Advances in Studies on Chemistry, Pharmacological Effect, and Pharmacokinetics of *Eurycoma longifolia*

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Abstract: *Eurycoma longifolia*, also known as Tongkat Ali in Malaysia, as one of traditional herbal medicines, is used for centuries in South-East Asia. With the discovery of anticancer and anti-HIV properties, this herbal medicine has attracted great attention recently. In this review, the following information on *E. longifolia*, including chemistry, bioactivities, pharmacokinetics, clinical studies, and side effects and safety, was introduced. Our results, to a certain extent, will provide scientific base for commercial utilization and clearance of the Tongkat Ali products with regard to consumers’ safety.

Key words: chemistry; clinical study; *Eurycoma longifolia*; pharmacokinetics; pharmacological effects

Introduction

*Eurycoma longifolia* Jack, also known as Tongkat Ali in Malaysia, is a shrub-tree that grows in Malaysia, Burma, Indochina, Thailand, Sumatra, Borneo, and Philippines (Kuo *et al*., 2003). It is traditionally used primarily as an aphrodisiac and for improving general health (Ang and Lee, 2002). Other traditional uses include the treatment of aches, persistent fever, malaria, dysentery, glandular swelling, bleeding, edema, hypertension, syphilitic sores, and ulcers (Kuo *et al*., 2004; Bedir *et al*., 2003). Tongkat Ali is called “Malaysia ginseng”, and it has been used for centuries to increase male virility and sexual prowess. The plant parts are rich in various bioactive compounds (such as eurycomaside, eurycolactone, eurycomalactone, eurycomanone, and pasakbumin-B) among which the alkaloids and quassinoids form a major portion. A large variety of constituents have been identified, including quassinoids, canthin-6-one alkaloids, β-carbolines, tirucallane-type triterpenes, squalene derivatives, and biphenylneolignans (Ang and Lee, 2002).

The plant parts have been traditionally used for their antimalarial, anticancer, antidiabetic, antipyretic, antimicrobial, and aphrodisiac activities which have also been proved scientifically. Recently *E. longifolia* has been the subject of exhaustive and clinical studies in Malaysia, Japan, and the United States of America.

Even though studies of toxicity and safety evaluation have been pursued, still a major gap exists in providing scientific base for commercial utilization and clearance of Tongkat Ali products with regard to consumers’ safety. *E. longifolia* plant has also been found to have anticancer and anti-HIV properties, and in the latest research, one of its compounds was found to have a direct action on assisting the facilitation of penile erection by directly inducing the vasodilatation and relaxation of penile corpus cavernosum (Bhat and Karim, 2010). In this review, the information on the chemistry and bioactivities of *E. longifolia* is introduced.

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Phytochemical investigation

The extract of Tongkat Ali, *E. longifolia* (Ali’s walking stick), contains the highest quality of glycosaponins (5%) and polysaccharides (10%). The plant parts are rich in various bioactive compounds (such as eurycomaoside, eurycolactone, eurycomalactone, eurycomanone, and pasakbumin-B) among which the alkaloids and quassinoids form a major portion. Quassinoid compounds are presented in *E. longifolia* (Simaroubaceae), identified by the local names Tongkat Ali in Malaysia and Pasakbumi in Indonesia.

Chemically, they are C-18, C-19, and C-20 quassinoids (Table 1, Figs. 1, 2, and 3). All quassinoids are believed to be biosynthesized through the triterpenoid biogenetic pathway (Kuo et al., 2003). The biosynthetic process begins with the degradation of triterpenes.

Phytochemical investigation of the stems of *E. longifolia* led to the isolation of two new canthin-6-one alkaloids, 4,9-dimethoxy-canthin-6-one and 10-hydroxy-11-methoxy-canthin-6-one, and a new tirucallane-type triterpenoid, 23,24,25-trihydroxytirucall-7-en-3,6-dione, along with 37 known compounds. Among these, 37 known compounds.

**Table 1** C-18, C-19, and C-20 quassinoids in *Eurycoma* plants

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Compound type</th>
<th>Compound</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td><em>E. longifolia</em></td>
<td>C-18</td>
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<td>Ang, Hitotsuyanagi, and Takeya, 2000; Takeya et al., 2002</td>
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<td>Itokawa et al., 1993a</td>
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<td>C-20</td>
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an oxa-squalenoid was isolated as a natural product for the first time. The structures of the isolates were elucidated by spectroscopic and mass spectrometric means. All the isolates were evaluated for their cytotoxic activities against an HT-1080 human fibrosarcoma cell line. Among them, 9,10-dime-thoxycanthin-6-one (14, \( IC_{50} = 5.0 \) µmol/L), 10-hydroxy-9-methoxycanthin-6-one (15, \( IC_{50} = 7.2 \) µmol/L), dihydroniloticin (18, \( IC_{50} = 8.2 \) µmol/L), and 14-deacetylleurylene (34, \( IC_{50} = 3.2 \) µmol/L) displayed stronger activity than the positive control 5-FU (\( IC_{50} = 9.2 \) µmol/L) (Miyake \textit{et al.}, 2010).

