households and traditional healers interviewed in the region of Peninsular Malaysia responded with names of several plants which have been used to treat malaria. Amongst the response, *E. longifolia* was the popular species mentioned often by most respondents. It can be said that *E. longifolia* has the potential to be developed as the source for novel drug to combat malaria based on preliminary researches that have been undertaken to determine the efficacy of this plant against this disease.

Several researchers tested the antimalarial activity of the extract of *E. longifolia*. Tran *et al*. (2003) and Nguyen-Pouplin *et al*. (2007) tested the antimalarial activity of plants that are used in Vietnam traditional medicine. Nguyen-Pouplin *et al*. (2007) discovered that *E. longifolia* extract showed high antimalarial activity while Tran *et al*. (2003) found *E. longifolia* able to inhibit the growth of chloroquine resistant *Plasmodium falciparum*. Butanol extracts were more potent than diethyl ether extracts but compared to chloroquine, both extracts were more potent against chloroquine-resistant Gombak A *Plasmodium falciparum* isolate (Chan *et al*., 2004). Hout *et al*. (2006) observed a very high anti-plasmodial activity of the plant aqueous extracts which is the preferred extraction method in traditional medicines and it was as active as dichloromethane extracts. As combination of treatment is said to result in increased efficacy, Ridzuan *et al*. (2007) compared anti-malarial activity of standardized extract alone which contained three major quassinoids (eurycomanone, 13, 21-dihydroeurycomanone and 13α (21) epoxyeurycomanone) and as well as combination of standardized extract with artemisinin. It was discovered that combination of the extract and the drug showed promising activity than standardized extract alone. Wernsdorfer *et al*. (2009) compared the anti-plasmodial activity of the

standardized extract with artemisinin activity. They reported that artemisinin showed a higher activity as compared to the standardized extract but the activity of the latter was relatively high to be ignored for further studies. Activity of the standardized extracts was higher than isolated quassinoids, consequently they claimed synergism between the combined three quassinoids. Astelbauer *et al*. (2012) compared the activity of plant derived compounds such as aglafolin, rocaglamid, against standardized quassinoid extract from *E. longifolia* for antiplasmodial activity. The result demonstrated that the standardized quassinoid extract showed higher activity than the two compounds.

The antimalarial activity of individual compounds from *Eurycoma longifolia* especially the various quassinoids has also been tested by several researchers. Ang *et al*. (1995) tested that three quassinoids (eurycomanol, eurycomano l2-O-beta-D-glucopyranoside and 13 beta, 18-dihydroeurycomanol) against chloroquine resistant *Plasmodium falciparum*, but chloroquine posed higher activity against the *P. falciparum* where the LC_{50} for the compounds were 1.231-4.899 μM, 0.389-3.498 μM and 0.504-2.343 μM, respectively, compared with 0.323-0.774 μM for chloroquine. Jiwajinda *et al*. (2002) discovered that LC_{50} of longilactone were from 5.5 to 13.7 μM, while 11-dehydroklaineanone, 15β-hydroxyklainone, 14,15 β-dihydroxyklaineanone, 15β-O-acetyl-14-hydroxyklaineanone showed LC_{50} of 5.5, 5.3, 5.0, 23.8 μM. Kuo *et al*. (2004) isolated eurycomanone and pasakbumin B. The compounds displayed potent antimalarial activity against the resistant *Plasmodium falciparum*. Chan *et al*. (2004) isolated four quassinoids (eurycomanone, 13, 21-dihydroeurycomanone, 13α(21)-epoxyeurycomanone, eurycomalactone) and an alkaloid (9-methoxycanthin-6-one). Using lactate dehydrogenase assay, the four quassinoids showed high activity against chloroquine resistant Gombak A isolate, but less active on chloroquine sensitive compared to chloroquine. Ultimately, all the quassinoids and chloroquine tested were found less potent than artemisinin.

As eurycomanones is considered to be the most potent anti-malarial compound in *E. longifolia* Chan *et al*. (2005), studied the anti-malarial activity against chloroquine-resistant is olate using semi synthetic forms of eurycomanone; referred as diacylated 1,15-di-O-isovaleryleurycomanone, 1,15-di-O-(3,3-dimethylacryloyl) eurycomanone, 1,15-di-O-benzoyleurycomanone and the monoacylated 15-O-isovaleryleurycomanone. The result showed that the monoacylated showed high activity and lower toxicity, while the diacylated showed lower activity, thus acylation only at the C-15 hydroxy group may be worthy of further antimalarial investigation. Mayer *et al*. (2009) studied the activity of Oxidized Hydroxyl of Cis Terpenone (OHCT) against chloroquine and artemisinin resistant. The result showed that OHCT was more active than chloroquine on chloroquine resistant but less active against chloroquine sensitive while OHCT and chloroquine exhibited similar potency against chloroquine resistant clone. The OHCT found even at low nano molar concentration, able to inhibit survival of artemisinin resistant isolates. It is therefore concluded that OHCT has the potential capabilities to be developed as anti-malarial lead compound especially as it is easily synthesized (Mayer *et al*., 2009).

In ensuring effective treatment, how the drug is administered in the body also plays its role. Ridzuan *et al*. (2007) discovered subcutaneous route of *Eurycoma longifolia* extract was more effective than oral administration. They claimed that was because with subcutaneous route, drugs are directly exposed and absorbed to the bloodstream with the rapid detection of drugs in plasma while with oral administration, the drug is absorbed through the digestive tract and passed through the liver before it is transported via the bloodstream causing inactivation and incomplete absorption. In determining which stage of *P. falciparum* is susceptible towards *E. longifolia* treatment, Sholikhah *et al*. (2008) discovered that trophozoites stage of *P. falciparum* was the weakest when treated with *E. longifolia*.

Studies on the anti-malarial effect of *E. longifolia* have been therefore exhaustively been carried out on its extract, isolated individual compounds or in combination as well as semi synthetic derived compounds. As most of the laboratory results demonstrated positive effects, further research i.e., clinical trial is recommended to evaluate the effect of this treatment on human and to determine whether compounds from this plant can therefore serve as another form of therapy other than the current drugs available for anti-malarial treatment.

Anti-parasitic effect: Besides malaria, the studies on *E. longifolia* potential as anti-parasitic have been carried out on other diseases such as schistosomiasis and toxoplasmosis. Schistosomiasis or bilharziasis is a tropical parasitic disease caused by blood-dwelling fluke worms of the genus Schistosoma. Jiwajinda *et al*. (2002) studied the inhibitory effect of three quassinoids (longilactone, 11-dehydroklaineanone and 14,15 β-dihydroxyklaineanone) on adult schistosomes movement and egg-laying of *S. japonicum*. All the three quassinoids showed significant inhibitory effect on *S. japonicum* but the activities were found to be weaker than the current drug for schistosoma treatment, the praziquantel.

Kavitha *et al*. (2010, 2012) investigated the anti-parasitic activities of *E. longifolia* on toxoplasmosis caused by *Toxoplasma gondii*, one of the most widespread protozoan parasites, chronically infecting approximately 30% of the global human population. Before studying the inhibitory effect, they first identified an appropriate host to support the growth of the parasite to ensure that the *E. longifolia* extract used not toxic to the host and affect host performance. Kavitha *et al*. (2010) tested the cytotoxicity of *E. longifolia* extract against two mammalian cell lines (Vero and HS27). One of the *E. longifolia* fraction did not show toxicity ($CC50>20\ \mu g\ mL^{-1}$) towards Vero cell, so the Vero cell was chosen as the host for anti-parasitic studies. The inhibitory study showed *E. longifolia* fraction significantly inhibited *T. gondii* growth even at concentration as low as

0.369 μg mL^{-1} (Kavitha *et al*., 2012). Further advantage is the *E. longifolia* fraction was less toxic to host cell than Clindamycin, the current drug for toxoplasmosis treatment.

Anti-bacterial and anti-fungal effects: Farouk and Benafri (2007), investigated the anti-bacterial properties of methanolic, ethanolic, acetone, aqueous extracts from different parts of *E. longifolia* against several bacteria. The alcoholic and acetone extracts of the leaves and stem were active against *Escherichia coli* and *Salmonella typhi* microbes while the aqueous leaves extracts were found to be active against *Staphylococcus aureus* and *Serratia marscesens*. However, the root extracts, which has been the preference in traditional medicines and modern researches, failed to show any antibacterial property. The bacterial strains used in this study has no significant clinical importance, however in another study done by Tzar *et al*. (2011), *E. longifolia* aqueous extracts were studied on bacterial strains that cause diseases which are difficult to be treated. The pathogenic bacterial strains were methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecium*, extended-spectrum beta lactamase-producing *Klebsiella pneumoniae*, group-1 beta lactamase-producing *Pseudomonas aeruginosa*, multidrug-resistant *Acinetobacter baumanii* and *Salmonella typhi*. The result was in agreement with Farouk and Benafri (2007), where anti-bacterial effects were only observed from the leaves and stems but not from the root extract. They also studied the anti-fungal effect as fungal infection is fast becoming an emerging infection especially among immuno compromised patients. Three species of *Candida* (*Candida albicans*, *Candida glabrata* and *Candida krusei*) were used. However, the *E. longifolia* extract did not show any antifungal activities at the concentrations tested in this study (10, 5, 2.5, 1.25 and 0.625 mg mL^{-1}).

Anti-cancer effect: Cancer has caused many deaths worldwide and cancer cases continue to increase. However, cancer therapies are limited due to the adverse effect and development of drug resistance thus there is a need to find newer anti-cancer treatments. Plants are considered as promising anti-cancer candidate as they contain large amount of pharmacologically active and generally safe compounds. Ideally, the new cancer medicine derived from plants should be selectively cytotoxic towards the cancer cells, able to stop its proliferation and consequently induce cell death. Several studies reported the potential of *E. longifolia* as anti-cancer agents.

Okano *et al*. (1995) assayed the anti-tumor promoting activity of quassinoids. Some of the quassinoids showed potent activity, with more than 50% inhibition. They also studied the mode of action of the quassinoid whereby it was discovered a methyleneoxy bridge and a side chain found to enhance the activity while a sugar moiety reduces the activity instead. Jiwajinda *et al*. (2002) discovered 14,15β-dihydroxyklaineanone (LC_{50} = 5 μM) has the highest anti-tumor promoting activity and the inhibitory potential was much higher than that of quercetin (LC_{50} = 23 μM) and β-carotene (LC_{50} = 30 μM) i.e., two common anti-tumor promoting natural agent. Dehydrolongilactone, longilactone, 11-dehydroklaineanone and 15β hydroklaineanone were comparable to quercetin or b-carotene, while 15b-O-acetyl-14-hydroxyklaineanone was classified altogether as a less active compound.

Kuo *et al*. (2004) isolated 65 compounds from *E. longifolia*. Some of the compounds; eurycomalactone, 6-dehydroxylongilactone, 9-methoxycanthin-6-one, canthin-6-one, longilactone, 14,15b-dihydroxyklaineanone, pasakbumin C and canthin-6-one-9-O-b-glucopyranoside demonstrated strong cytotoxicity toward human lung cancer (A-549) cell lines while eurycomalactone, 6-dehydroxylongilactone, 9-methoxycanthin-6-one, 14,15b-dihydroxyklaineanone, eurycomanone, pasakbumin B and pasakbumin C exhibited strong cytotoxicity toward human breast cancer (MCF-7) cell lines. This research proved that eurycomanone pose cytotoxicity towards breast cancer cells while Chuen and Pihie (2004) substantiated this finding by showing that their semi purified eurycomanone also confer toxicity towards breast cancer cell. Moreover, it was discovered this compound was less toxic on non-cancerous breast cell (MCF10A) with EC value of 30.90±0.99 μg mL^{-1}, compared to Tamoxifen, the drug for breast cancer treatment with EC value of 2.59±0.11 μg mL^{-1}. Chuen and Pihie (2004) also elucidated the mode of action of the semi-purified eurycomanone where they discovered the treatment resulted in apoptotic cell death of MCF-7 breast cancer cells involving down-regulation of an apoptosis regulator protein (BCL-2).

Previous studies tested the anti-proliferative property of isolated compounds (Okano *et al*., 1995; Jiwajinda *et al*., 2002; Kuo *et al*., 2004) and synthetic compounds (Chuen and Pihie, 2004). Nurhanan *et al*. (2005) evaluated the cytotoxic effect of *E. longifolia* root extract (methanol, n-butanol, chloroform and water) against human papillomavirus (KB), human prostate cancer (DU-145), human rhabdomyosarcoma (RD), breast cancer (MCF-7), ovarian cancer (CaOV-3), normal kidney (MDBK) cell lines. The study showed all of the root extracts except the water extract gave significant cytotoxic effect on all the cell lines except MDBK (kidney) normal cell line. Tee and Azimahtol (2005) tested the anti-tumor effect of *E. longifolia* chromatographic fraction on breast cancer cell lines (MCF7) as they claimed multi composition of herbal medicines were more effective than single compound, probably due to the synergism of the contents. They discovered methanolic extract has higher anti-proliferative activity than the aqueous extract towards MCF7 cells. Three fractions showed high toxicity towards MCF7 and significantly increased apoptosis in MCF 7 cell, but only one fraction was chosen for further purification as the two fractions contained eurycomanone which has been well studied. Mode of action was elucidated and it was discovered that the anti- apoptotic protein, which is the Bcl-2 was down regulated in the fraction treated MCF7 cells. Following this result, Tee *et al*. (2007) elucidated further the mode of action of the fraction. They discovered Bcl-2 protein was reduced followed by the cleavage and activation of caspase-7, which is

an executioner protein of apoptosis. Mahfudh and Pihie (2008) also discovered the same mode of action of eurycomanone against cervical cancer HeLa cells. The apoptosis triggered by eurycomanone involved the up-regulation of p53 tumor suppressor protein and the increase of pro-apoptotic Bax protein. Besides that, in the study, eurycomanone also reduced the viability and proliferation against ovarian cancer cells (CaOv-3), immortal cell line (HeLa), liver hepatocellular cells (HepG2), human melanoma (HM3KO), breast cancer cells (MCF-7) and less toxic on normal cells (MDBK, Vero). Zakaria *et al*. (2009) also discovered that apoptosis through the up regulation of p53 and Bax protein, with the down regulation of Bcl-2 protein was the mode of cell death in liver cancer (HepG2) cells treated with eurycomanone. Within four cancer cell lines (HepG2, Hela cells, CaOV3, HM3KO) tested by Zakaria *et al*. (2009), crude extract of *E. longifolia* was the most potent towards cancerous liver cell (HepG2) and showed very little toxicity towards normal cells (Chang's liver and WLR-68). Tamoxifen, a standard drug showed great toxicity towards all the cancer cell lines including the normal cells.

Most researches on *E. longifolia* were conducted on the root extract, however, Miyake *et al*. (2010) studied the phytochemical property of the stem extracts. Isolates from the stem extracts were evaluated for their anti-cancer activity against human fibrosarcoma (HT-1080) cell line. Among the isolates, 9, 10-dimethoxycanthin-6-one, 10-hydroxy-9-methoxycanthin-6-one and dihydroniloticin, showed strong toxicity and an isolate, 14-deacetyleurylene displayed stronger activity than standard drug, the Fluorouracil. Wong *et al*. (2012) studied the effect of eurycomanone on lung cancer cells (A549) proliferation, clonogenic cell growth, expression of lung cancer markers and cancer related genes. The result showed that eurycomanone inhibited lung cancer cell proliferation in a dose dependent manner in a concentration ranging from 5 to 20 μg mL^{-1} and the concentration that inhibited 50% cell growth was 5.1 μg mL^{-1}. Cisplatin, the standard drug, displayed GI50 at 0.58 μg mL^{-1}, proving that Cisplatin was at least 10 folds more potent than eurycomanone. However, cisplatin come with adverse effect, thus, eurycomanone at the viable therapeutic concentrations (5-20 μg mL^{-1}) can inhibit lung cancer cell proliferation. After the eurycomanone removal, the proliferation activity of the A549 was not fully restored. The irreversible inhibition is good for anti-tumor activity especially if the half life of the anti-tumor compound in plasma is short. The treatment with eurycomanone also resulted in down regulation of cancer cell growth associated genes, which were the prohibition and endoplasmic reticulum luminal protein (ERp28).