**Biological activities**

Tongkat Ali is primarily used as a male aphrodisiac. In Malaysia, it is used to increase virility and sexual prowess and is claimed to improve strength and power during sexual activities (Ang and Lee, 2002; Ang, Cheang, and Yusof, 2000). Many animal studies, both in rats and mice, have found administration of \( E. \ longifolia \) extracts to increase sexual arousal and motivation and frequency of sexual activity (Ang and Lee, 2002; Ang, Cheang, and Yusof, 2000; Ang, Lee, and Kiyoshi, 2003). These effects are similar to those caused by administration of testosterone, although the effect of Tongkat Ali is not as strong (Ang, Cheang, and Yusof, 2000).

**Antitumor activity**

The antitumor activity is one of the most impressive medicinal properties of quassinoids and has been intensively studied (Miyake \textit{et al.}, 2010; Jiwajinda \textit{et al.}, 2002). Many quassinoids display antitumor activity in different potencies. The mechanism of the action is believed to be that quassinoids can inhibit the protein synthesis by inhibiting the ribosomal peptidyl transferase activity and leading to the termination of the chain elongation.
The cytotoxicity of eurycomanone from *E. longifolia* was evaluated using MTT assay and the mode of cell death was detected by Hoechst 33258 nuclear staining and flow cytometry with Annexin-V/propidium iodide double staining. The findings suggested that eurycomanone was cytotoxic on
cancerous liver cell, HepG2, and less toxic on normal cells, such as Chang’s liver and WLR-68. Furthermore, various methods proved that apoptosis was the mode of death in eurycomanone-treated HepG2 cells. The characteristics of apoptosis including chromatin condensation, DNA fragmentation, and apoptotic bodies were found following eurycomanone treatment. This study also found that apoptotic process triggered by eurycomanone involved the up-regulation of p53 tumor suppressor protein. The up-regulation of p53 was followed by the increasing of pro-apoptotic Bax and decreasing of anti-apoptotic Bcl-2. The increase of cytochrome C level in cytosol also results in induction of apoptosis (Zakaria et al., 2009).

In antitumor activity, there was no significant difference within the G1 peak for untreated cells at 24 and 48 h with percentage cell count of 72.5% and 73.6%, respectively. Besides, at 72 h of treatment duration, a bit decrease of cell population to 67.5% was observed. Cells treated by eurycomanone showed an accumulation in G2/M phase following 48 h of exposure to 20.8% of the cell population compared to 24 h with 16.58%. At 72 h, the significantly increased percentage of cells in G2/M phase was observed and 39.9% of cells accumulated was recorded. The amount of apoptotic cells was calculated based on the appearance of cells in G0. G0 phase corresponds to apoptotic cells. Although there was an increase of apoptosis in eurycomanone-treated cells, the magnitude of change was relatively small in comparison to the growth inhibition. This is also evident that the G2/M phase could become the dominant phase in cells treated with 5 µg/mL of eurycomanone in a time-dependent manner with a late apoptosis. These findings indicate that at the range of concentration studied, the antiproliferative effect of eurycomanone on HepG2 cells could be attributed primarily to the induction of G2/M arrest, with less contribution of cell division rather than DNA synthesis. Following vinblastine sulfate treatment, the G2/M peak dramatically decreased cell population of G1 phase (2.5%) and increased percentage of cells in G2/M phase (80.7%) at 24 h. The significant accumulation of cells clearly indicates that vinblastine sulfate efficiently arrested the cell cycle progress in HepG2 cells at the G2/M phase. The study suggested that eurycomanone was cytotoxic on HepG2 cells by inducing apoptosis through the up-regulation of p53 and Bax and down-regulation of Bcl-2 (Zakaria et al., 2009). The cytotoxic activity of extract from E. longifolia to human cell lines, Hep2 and HFL1, was with IC50 ranging from 11 to 55 µg/mL, and the most cytotoxic activity of extract from E. longifolia was with IC50 of 11 and 13 mg/mL, respectively (Mohd-Fuat, Kofi, and Allan, 2007).