The anti-tumor activity shown by *E. longifolia* probably is contributed by the free radical scavenging property of the plant. This is because one of the fundamental steps of cancer is the presence of free radicals that are damaging to DNA, leading to cancer. Free radical scavengers can get rid of free radicals, help in preventing and fighting cancer. Purwantiningsih *et al*. (2011) investigated the free radical scavenging activity of the standardized ethanolic extract of *E. longifolia* (TAF-273) related to its total phenolic and flavonoid contents. They discovered free radicals scavenging activity increased in correlation with total flavonoid and phenolic contents. However, the activity was lower than galic acid, which is the standard anti-oxidant or free radical scavenger.

It can be concluded here that the anti-tumor effect of *E. longifolia* has been thoroughly tested on the leave, stem and root extract as well as on isolated bioactive compounds. Promising results were shown by the studies. To a certain extend mode of action has been elucidated and it was observed that apoptosis process was involved whereby down regulation of anti-apoptotic and up-regulation of pro apoptotic occurred. If utilization of *E. longifolia* to treat cancer disease is to be applied, clinical trials are warranted.

Toxicity: Razak and Aidoo (2011) studied the toxicity and mutagenicity of three types of *E. longifolia* products in the market; products containing mixture of *E. longifolia* with other herbs, product which contains only *E. longifolia* and an authenticated *E. longifolia*. They discovered, all extracts, except one product which is mixture of *E. longifolia* and another herb was not mutagenic and they concluded there is a risk of increased cytotoxicity and mutagenicity of extract of *E. longifolia* based remedies compared to the toxicity of remedies containing solely *E. longifolia*.

Wiart (2012) in a letter to the editors of publication claimed that based on several publications that he read, *E. longifolia* can have adverse effects on long term consumption such as cancer, diabetes, obesity, depression and aggressivity, fatal pulmonary haemorrhages, breathing problems and seizures. He claimed that the effects are caused by raised testosterone level, a well-known effect of *E. longifolia* treatment. It has to be noted here that his readings are not based on testosterone raised by *E. longifolia* but related to the alarming increased in testosterone level due to synthetic testosterone supplementation. *E. longifolia* does not work by increasing the amount of testosterone directly i.e., it does not contain testosterone, but instead regulate the de novo synthesis of testosterone in the body.

Other activities: Tada *et al*. (1991) studied the anti-ulcer on two quassinoids (Pasak bumin A and Pasak bumin B) and they were found to show potent anti-ulcer activity. The anti-ulcer property prompted Ang and Cheang (1999) to investigate the *E. longifolia* fraction on rats of another anti-stress related disorder, which was the anxiolytic effect. The anxiolytic effect studied showed positive with increased of square crossed, inhibit fighting behavior, decreased immobility and fecal pellets, increases number of entries and time spent in the elevated plus maze. There were not much difference of the effect between different fractions (chloroform, methanol, water, butanol) of the extracts, however, the effect was consistent with the effect of diazepam, a drug used for the relief of symptoms related to anxiety disorders.

Traditionally *E. longifolia* has been used to increase health and general wellbeing. The plants are among the popular herbs used to enhance exercise and sports performance

(Chen *et al*., 2012). Muhamad *et al*. (2010) tested the ergogenic effect, which is the effect of increased performance and stamina in high intensity exercise. The ergogenic effect is signified by athletes' endurance running capacity and physiological responses in the heat. In the study, after supplementation with *E. longifolia* capsule for seven days, it was found unfortunately to have no significant difference on the endurance of their running capacity and physiological responses in the heat when compared with placebo controlled. They suggested longer duration of supplementation and higher dosage to evaluate further the potential of *E. longifolia* as ergogenic aids.

Eurycoma longifolia is mostly used for its aphrodisiac effects, believed to be contributed by its ability in raising testosterone levels. Shuid *et al*. (2012) found the testosterone raising ability of EL extract effects mostly on the regeneration of bone resorption in aged man. The main cause of osteoporosis in men is androgen (e.g., testosterone) deficiency due to natural aging. Serum testosterone levels were measured in orchidectomised rats, fed with *E. longifolia* extract capsule. It was reported that supplementing the orchidectomised rats with *E. longifolia* extract elevated the testosterone levels, reduced the bone resorption marker and up-regulated the gene expression of osteoprotegerin, which is a protein that affect bone resorption.

Cytoselectivity: To avoid the site effects of treatment, anti-parasite medicine that is given should only be toxic towards the parasite but not on the healthy host cells. There are several studies reporting the selectivity of *E. longifolia* towards parasite and normal cells. Chan *et al*. (2005) revealed that monoacylated eurycomanone displayed anti-plasmodial potency but toxicity was low in brine shrimp assay test. Mayer *et al*. (2009) reported that hydroxy cis terpenone from *E. longifolia* displayed strong growth inhibitory against all stages of *P. falciparum* but not toxic to cultured human liver cells. There was a discouraging report by Hout *et al*. (2006) where they revealed *E. longfolia* extracts showed low selectivity between human and plasmodium cells. Kavitha *et al*. (2012) in their studies on the treatment of toxoplasmosis, compared the toxicity of several *E. longifolia* extracts and standard drug Clindamycin. Results showed that one extract did not show any toxicity towards Vero cells while Clindamycin showed toxicity at CC50<20 μg mL^{-1}.

One of the adverse effect for current cancer treatment is that cancer drug is also toxic towards normal cells. To reduce side effect during treatment, anti-tumor medicine should have cytoselective property, which is highly selective towards cancer cells and ineffective towards normal cells. There are several studies reporting the toxicity of *E. longifolia* on normal and cancer cells.

Chan and Choo (2002) studied the toxicity of diethyl ether, n- butanol and water fraction of *E. longifolia* that were fed on mice and brine shrimp assay. The result showed that butanol fraction was the most toxic and eurycomanone was identified as the most toxic compounds in the butanol fraction. Structure activity relationship study showed that a C20 type quassinoid, an alpha, beta-unsaturated ketone in ring A, an exomethylene function at C-13 and an oxymethylene bridge connecting C-8 and C-11 of ring C contributed to increased toxicity. In one of their experiments, Chuen and Pihie (2004) tested the cytoselectivity of semipurified eurycomanone on non-cancerous breast cells (MCF-10A). The results were encouraging as the semi purified eurycomanone is cytoselective where it inhibited the proliferation of cancer cells (MCF7) but not the normal cells. In contrast to the standard drug Tamoxifen, the drug was toxic to both normal and cancer cells. Tee and Azimahtol (2005) in their studies on *E. longifolia* fraction also discovered that the fraction only toxic on cancerous cell (MCF-10A) and spare the normal cell (MCF-10A) from the toxic effect. Cytotoxic effect of root extracts (water, methanol, n-butanol, chloroform) against carcinoma (KB), human prostate cancer cell line (DU-145), human embryo rhabdomyosarcoma (RD), breast cancer cell line (MCF-7), ovarian cancer cell line (CaOV-3) and normal cell lines (MDBK) was studied by Nurhanan *et al*. (2005). All the extracts except water showed high cytotoxic effect on the cancer cell lines and not toxic on normal cell lines. Cytoselectivity of eurycomanone on cancerous cells (CaOv-3, HeLa, HepG2, HM3KO, MCF-7) and normal cells was studied by Mahfudh and Pihie (2008). The results revealed that eurycomanone could act as cytoselective agent as it inhibited the proliferation of cancer cells but not normal cells. Zakaria *et al*. (2009) compared the cytoselectivity of Tamoxifen and eurycomanone on normal cells (Chang's liver and WLR-68). It was revealed that Tamoxifen was 12 times more toxic on normal cells as it inhibited 50% cells viability at 1.4±0.31 μg mL^{-1} while eurycomanone gave IC50 value of 17±0.15 μg mL^{-1}. For WLR-68 cells, eurycomanone gave IC50 value of 20±0.22 μg mL^{-1}. This finding supported the fact that eurycomanone has less cytotoxic effect on normal cell.

Most of the studies above showed that *E. longifolia* compounds were cytoselective on parasite or cancer cells but were relatively non-toxic on normal cells and cell host. It showed the prospect of *E. longifolia* as an alternative in treating parasitic disease and cancer.

Phytochemical test: The pharmacological activities displayed by *E. longifolia* are contributed by the presence of bioactive metabolites in the plant. Majority of these metabolites are from the class of quassinoids, squalene derivatives, biphenylneolignans, beta-carboline alkaloids, tirucallane-type triterpenes and canthin-6-one alkaloids. Among the metabolites, quassinoids present as the highest amount. Chua *et al*. (2011) stated more than 85 compounds have been identified from *E. longifolia*. Kuo *et al*. (2004), Miyake *et al*. (2010) and Chua *et al*. (2011) listed known compounds that have been identified in their research and previous studies.

In the studies of pharmacological activities and in the product development of *E. longifolia*, more researches have been conducted to find new and novel compounds from this plant. This section discusses the new compounds discovered from this plant, based on researches conducted from the year

2000 onwards. Ang *et al*. (2000) isolated three novel quassinoids; eurycolactones A, eurycolactones B and eurycolactones C from the chloroform fraction of *E. longifolia* roots. Bedir *et al*. (2003) discovered new quassinoid type glycoside from the roots of *E. longifolia*, where the C (1)-glycosidation site in the quassinoid framework was encountered for the first time. From the roots of *E. longifolia*, Kuo *et al*. (2004) isolated four quassinoid diterpenoids; eurycomalide A, eurycomalide B, 13β, 21-dihydroxyeurycomanol and 5α, 14β, 15β trihydroxy klaineanone. Those quassinoids were reported to be isolated from natural resources for the first time. From the roots of *E. longifolia*, Teh *et al*. (2010), isolated a novel quassinoid, 2,3-dehydro-4α-hydroxy-longilactone. The compound is reported to be the first C19 quassinoid from Simaroubaceae family possessing an unsubstituted vinyl function and C4 methyl group of β configuration in ring A. A phenyl propanoid; scopolin, was isolated from this plant for the first time.

Miyake *et al*. (2010), in their phytochemical investigation of the stems of *E. longifolia* isolated a new tirucallane-type triterpenoid; 23,24,25-trihydroxytirucall-7-en-3,6-dione while two new canthin-6-one alkaloids; 4,9-dimethoxycanthin-6-one and 10-hydroxy-11-methoxycanthin-6-one were isolated.

Lots of compounds have been isolated from *E. longifolia* and most of these compounds are from quassinoid class. On the pharmacological effect of these compounds, the research is still limited.

Eurycoma. longifolia or commonly known in Malaysia and internationally as Tongkat Ali is most famous and notorious for its sexual enhancing property, however, in literatures, the compound that is responsible for the property is surprisingly unknown yet. As such most of the studies are conducted on extracts only (Ang and Sim, 1998; Ang *et al*., 2001, 2003, 2004a,b; Wahab *et al*., 2010; Abdulghani *et al*., 2012), root powder (Zanoli *et al*., 2009) and quassinoid rich fraction (Low *et al*., 2013). However, Sambandan *et al*. (2006) and Asiah *et al*. (2007) identified a bioactive peptide of 4.3 kDA, labelled as eurypeptide, which enhance synthesis of various androgens. The bioactive peptide (4.3 kDa) (patented: PI 20003988, MAL; 10/362697, USA; 01920972.5, EUROPE and 2002-522919, JAPAN) isolated from *E. longifolia* is a potential phytoandrogen, which has been reported to increase the testosterone level in rat leydig cells. Therefore, it is a scientific and commercial interest to study this protein further.

Issues and solutions relevant to *E. longifolia* herbal industries: *E. longifolia* contains large amount of biologically active compounds and due to its well-known aphrodisiac potential, the product development using this plant is expanding rapidly and becoming a lucrative business. The standardized water soluble extract has been patented in United States (US2004/0087493 A1) (Tambi, 2009). Currently in the health food market, the products come in raw crude powder or standardized extract in the form of capsule. It is also available as energy drinks, single or mixed with other herbs. Besides that, *E. longifolia* available as an additive brewed with coffee and even canned processed drinks (Effendy *et al*., 2012). Due to rapid development and increased consumption of *E. longifolia* products, safety issues need to be addressed. In Malaysia, registration criteria for traditional medicines involves evaluation of heavy metals, microbial contamination, steroids and adulterants, disintegration time and claimed indications (Medicines Act, Advertisement and Sale, 1956 Revised 1983) (Ang, 2004).

According to Zhang *et al*. (2012), the quality of herbal medicine can be classified to two types; external and internal. One of the external issues is contamination with microbes, mycotoxins, pesticides and heavy metals. Ang *et al*. (2004b) using cold vapor atomic absorption spectrophotometer in their screening of 100 products containing *E. longifolia*, discovered that 36% of the products possessed 0.52-5.30 ppm of mercury and the amount do not comply with requirement for traditional medicines in Malaysia where mercury contents should not exceed 0.5 ppm.

If herbal products are not dried properly during preparations, mycotoxins contamination can occur and Ali *et al*. (2005) discovered low level of aflatoxin in *E. longifolia* products. Other external issue is adulteration. If contamination can occur unintentionally, adulteration is deliberately done by the manufacturer (Zhang *et al*., 2012) for cost saving measure. Usually adulteration is done by adding undeclared drugs that will give the same effect of the herbal products. This situation is dangerous as the drugs may pose adverse effect. Bogusz *et al*. (2006) analyzed an *E. longifolia* product and discovered the product contained sildenafil, an erectile dysfunction treatment drug. Using HPLC-DAD and HPLC-MS, Venhuis *et al*. (2012) investigated an *E. longifolia* product that was suspected to be adulterated with drug substance. The results are interesting where the capsule content did not show traces of adulterant, but the capsule shell was found to content tadalafil, a drug for treating erectile dysfunction. They used microscopy and RAMAN spectroscopy and the presence of tadalafil was shown throughout the gelatine matrix as particles and dissolved into the matrix.

Misidentification of one herb with another herb is another external issue. This false authentication usually occurs with incorrect labeling and similar appearance of herbal materials (Zhang *et al*., 2012). *Eurycoma longifolia* has a unique morphology with its long, straight root, so the problem of misidentification with other herbs probably is unlikely.

Internal issues affecting quality of herbal products are complex phytochemical and non-uniform ingredient. Varying planting conditions and geographical factor will cause different types and concentration of metabolites. Quality control for herbal products is more difficult than drugs analysis as herbal products contain large range of active compounds. It will also cause inconsistency of the products between batches to batches. For standardization purpose, consistency of the phytochemicals needs to be ensured.

Some of the *E. longifolia* herbal products are counterfeit products, which do not even contain any bioactive compounds

of *E. longifolia*, so the ability to determine the presence of active ingredient will be useful. Abdul Rahman *et al*. (2004) developed microcontroller based electronic taste sensing system that is capable of discriminating between liquid samples containing *E. longifolia* and those that do not. An embedded microcontroller controls the overall system, using specially fabricated disposable screen-printed array of non-specific lipid-membrane. They achieved 100% recognition rate in all samples tested and claimed the system is robust, reliable, flexible and easily applied for other herbal samples. Another most common problem of herbal products in the market is adulteration with synthetic adulterant or drugs having the same pharmacological property of the natural herbal. Using high-pressure liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS-MS), Bogusz *et al*. (2006) developed a procedure for detection of most common synthetic adulterant in herbal remedies. Eighty drugs belonging to various pharmacological classes were included in the study and they identified several drugs like sildenafil, tadalafil and testosterone in the herbal products. Analysis of aflatoxins in herbs can be difficult due to complex matrices consisting of phytochemicals present in herbs. Ali *et al*. (2005) determines aflatoxins content using immunoaffinity column (IAC) clean-up and HPLC with trifluoroacetic acid (TFA) pre-column derivatization and detection by fluorescence detector, which is more accurate and selective than Thin Layer Chromatography (TLC). They modified and evaluated the method to separate aflatoxins from matrices of other chemicals in the samples.

Vejayan *et al*. (2013) utilized protein to be the marker in identifying Tongkat Ali products. They detected two distinctive protein markers using two dimensional electrophoresis in a standardized extract and in several Tongkat Ali products sold in Malaysia. The marker, which was estimated to be approximately 14kDa in molecular weight, was not detected in a selected herbal product devoid of *E. longifolia* extract.