Plant cell culture technology is potentially useful in producing high-valued secondary metabolites. The extracts from E. longifolia roots are consumed as health tonic but more popularly used as aphrodisiac. Studies on the aphrodisiac properties and the possible compounds involved have been widely carried out. There are many potentially useful compounds reported on the root extracts from E. longifolia. However, studies on the in vitro production of useful compounds from this plant have not been reported. This chapter will describe the methods of callus induction and extraction of 9-methoxycaanthin-6-one from E. longifolia explants with emphasis on the tap and fibrous roots. This compound, known to have antitumor activity, is present in intact plant parts and in callus tissues of different explants (Maziah and Rosli, 2009).

Twenty-four quassinoids isolated from E. longifolia were investigated for their cytotoxicity against a panel of four different cancer cell lines which included three murine cell lines, colon 26-L5 carcinoma (colon 26-L5), B16-BL6 melanoma (B16-BL6), and Lewis lung carcinoma (LLC), and a human lung A549 adenocarcinoma (A549) cell line. Among the tested compounds, eurycomalanetone displayed the most potent activity against all the tested cell lines: colon 26-L5 (IC50 = 0.70 µmol/L), B16-BL6 (IC50 = 0.59 µmol/L), LLC (IC50 = 0.78 µmol/L), and A549 (IC50 = 0.73 µmol/L). These activities were comparable to clinically used anticancer agent Doxorubicin (colon 26-L5, IC50 = 0.76 µmol/L; B16-BL6, IC50 = 0.86 µmol/L; LLC, IC50 = 0.80 µmol/L; A549, IC50 = 0.66 µmol/L) (Miyake et al., 2010). Ten new structurally diverse quassinoids and 14 known compounds were isolated from the stems of E. longifolia. The new compounds were two eurycomanone-type C-20 quassinoids, one klaineanone-type C-20 quassinoid, one C-19 quassinoid with a 1,2-seco-1-nor-6-(5,10)-
aboe-picrasan-2,5-olide skeleton, and six eurycomalactone-type C-19 quassinoids. Compounds 5 and 6 both possessed a 3,4-epoxy group observed for the first time in eurycomalactones. Compound 1 had an α-oriented OH group at C-14 that had not been reported previously in eurycomanone-type quassinoids. All of the isolates were evaluated for cytotoxicity toward the highly metastatic HT-1080 human fibrosarcoma cell line, and compounds 11, 23, and 24 showed potent cytotoxicity (Miyake et al., 2009).

**Antimalarial activity**

It has been considered as a great discovery that several quassinoids possess potent antimalarial activity, especially the activity against the chloroquine-resistant *Plasmodium falciparum* (Maziah and Rosli, 2009; Miyake et al., 2010). IC₅₀ values of bruceantin and glaucarubinone are at nmol/L level and are much more potent than that of chloroquine. The mechanism of the action is also the inhibition of protein synthesis (Kirby et al., 1989). However, it seems to be different from that of cytotoxicity, since some quassinoids have shown greater selectivity against *P. falciparum* than against KB cells (Anderson et al., 1991). For instance, the cytotoxic activity of glaucarubinone against KB cells is 285 times of its activity against *P. falciparum*. This result suggests that it may be a possible way to develop more selective quassinoid derivatives in the future (Wright et al., 1993).

Kirby et al. (1989) utilized the incorporation of ³H-isoleucine into acid-insoluble products as the protein synthesis index and the incorporation of ³H-hypoxanthine into acid-insoluble products as the nucleic acid synthesis index to determine the inhibitory activities of several quassinoids on both syntheses. According to the results, all nucleic acid synthesis in *P. falciparum* infected human erythrocytes, and the inhibition on nucleic acid synthesis was observed following the failure of protein synthesis. In the malarial parasite, as in eukaryote model, quassinoids are rapid and potent inhibitors of protein synthesis, most likely due to effects upon the ribosome other than upon nucleic acid metabolism (Kirby et al., 1989). Studies have shown that the chance of cross-resistance of malaria between quassinoids and chloroquine was less, since chloroquine did not affect protein synthesis. Quassinoids may be presumed to act upon the malarial parasite through a fundamentally different mechanism to that of chloroquine. Using the inhibition of incorporation of ³H-hypoxanthine as an index, Ekong et al. (1990) has proved that a chloroquine-sensitive strain of *P. falciparum* and a chloroquine-resistant strain did not differ in their sensitivities to the quassinoids; Therefore, these triterpenoids offer a promising source for the development of new antimalarial drugs against chloroquine-resistant malaria.

**Anti-inflammatory activities**

In contrast to other activities of quassinoids, quassin, which is inactive in most other biological activities, is relatively active as an aphid antifeedant and is not phytotoxic at the concentration of 0.05% (Ekong et al., 1990). Certain quassinoids display *in vitro* antiviral activity, but usually at relatively high concentration. A recent report showed that some quassinoids possessed anti-HIV activity. Among the eighteen quassinoïd glycosides and nine quassinoids tested, the shinjulactone C demonstrated the highest anti-HIV activity (EC₅₀ = 10.6 mmol/L) with a therapeutic index of greater than 25 (Polonsky et al., 1989). Quassinoids were also reported to possess anti-inflammatory activity (Okano et al., 1996).

Four tested quassinoids, pasakbumins A, B, C, and D, showed up to 92.4% inhibition at dose of 1.0 mg/kg (Satayavivad et al., 1998). Eurycomanone displayed comparable potency with Tamoxifen but was more potent than 13α,21-dihydroeurycomanone in the anti-estrogenic effect against 17α-ethynylestradiol (EE)-induced uterotrophy of immature rats (Teh et al., 2011).

**Anti-osteoporotic effect**

*E. longifolia* extract acts as a potential agent for reversing the effects of estrogen by increasing spermatogenesis and sperm counts in rats after 14-day consecutive treatment (Wahab et al., 2010). In a study, *E. longifolia*, a plant with androgenic effects, was supplemented to an androgen-deficient osteoporotic aged rat. Elderly men with androgen deficiency are exposed to osteoporosis and can be treated with testosterone replacement. After six weeks of treatment, serum osteocalcin, serum terminal C-telopeptide type 1 collagen (CTX), and the fourth lumbar bone calcium were measured. There were no significant differences in the osteocalcin levels before and after treatment in all groups. The CTX levels were also similar for all groups.
before treatment. However, after treatment, orchidectomy had caused significant elevation of CTX compared to normal control rats. Testosterone replacements in orchidectomised rats were able to prevent the rise of CTX. Orchidectomy had also reduced the bone calcium level compared to normal control rats. Both testosterone replacement and *E. longifolia* supplementation to orchidectomised rats were able to maintain the bone calcium level, with the former showing better effects. As a conclusion, *E. longifolia* prevented bone calcium loss in orchidectomised rats and therefore has the potential to be used as an alternative treatment for androgen deficient osteoporosis (Shuid *et al*., 2010).

### Side effects and safety

A related property that Tongkat Ali is reputed to have is a testosterone-increasing effect (Ang and Lee, 2002). Tongkat Ali does have androgenic effects in male rats, either directly or indirectly, such as increasing the weight of sexual accessories (Ang, Cheang, and Yusof, 2000; Ang and Cheang, 2001). *In vitro*, ethanolic extracts of *E. longifolia* increased human chorionic gonadotropin (hCG)-induced production of testosterone by rat Leydig’s cells (Kuo *et al*., 2004).

There are many anecdotal reports on the internet where people indicate that they had testosterone levels tested before and during Tongkat Ali supplementation, which caused an increase of testosterone. The present evidence indicates that this is a likely property of this plant, but how great the effect is, dose-dependency, and whether or not it contributes significantly to the aphrodisiac qualities of Tongkat Ali will only be established with more studies. Until then, Tongkat Ali should not be treated as a reliable way to increase testosterone levels. Many other possible benefits have been identified in the experimental studies. An animal study found that Tongkat Ali had anxiety-reducing effects in the open field, elevated plus-maze, and antifighting tests (Ang and Cheang, 1999).

It also has antimalarial activity *in vitro*, an effect which is due to multiple constituents of the plant, and which multiple studies have replicated (Kuo *et al*., 2004). In an animal study, Tongkat Ali had the ability to improve survival in infected animals, but the effective dose was near-toxic. It was commented that
the history of human use as an antimalarial agent might be explained by differences between rodents and humans, which caused the toxic effect to be reduced in the second group, but studies are needed to be confirmed (Satayavivid et al, 1998). Tongkat Ali also has anticancer effects against multiple cancer cell lines and is effective against multiple parasites in vitro (Ang and Lee, 2002; Jiwajinda et al, 2002).