As varying planting condition cause difference in phytochemical contents, Choo and Chan (2002) developed a reversed Phase-high Performance Liquid Chromatography (HPLC) method with a photodiode array detector to determine three major alkaloids: 9-methoxycanthin-6-one, 3-methylcanthin-5,6-dione and its 9-methoxy analogue in *Eurycoma longifolia* obtained from different sources. Besides geographical factor, concentration of metabolites is dependent on the processing temperature. For standardization, it is important to ensure consistency of the phytochemical content. To detect small metabolites, high sensitivity and high mass accuracy tandem mass spectrometer is required to produce highly reliable data. Chua *et al*. (2011) used LCMS to profile metabolites (quassinoids, alkaloids, triterpene and biphenylneolignans) of *E. longifolia* collected from the state of Perak and Pahang in Malaysia and extracted at different temperatures (35 and 100°C). Using three LC-MS/MS hybrid systems (QTof, TripleTof and QTrap), quassinoids particularly eurycomanone and its derivatives were detected to be at highest concentration than other metabolites. However, the concentration of canthin- 6-one and β-carboline alkaloids was significantly increased when the roots of the plant samples were extracted at 100°C. Extracts that are prepared at 35 and 100°C could be differentiated by a small peptide of leucine (m/z 679) and a new hydroxyl methyl β-carboline propionic acid respectively. 16-α-o-methylneoquassin could only be detected in the room temperature extract in small amount. In terms of geographical factor, 3,4ε -dihydroeurycomanone and eurylene could only be detected in the Pahang extract, while canthin-6-one-3N-oxide could only be detected in the Perak extract. Concentration of longilactone, chaparrinone, 3,4ε -dihydrochaparrinone and canthin-6-one in the Pahang extract was significantly higher than Perak extract at both temperatures. Quassinoids present in high amount in *E. longifolia*, thus quassinoids can be a marker for *E. longifolia* products.

Quassinoids analysis usually are carried out using LC method using UV, photodiode array or fluorescence detections. However, these methods were not sensitive to detect non-chromophoric constituents; eurycomanol and eurycomanol-2-O-β-D-glucopyranoside present in *E. longifolia*. Teh *et al*. (2011) developed and validated a HPLC method using electrospray ionization (ESI) and atmospheric pressure chemical (APCI) in positive and negative ion modes for the simultaneous determination of five quassinoid markers; eurycomanone, 13α(21)-epoxyeurycomanone, eurycomanol, eurycomanol-2-O-β-dglucopyranoside and 13,21-dihydroeurycomanone for standardization of manufactured batches of *E. longifolia* extracts as anti-malarial medicaments. The results showed these product batches varied in range of each constituents concentration probably due to the age of harvesting and growing conditions. In comparing ESI and APCI, positive ion ESI provided the highest response for the test compounds and the use of methanol-water (9:91, v/v) as mobile phase was preferred rather than acetonitrile-water (4:96, v/v). They established a new analytical LC-ESI-MS method and validated the method, which was found to be precise and accurate.

Volatile compounds will give herbs their own characteristic smell. Usually volatile compounds are analyzed using Gas Chromatography Mass Spectrometry (GCMS), which is expensive for routine analysis. Human sensory analysis is limited to subjectivity and sensitivity of the human panels. As alternatives, Islam *et al*. (2006) used electronic nose, which is simpler and cheaper than GCMS. This electronic nose consisted of chemical imaging and multi-parameter sensing systems. They used lipids and gas chromatography stationary phase materials with different polarities as sensing membranes and developed a quartz crystal microbalance smell sensor array and the volatile compounds were analyzed by the smell sensor and GC-MS. The volatile vapors present in the headspace interact with the array sensor and produce a chemical fingerprint or pattern characteristic to the vapor. Based on the analysis, 133 volatile compounds were found, freeze dried extract was found to contain maximum number of 83 compounds while spray dried contained maximum of 28 compounds.

Herbal product industry is blooming. However, the main pitfall of this industry is the production of the products mainly is based on traditional method which will lead to high losses and low yield. To overcome the problem, processing method need to be optimized. The most important ingredient in herbal products is the bioactive compounds, thus, phytochemical extract processing method must be developed to achieve maximum yield of bioactive contents. Kumaresan and Sarmidi (2003), studied the effect of processing on *E. longifolia* water extract yield. Particle size, extraction time, solvent ratio were the processing parameters. The results showed that extraction time and solvent ratio need to be increased to produce high yield, while the effect of particle size on the yield was inconclusive.

Athimulam *et al*. (2006) modeled and optimized an economically viable water extract production for *E. longifolia* products, with the use of Computer Aided Process Design (CAPD) and simulation tools based on existing pilot scale manufacturing setup. Four alternative production schemes were further developed with several debottlenecking and optimization strategies. Spray drying process was recognized as bottleneck in the manufacturing process, so drying operation time was reduced as the debottlenecking scheme. The existing pilot scale produced 390 kg of extract. After optimization, the yield improved to 1137.72 kg while minimum batch cycle time was reduced from 24.44-8.32 h. The proposed alternative production scheme was estimated to give annual revenue of RM6.32 million and payback period in less than two years.

Besides optimizing yield of the product processing, the impact on the environment should be considered. The overall process- yield of *E. longifolia* products was estimated to be 3% of the raw material feed by weight. Significant fibrous residues were generated which pose disposal problems. Kuan *et al*. (2007) conducted a streamlined lifecycle assessment for the analysis on the environmental impacts on the processing of *E. longifolia* products based on the utilization of fibrous residue from water extract production. The result showed that using the residue as process fuel was the most environmental friendly option where it produced the least emissions and reduced resources usage per unit. Based on the works done by Athimulam *et al*. (2006), Kuan *et al*. (2007) and Tjan *et al*. (2010) applied graphical technique based on pinch analysis to determine strategies for carbon footprint improvement. The analysis estimated that the maximum carbon foot print reduction is 1.2 t, which is 8.8% of the total carbon footprint of the overall process.

In the phytochemical based industry, the raw materials are heavily collected from natural forest. The exploitation can resulted in the extinction of a plant species. Plucking the leaves, pruning the stems may leave a plant to remain alive, but possessing the roots require the uprooting the root from the soil which can kill a tree. For *E. longifolia* plant, the root is the most sought part as it contains many bioactive compounds. The indiscriminate practice of uprooting the roots from the habitat can result in depleting natural resources. Moreover, *E. longifolia* is a plant with late maturity, less flowering and bears little fruit, so, the use of seedling are the main mode of plantings. Since this plant is a valuable resource and has the potential in pharmaceutical industry, there is a need to ensure an adequate supply and sustainability of this plant. To support conservation of this species, suitable genetic marker would be useful. Osman *et al*. (2003) assessed genetic variation within and between populations of *E. longifolia*. To investigate the genetic diversity, they applied a genome complexity reduction strategy to identify a series of Single Nucleotide Polymorphisms (SNPs) within the genomes of several *E. longifolia* accessions. That was due to the property of SNPs which were more abundant in the genome and are much more stably inherited. They discovered SNPs could differentiate the plants from different natural populations as SNPs reflects the geographic origins of individual plants. Tissue culture technology through somatic embryogenesis can play important role in preserving plant species. However, the problem with somatic embryogenesis is the quality of the embryo where the developed embryos mostly are asynchronous and malformed (Hussein *et al*., 2006). In selecting which cultures should be optimized for further regeneration, Hussein *et al*. (2006) used biochemical marker for early identification of embryogenic cultures. Based on the result, peroxidase, an isozyme was recognized as marker that was closely associated with the regeneration capability of *E. longifolia.* The main mode of planting of *E. longifolia* is through the plantlet where it is time consuming. Rapid propagation is useful as demand for *E. longifolia* is increasing. Hassan *et al*. (2012) successfully propagated *E. longifolia* via tissue culture method. They compared the production of *E. longifolia* compounds in roots of tissue culture plantlets and wild plants. Marker compounds from *E. longifolia*, eurycomanone, 9-methoxycanthin-6-one and canthin-6-one compounds were detected in roots of tissue culture plantlets. Rosli *et al*. (2009) optimized the medium composition of tissue culture to improve the production of 9-methoxycanthin-6-one, which is a potential anti-tumor compound. Medium at pH 5.5, addition of 2% fructose as carbon source, 2.0 mg L^{-1} dicamba and 1×10^{-1} μM phenylalanine resulted in better yield of 9-methoxycanthin-6-one.

The popularity of *E. longifolia* based products in herbal or drug form is growing tremendously due to wide spectrum of its pharmacological properties. To ensure the safety and efficacy of those products, the mechanism of adsorption and distribution of the pharmaceutical compounds need to be studied. Tan *et al*. (2002) developed HPLC analysis of 9-methoxycanthin-6-one, an active compound of *E. longifolia* in rat and human plasma. The result showed that the compound is better absorbed if administered intravenously than orally. Low *et al*. (2005) discovered the same result in their studies on the bioavailability and pharmacokinetics of eurycomanone in rat plasma following oral and intravenous administration. Higher plasma concentration of eurycomanone was detected after intravenous injection than oral administration despite the oral dose was 5 times higher. They discussed that the poor oral bioavailability was not because pH instability, but due to poor membrane permeability. Low *et al*.

(2011) studied the bioavailability and pharmacokinetic properties of major quassinoids; 13α(21)-epoxyeurycomanone, eurycomanone, 13α,21-dihydroeurycomanone and eurycomanol in standardized *E. longifolia* extract following oral and intravenous administration for application in antimalarial activity. They discovered 13α(21)-epoxyeurycomanone and eurycomanone were the only quassinoids that were stable upon oral administration.

Concluding remarks and future perspectives: This paper reviews the scientific credential of *E. longifolia*. To understand the therapeutic effect of this valuable natural product, various researches have been conducted and majority of the literatures supported the traditional uses of this plant. Effort has been devoted in phytochemical analysis of this plant where about 80 compounds have been isolated from this plant. Most of new compounds are from quassinoids and alkaloid groups. These new isolated compounds gave promising effect in toxicological and pharmacological studies.

The potential pharmacological effects of *E. longifolia* mostly are confirmed in anti-tumor, anti-malarial and aphrodisiac property. For aphrodisiac effect, the results are promising where most of the studies gave positive results. The clinical trials on the effect of *E. longifolia* on sexual performance were carried out but none of the studies were reported in published journal, but on conference papers. In making the results more convincing, it is suggested more clinical trials should be carried out and the findings to be reported in published journal. Most of the studies of aphrodisiac effect were tested on the *E. longifolia* water extract instead of isolated compounds, mimicking the traditional method where decoction of the water extract is consumed. To understand further the aphrodisiac role of this plant, standardized extract and isolated compounds should be tested whether it will yield consistent pharmacological activity and much more researches is needed to determine the side effect of long term consumption.

Available studies demonstrated that it is worth testing *E. longifolia* as anti-cancer and anti-malarial agent as the effect of using *E. longifolia* were comparable as using current drugs. However, the tests were carried out on rodents and no human studies were carried out yet on these tests, so future studies should allow better understanding whether the treatment will give the same response if it is to be applied in human. Moreover, most of the tests were carried out on the *E. longifolia* extract. It is a known fact that bioactive compounds, once isolated in pure state will give effects that differ significantly than the whole extracts and possibly giving deleterious results. So the possible effect of concentrations that are biologically effective should be taken into consideration. *E. longifolia* species is facing the problem of extinction due to indiscriminate harvesting from its habitat. In the processing of *E. longifolia* products, the extract yield is small, resulting in expensive price. Quality control in herbs product also is claimed to be difficult. Scientific researchers have been carried out to overcome the problem of extinction such as using tissue culture method. To help conserve this plant, the genetic diversity has been investigated by profiling the DNA of this plant using single nucleotide polymorphisms (SNPs). To improve the extract yield in the processing of *E. longifolia* products, some researchers optimized the production by using method such as streamlined lifecycle assessment, pinch analysis and suggesting debottlenecking scheme. In quality control, most of the studies improved and validated some analytical equipment such as spectrometer and high performance liquid chromatography.

Based on the scientific studies conducted, *E. longifolia* is demonstrated to have promising therapeutic effect. More clinical studies should be carried out to confirm the efficacy and safety of *E. longifolia* in treating ailment in human. Approaches should be attempted in improving the practical use as *E. longifolia* by enhancing its bioavailability. This is because several studies showed that *E. longfolia* compounds gave poor absorption if administered orally. Elucidating the structure activity relationship, structure modification and synthesize method will be rewarding in tackling this problem.

The main obstacle in applying *E. longifolia* as alternative to current treatment is its poor solubility, absorption and bioavailability. The data gap in human studies is also big. Once these obstacles have been overcome, *E. longifolia* can become desirable nutraceutical alternatives in treating ailments.

ACKNOWLEDGMENT

The authors would like to acknowledge University Malaysia Pahang for providing financial support in the form of grants bearing internal numbers of RDU130396, RDU140305 and GRS120332.

REFERENCES

Ab Rahman, A.A., N. Al-Sadat and Y.L. Wah, 2011. Help seeking behaviour among men with erectile dysfunction in primary care setting. J. Men's Health, 8: S94-S96.

Abdul Rahman, A.S., M.M.S. Yap, A.Y.M. Shakaff, M.N. Ahmad, Z. Dahari, Z. Ismail and M.S. Hitam, 2004. A microcontroller-based taste sensing system for the verification of *Eurycoma longifolia*. Sensors Actuator. B: Chem., 101: 191-198.

Abdulghani, M., A.H. Hussin, S.A. Sulaiman and K.L. Chan, 2012. The ameliorative effects of *Eurycoma longifolia* Jack on testosterone-induced reproductive disorders in female rats. Reprod. Biol., 12: 247-255.

Adnan, N. and N. Othman, 2012. The relationship between plants and the Malay culture. Procedia-Social Behav. Sci., 42: 231-241.

Al-Adhroey, A.H., Z.M. Nor, H.M. Al-Mekhlafi and R. Mahmud, 2010. Ethnobotanical study on some Malaysian anti-malarial plants: A community based survey. J. Ethnopharmacol., 132: 362-364.

Ali, N., N.H. Hashim, B. Saad, K. Safan , M. Nakajima and T. Yoshizawa, 2005. Evaluation of a method to determine the natural occurrence of aflatoxins in commercial traditional herbal medicines from Malaysia and Indonesia. Food Chem. Toxicol., 43: 1763-1772.

Ang, H.H., 2004. An insight into Malaysian herbal medicines. Trends Pharmacol. Sci., 25: 297-298.

Ang, H.H., K.L. Chan and J.W. Mak, 1995. *In vitro* antimalarial activity of quassinoids from *Eurycoma longifolia* against Malaysian chloroquine-resistant *Plasmodium falciparum* isolates. Planta Med., 61: 177-178.

Ang, H.H. and M.K. Sim, 1998. *Eurycoma longifolia* increases sexual motivation in sexually naive male rats. Arch. Pharm. Res., 21: 779-781.

Ang, H.H. and H.S. Cheang, 1999. Studies on the anxiolytic activity of *Eurycoma longifolia* Jack roots in mice. Japan J. Pharmacol., 79: 497-500.

Ang, H.H., Y. Hitotsuyanagi and K. Takeya, 2000. Eurycolactones A-C, novel quassinoids from *Eurycoma longifolia*. Tetrahedron Lett., 41: 6849-6853.

Ang, H.H., S. Ikeda and E.K. Gan, 2001. Evaluation of the potency activity of aphrodisiac in *Eurycoma longifolia* Jack. Phytother. Res., 15: 435-436.

Ang, H. H., T.H. Ngai and T.H. Tan, 2003. Effects of *Eurycoma longifolia* Jack on sexual qualities in middle aged male rats. Phytomedicine, 11: 590-593.

Ang, H.H., K.L. Lee and M. Kiyoshi, 2004a. Sexual arousal in sexually sluggish old male rats after oral administration of *Eurycoma longifolia* Jack. J. Basic Clin. Physiol. Pharmacol., 15: 303-309.

Ang, H.H., E.L. Lee and H.S. Cheang, 2004b. Determination of mercury by cold vapor atomic absorption spectrophotometer in tongkat ali preparations obtained in Malaysia. Int. J. Toxicol., 23: 65-71.

Asiah, O., M.Y. Nurhanan and A.M. Ilham, 2007. Determination of bioactive peptide (4.3 kDa) as an aphrodisiac marker in six Malaysian plants. J. Trop. For. Sci., 19: 61-63.