Few studies have been done on the safety of Tongkat Ali in humans. An animal study found that the LD$_{50}$ was 1500—2000 mg/kg of the alcohol extract and 3000 mg/kg of the water extract. A subacute toxic study with the alcohol extract indicated that 600 mg/kg daily was associated with signs of toxicity while 200 mg/kg daily was not, and another study found no toxic effects at 270—350 mg/kg daily but toxic effects were observed at 430 mg/kg daily. The subacute signs of toxicity were increased weights of liver, kidneys, spleen, and testes (Satayavivid et al, 1998). Most consider 1 g daily to be the maximum dose for supplemental use, although it depends on the potency of the product. Tonkgat Ali should not be taken by methods other than ig administration, as this increases toxicity by approximately 100-fold, indicating that the digestive tract filters out many toxic compounds (Satayavivid et al, 1998).

This supplement has been traditionally used in many countries without reports of serious side effects. However, it has not been studied much in humans, so it is advisable to use this supplement with caution. Using more than 1 g daily is not advisable, and it should not be used continuously for more than a month before taking an equivalent amount of time off. Eurycoma has androgenic effects when administered to rodents. Anabolic side effects, such as prostate enlargement, may be a possibility. Many users report insomnia from taking this supplement (Satayavivid et al, 1998).

Pharmacokinetics

A placebo-controlled randomized single-blinded crossover study on the effect of a water-based extract from E. longifolia by the pharmacokinetics of a single dose of Propranolol in 14 healthy non-smoker young males was carried out. When Propranolol was administered with E. longifolia, its bioavailability (AUC$_{0-\infty}$) decreased by 29% while $C_{\text{max}}$ was reduced by 42% and $T_{\text{max}}$ was significantly prolonged by almost 86%. The terminal elimination half-life, however, was not significantly affected. The bioavailability of Propranolol is significantly decreased when consumed together with E. longifolia. The interaction is due to a reduction in absorption, rather than an increase in Propranolol’s metabolism. Although the pharmacodynamics of Propranolol was not affected in healthy volunteers, caution is still advisable with co-administration of the drug and the herb (Salman et al, 2010).

Clinical studies

E. longifolia, which is thought to enhance male fertility with regard to higher semen volumes, sperm concentrations, the percentage of normal sperm morphology, and sperm motility in male partners of sub-fertile couples with idiopathic infertility. A total of 350 patients were given 200 mg of the extract daily and follow-up semen analyses were performed every three months for nine months. Of these 350 patients, 75 patients completed one full cycle of three months. Follow-up semen analyses in these patients showed significant improvement in all semen parameters. The proprietary extract of E. longifolia significantly improved the sperm quality in these patients, allowing for 11 (14.7%) spontaneous pregnancies (Tambi and Imran, 2010).

References


195


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**Congratulations on Chinese Traditional and Herbal Drugs Winning the 2nd Chinese Government Award for Publishing**

*Chinese Traditional and Herbal Drugs* (CTHD), published by Tianjin Press of Chinese Herbal Medicines, has just won the highest honor of publishing field in China—the Chinese Government Award for Publishing. The 2nd award has just been promulgated and 20 journals (10 belongs to science and technology journals and other 10 to social science) were honored for the first time.

CTHD was first published in 1970 and has been playing a great role for Chinese materia medica (CMM), especially in its present modernization. CTHD has successively won several honors in recent years, such as the 2nd State Journal Award (the highest award of journals) at the beginning of 2003; the nominated Third State Journal Award in 2005; Excellent Science and Technology Journals of China in 2008; the honor of Top 100 Excellent Academic Periodicals of China for consecutive six times from 2005 to 2010; The Most Effective Journal of The Past 60 Years in China in 2009; and the award—the 2nd Chinese Government Award for Publishing in 2011.

Nowadays, there are four journals published in Tianjin Press of Chinese Herbal Medicines, they are CTHD, *Chinese Herbal Medicines* (CHM), *Drugs & Clinic* (DC), and *Drug Evaluation Research* (DER). The initial issue of CHM was first published in 2009 with the purpose to provide a forum for the studies on Chinese herbal medicines, traditional medicines, and natural products, as well as greatly promote the internationalization of CMM. DER provides a forum for the studies on evaluation research of drug criterion, drug safety, pharmacodynamics, pharmacokinetics, toxicology, clinical, and marketed drugs, etc. DC mainly focuses on the domestic and foreign botanical medicine development, biological medicine, chemistry medicine, and the Chinese nature medicine, and integrates the medicinal research and the clinical medication.

Taking CTHD as a good example, the four journals publishing in Tianjin Press of Chinese Herbal Medicines will follow the step of CTHD and make the press more influential both at home and abroad!