Astelbauer, F., M. Gruber, B. Brem, H. Greger and A. Obwaller *et al*., 2012. Activity of selected phytochemicals against *Plasmodium falciparum*. Acta Trop., 123: 96-100.

Athimulam, A., S. Kumaresan, D.C.Y. Foo, M.R. Sarmidi and R.A. Aziz, 2006. Modelling and optimization of *Eurycoma longifolia* water extract production. Food Bioprod. Process., 84: 139-149.

Bedir, E., H. Abou-Gazar, J.N. Ngwendson and I.A. Khan, 2003. Eurycomaoside: A new quassinoid-type glycoside from the roots of *Eurycoma longifolia*. Chem. Pharm. Bull., 51: 1301-1303.

Bhat, R. and A .A. K arim, 2010. Tongkat Ali (*Eurycoma longifolia* Jack): A review on its ethnobotany and pharmacological importance. Fitoterapia, 7: 669-679.

Bogusz, M.J., H. Hassan, E. Al-Enazi, Z. Ibrahim and M. Al-Tufail, 2006. Application of LC-ESI-MS-MS for detection of synthetic adulterants in herbal remedies. J. Pharm. Biomed. Anal., 41: 554-564.

Chan, K.L. and C.Y. Choo, 2002. The toxicity of some quassinoids from *Eurycoma longifolia*. Planta Med., 68: 662-664.

Chan, K.L., C.Y. Choo, N.R. Abdullah and Z. Ismail, 2004. Antiplasmodial studies of *Eurycoma longifolia* Jack using the lactate dehydrogenase assay of *Plasmodium falciparum*. J. Ethnopharmacol., 92: 223-227.

Chan, K.L., C.Y. Choo and N.R. Abdullah, 2005. Semisynthetic 15-O-acyl- and 1,15-di-O-acyleurycomanones from *Eurycoma longifolia* as potential antimalarials. Planta Med., 71: 967-969.

Chen, C.K., A.S. Muhamad and F.K. Ooi, 2012. Herbs in exercise and sports. J. Physiol. Anthropol., Vol. 31.

Choo, C.Y. and K.L. Chan, 2002. High performance liquid chromatography analysis of canthinone alkaloids from *Eurycoma longifolia*. Planta Med., 68: 382-384.

Chua, L.S., N.A.M. Amin, J.C.H. Neo, T.H. Lee, C.T. Lee, M.R. Sarmidi and R. Abdul Aziz, 2011. LC-MS/MS-based metabolites of *Eurycoma longifolia* (Tongkat Ali) in Malaysia (Perak and Pahang). J. Chromatogr. B, 879: 3909-3919.

Chuen, C.S. and A.H.L. Pihie, 2004. [BIO04] Eurycomanone exerts antiproliferative activity via apoptosis upon MCF-7 cells. Proceedings of the 4th Annual Seminar National Science Fellowship (NSF), December 20-21, 2004, Penang, Malaysia, pp: 13-18.

Effendy, N.M., N. Mohamed, N. Muhammad, I.N. Mohamad aned A.N. Shuid, 2012. *Eurycoma longifolia*: Medicinal plant in the prevention and treatment of male osteoporosis due to androgen deficiency. Evidence-Based Complement. Altern. Med. 10.1155/2012/125761

Farouk, A.E. and A. Benafri, 2007. Antibacterial activity of *Eurycoma longifolia* Jack. A Malaysian medicinal plant. Saudi Med. J., 28: 1422-1424.

Hassan, N.H., R. Abdullah, L.S. Kiong, A.R. Ahmad and N. Abdullah *et al*., 2012. Micropropagation and production of eurycomanone, 9-methoxycanthin-6-one and canthin-6-one in roots of Eurycoma longifolia plantlets. Afr. J. Biotechnol., 11: 6818-6825.

Hout, S., A. Chea, S.S. Bun, R. Elias and M. Gasquet *et al*., 2006. Screening of selected indigenous plants of Cambodia for antiplasmodial activity. J. Ethnopharmacol., 107: 12-18.

Hussein, S., R. Ibrahim and A.L.P. Kiong, 2006. Potential biochemical markers for somatic embryos of *Eurycoma longifolia* jack. J. Plant Biol., 49: 97-101.

Islam, A.K.M.S., Z. Ismail, B. Saad, A.R. Othman, M.N. Ahmad and A.Y.M. Shakaf, 2006. Correlation studies between electronic nose response and headspace volatiles of *Eurycoma longifolia* extracts. Sensor Actuators B: Chem., 120: 245-251.

Jiwajinda, S., V. Santisopasri, A. Murakami, M. Kawanaka and H. Kawanaka *et al*., 2002. *In vitro* anti-tumor promoting and anti-parasitic activities of the quassinoids from *Eurycoma longifolia*, a medicinal plant in Southeast Asia. J. Ethnopharmacol., 82: 55-58.

Kavitha, N., R. Noordin, K.L. Chan and S. Sasidharan, 2010. Cytotoxicity activity of root extract/fractions of *Eurycoma longifolia* Jack root against vero and Hs27cells. J. Med. Plants Res., 4: 2383-2387.

Kavitha, N., R. Noordin, K. Chan and S. Sasidharan, 2012. *In vitro* anti-toxoplasma gondii activity of root extract/fractions of eurycoma longifolia jack. BMC Complem. Altern. Med., Vol. 12. 10.1186/1472-6882-12-91

Kuan, C.K., D.C.Y. Foo, R.R. Tan, S. Kumaresan and R. Abdul Aziz, 2007. Streamlined life cycle assessment of residue utilization options in Tongkat Ali (*Eurycoma longifolia*) water extract manufacturing process. Clean Technol. Environ. Policy, 9: 225-234.

Kumaresan, S. and M.R. Sarmidi, 2003. A preliminary study into the effect of processing on *Eurycoma longifolia* water extract yield. Proceedings of the International Conference on Chemical and Bioprocess Engineering, August 27-29, 2003, Sabah, Malaysia, pp: 750-754.

Kuo, P.C., A.G. Damu, K.H. Lee and T.S. Wu, 2004. Cytotoxic and antimalarial constituents from the roots of *Eurycoma longifolia*. Bioorg. Med. Chem., 12: 537-544.

Low, B.S., B.H. Ng, W.P. Choy, K.H. Yuen and K.L. Chan, 2005. Bioavailability and pharmacokinetic studies of eurycomanone from *Eurycoma longifolia*. Planta Med., 71: 803-807.

Low, B.S., C.H. Teh, K.H. Yuen and K.L. Chan, 2011. Physico-chemical effects of the major quassinoids in a standardized *Eurycoma longifolia* extract (Fr 2) on the bioavailability and pharmacokinetic properties and their implications for oral antimalarial activity. Nat. Prod. Commun., 6: 337-341.

Low, B.S., P.K. Das and K.L. Chan, 2013. Standardized quassinoid-rich *Eurycoma longifolia* extract improved spermatogenesis and fertility in male rats via the hypothalamic-pituitary-gonadal axis. J. Ethnopharmacol., 145: 706-714.

Low, W.Y. and H.M. Tan, 2007. Asian traditional medicine for erectile dysfunction. J. Men Health Gender, 4: 245-250.

Mahfudh, N. and A.H.L. Pihie, 2008. Eurycomanone induces apoptosis through the up-regulation of p53 in human cervical carcinoma cells. J. Cancer Mol., 4: 109-115.

Mayer, D.C.G., M. Bruce, O. Kochurova, J.K. Stewart and Q. Zhou, 2009. Antimalarial activity of a *cis*-terpenone. Malaria J., Vol. 8. 10.1186/1475-2875-8-139

Miyake, K., Y. Tezuka, S. Awale, F. Li and S. Kadota, 2010. Canthin-6-one alkaloids and a tirucallanoid from *Eurycoma longifolia* and their cytotoxic activity against a human HT-1080 fibrosarcoma cell line. Nat. Prod. Commun., 5: 17-22.

Muhamad, A.S., C.C. Keong, O.F. Kiew, M.R. Abdullah and C.K. Lam, 2010. Effects of *Eurycoma longifolia* jack supplementation on recreational athletes endurance running capacity and physiological responses in the heat. Int. J. Applied Sports Sci., 22: 1-19.

Nguyen-Pouplin, J., H. Tran, H. Tran, T.A. Phan and C. Dolecek *et al*., 2007. Antimalarial and cytotoxic activities of ethnopharmacologically selected medicinal plants from South Vietnam. J. Ethnopharmacol., 109: 417-427.

Nurhanan, M.Y., L.P.A. Hawariah , A.M. Ilham and M.A.M. Shukri, 2005. Cytotoxic effects of the root extracts of *Eurycoma longifolia* jack. Phytother. Res., 19: 994-996.

Okano, M., N. Fukamiya, K. Tagahara, H. Tokuda, A. Iwashima, H. Nishino and K.H. Lee, 1995. Inhibitory effects of quassinoids on Epstein-Barr virus activation. Cancer Lett., 94: 139-146.

Ong, H.C. and J. Norzalina, 1999. Malay herbal medicine in gemencheh, Negri Sembilan, Malaysia. Fitoterapia, 70: 10-14.

Osman, A., B. Jordan, P.A. Lessard, N. Muhammad and M.R. Haron *et al*., 2003. Genetic diversity of *Eurycoma longifolia* inferred from single nucleotide polymorphisms. Plant Physiol., 131: 1294-1301.

Purwantiningsih, A., H. Hussin and K.L. Chan, 2011. Free radical scavenging activity of the standardized ethanolic extract of *Eurycoma longifolia* (TAF-273). Int. J. Pharm. Sci., 3: 343-347.

Razak, M.F.A. and K.E. Aidoo, 2011. Toxicity studies of *Eurycoma longifolia* (Jack)-Based remedial products. Asian J. Pharm. Clin. Res., 4: 23-27.

Ridzuan, M.A.R.M., A. Sow, A.N. Rain, A.M. Ilham and I. Zakiah, 2007. *Eurycoma longifolia* extract-artemisinin combination: Parasitemia suppression of *Plasmodium yoelii*-infected mice. Trop. Biomed., 24: 111-118.

Rosli, N., M. Maziah, K.L. Chan and S. Sreeramanan, 2009. Factors affecting the accumulation of 9-methoxycanthin-6-one in callus cultures of *Eurycoma longifolia*. J. For. Res., 20: 54-58.

Sambandan, T.G., C. Rha, N. Aminudim, J.M. Saad and A.A. Kadir, 2006. Bioactive fraction of *Eurycoma longifolia*. U.S. Patent No. WO0217946, Washington, DC., USA.

Samuel, A.J.S.J., A. Kalusalingam, D.K. Chellappan, R. Gopinath and S. Radhamani *et al*., 2010. Ethnomedical survey of plants used by the Orang Asli in Kampung Bawong, Perak, West Malaysia. J. Ethnobiol. Ethnomed., Vol. 6. 10.1186/1746-4269-6-5

Sholikhah, E.N., M.A. Wijayanti, L.H. Nurani and Mustofa, 2008. Stage specificity of pasak bumi root (*Eurycoma longifolia* jack) isolate on *Plasmodium falciparum* cycles. Med. J. Malaysia, 63: 98-99.

Shuid, A.N., E. El-Arabi, N.M. Effendy, H.S.A. Razak, N. Muhammad, N. Mohamed and I.N. Soelaiman, 2012. *Eurycoma longifolia* upregulates osteoprotegerin gene expression in androgen-deficient osteoporosis rat model. BMC Complem. Altern. Med., Vol. 12. 10.1186/1472-6882-12-152

Tada, H., F. Yasuda, K. Otani, M. Doteuchi, Y. Ishihara and M. Shiro, 1991. New antiulcer quassinoids from *Eurycoma longifolia*. Eur. J. Med. Chem., 26: 345-349.

Tambi, M.I., 2009. Nutrients and botanicals for optimizing men's health. Examining the evidence for *Eurycoma longifolia* Jack, the Malaysian Ginseng in men's health. Asian J. Androl., 11: 37-38.

Tambi, M.I.B.M. and M.K. Imran, 2010. *Eurycoma longifolia* Jack in managing idiopathic male infertility. Asian J. Androl., 12: 376-380.

Tan, S., K.H. Yuen and K.L. Chan, 2002. HPLC analysis of plasma 9-methoxycanthin-6-one from *Eurycoma longifolia* and its application in a bioavailability/pharmacokinetic study. Plant. Med., 68: 355-358.

Tee, T.T. and H.L.P. Azimahtol, 2005. Induction of apoptosis by *Eurycoma longifolia* Jack extracts. Anticancer Res., 25: 2205-2213.

Tee, T.T., Y.H. Cheah and L.P.A. Hawariah, 2007. F16, a fraction from *Eurycoma longifolia* jack extract, induces apoptosis via a caspase-9-independent manner in MCF-7 cells. Anticancer Res., 27: 3425-3430.

Teh, C.H., H. Morita, O. Shirota and K.L. Chan, 2010. 2,3-Dehydro-4α-hydroxylongilactone, a novel quassinoid and two known phenyl propanoids from *Eurycoma longifolia* Jack. Food Chem., 120: 794-798.

Teh, C.H., V. Murugaiyah and K.L. Chan, 2011. Developing a validated liquid chromatography-mass spectrometric method for the simultaneous analysis of five bioactive quassinoid markers for the standardization of manufactured batches of *Eurycoma longifolia* Jack extract as antimalarial medicaments. J. Chromatogr. A, 1218: 1861-1877.

Tjan, W., R.R. Tan and D.C.Y. Foo, 2010. A graphical representation of carbon footprint reduction for chemical processes. J. Cleaner Prod., 18: 848-856.

Tran, Q.L., Y. Tezuka, J.Y. Ueda, N.T. Nguyen and Y. Maruyama *et al*., 2003. *In vitro* antiplasmodial activity of antimalarial medicinal plants used in Vietnamese traditional medicine. J. Ethnopharmacol., 86: 249-252.

Tzar, M.N., Y.Hamidah, S. Hartini, M. Marianayati and A.S. Nazrun, 2011. The antibacterial or antifungal effects of *Eurycoma longifolia* root extract. Internet J. Herbal Plant Med., Vol. 1. 10.5580/28a7

Vejayan, J., V. Iman, F. Siew-Liang and H. Ibrahim, 2013. Protein markers useful in authenticating *Eurycoma longifolia* contained herbal aphrodisiac products. Malaysian J. Sci., 32: 15-23.

Venhuis, B.J., J. Tan, M.J. Vredenbregt, X. Ge, M.Y. Low and D. de Kaste, 2012. Capsule shells adulterated with tadalafil. Forensic Sci. Int., 214: e20-e22.

Wahab, N.A., M.M. Norfilza, W.N.H.A. Halim and S. Das, 2010. The effect of *Eurycoma longifolia* Jack on spermatogenesis in estrogen-treated rats. Clinics, 65: 93-98.

Wernsdorfer, W.H., S. Ismail, K.L. Chan, K. Congpuong and G. Wernsdorfer, 2009. Activity of Eurycoma longifolia root extract against *Plasmodium falciparum in vitro*. Wiener klinische Wochenschrift, 121: 23-26.

Wiart, C., 2012. A note on the relevance of *Eurycoma longifolia* Jack to food and food chemistry. Food Chem., 134: 1712-1712.

Wong, P.F., W.F. Cheong, M.H. Shu, C.H. Teh, K.L. Chan and S. AbuBakar, 2012. Eurycomanone suppresses expression of lung cancer cell tumor markers, prohibitin, annexin 1 and endoplasmic reticulum protein 28. Phytomedicine, 19: 138-144.

Zakaria, Y., A. Rahmat, A.H.L. Pihie, N.R. Abdullah and P.J. Houghton, 2009. Eurycomanone induce apoptosis in HepG2 cells via up-regulation of p53. Cancer Cell Int., 9: 1-21.

Zanoli, P., M. Zavatti, C. Montanari and M. Baraldi, 2009. Influence of *Eurycoma longifolia* on the copulatory activity of sexually sluggish and impotent male rats. J. Ethnopharmacol., 126: 308-313.

Zhang, J., B. Wider, H. Shang, X. Li and E. Ernst, 2012. Quality of herbal medicines: Challenges and solutions. Complementary Ther. Med., 20: 100-106.