



A narrative review on pharmacological significance of *Eurycoma longifolia* jack roots

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Objective: This review is focused on the pharmacological properties of *Eurycoma longifolia* which is a popular herb and widely used by local folks in Southeast Asia including Malaysia.

Background: The wide application of the herb is mainly contributed by its efficacy in treating many chronic diseases and promoting wellbeing. With the remarkable ethnomedicinal value, the plant, especially its root extracts have been intensively investigated for pharmacological significance in a systematic manner for decades. This includes both *in vitro* and *in vivo* studies focusing on the aphrodisiac, anticancer, antimalarial, antiosteoporotic and antidiabetic properties using different assays.

Methods: A narrative review of previously reported data on the pharmacological importance of *E. longifolia* was systematically carried out. This covers data published in literature retrieved from the search engine of Google from 1980s until to date. The findings of the previous studies are gathered, organized and presented with the support of technical evidences and explanation.

Conclusions: *E. longifolia* root extract was mostly reported to exhibit anticancer properties using different human cancerous cell lines, particularly breast cancer cells. Many reports also revealed the properties could be due to the presence of phytochemicals such as quassinoids, canthin-6-one and carboline alkaloids in the plant extracts. Eurycomanone and eurycomanol were likely to be compounds of quassinoids in promoting the other frequently reported activities such as antimalarial and anti-hyperglycaemic potentials. Interestingly, the water extract of the plant was not toxic with the acute oral LD₅₀ up to 6 g/kg body weight of rats. The toxicity was most probably attributed to eurycomanone at high LD₅₀ (5.27 g/kg). Most studies were focused on crude extract which was not properly characterized in term of phytochemical composition. It is important to well characterise plant extract in order to relate to its pharmacological significance. Possibly, optimized or standardized plant extract is used for subsequent experiments to ensure continuity in research activities. Plant species authentication is of utmost importance prior to any experimental works.

Keywords: *Eurycoma longifolia*; eurycomanone; antimalaria; aphrodisiac; anti-diabetes

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Introduction to *Eurycoma longifolia*

Eurycoma longifolia Jack is a slender tree from the family Simaroubaceae. This plant is widely distributed in the tropical forest of Southeast Asia. It is popularly known as the king of herbs and locally recognized as 'Tongkat Ali' which literally means "Ali's walking stick". The plant gets the name because it has long-twisted roots (1). Sometimes, the plant is called as Malaysian Ginseng, and the other names include Payung Ali, Penawar Pahit, Setunjang Bumi, Bedara Pahit, Tongkat Baginda, Pokok Syurga, Tongkat Ali Hitam, Pokok Jelas and Jelaih in Malaysia (2). In South East Asian, the plant is commonly known as 'Pasakbumi' in Indonesia, 'Cay ba binh' in Vietnam and 'Ian-don' in Thailand.

The roots of *E. longifolia* has been well documented for its medicinal values such as antimalarial, anti-inflammatory and aphrodisiac effects. The ethnomedicinal knowledge of *E. longifolia* has been continuously and widely applied by indigenous people till to date. In particular, the plant roots are believed to have higher therapeutic effects than other parts of the plant. Recent scientific studies have also proven the remarkable pharmacological properties of the plant, mostly the plant roots. This can be seen from the previous reported findings which have been gathered and systematically compiled in *Table 1*. The chemical structures of the reported compounds from *E. longifolia* extracts are presented in *Figure 1*. The data reveals that scientific studies on the pharmacological properties of the plant have been extensively carried out since 1980s. The promising results have also boosted the application of the plant extracts and the acceptance of public using *E. longifolia* in health promoting interest. The technical evidences explained that the ethnopharmacological applications were mainly contributed by phytochemicals in the plant roots. The major classes of phytochemicals, particularly quassinoids, canthin-6-one alkaloids, β -carboline alkaloids, squalene derivatives and biphenylneolignans have been reported to possess the pharmacological significance (42). Most of the reported compounds in *E. longifolia* belong to the group of quassinoids or degraded triterpenes. One of the commonly known quassinoids is eurycomanone which is also a C 20-type quassinoids and the marker compound of the plant.

Therefore, the objective of this narrative review is to draw conclusive information from all published data on the pharmacological significance of *E. longifolia*. Previous review articles on *E. longifolia* have been published from 2010 onwards, they mostly report on individual pharmacological

properties and clinical studies. The present review is mainly focused on a broad range of reported pharmacological significance in relation with phytochemicals. The well-organized information is very important to rationale future studies, and leading to the drug development based on the potentials of phytochemicals in the plant extract. We present the following article in accordance with the Narrative Review checking checklist (available at <https://dx.doi.org/10.21037/lcm-21-32>).

Pharmacological properties of *Eurycoma longifolia*

Aphrodisiac effects of Eurycoma longifolia

E. longifolia has been traditionally gained the attention of local folks because of its promising efficacy in sexual enhancement. Research findings have also proved its potency to treat male sexual disorder and improve overall sexual wellbeing in men. Many experimental works on the aphrodisiac effect of *E. longifolia* extract were performed in rodents, mostly in sexually normal male rats (43), in sexually naïve male rats and mice (44,45), in middle-aged rats and mice (46-48) and sexually sluggish old rats (49). The observation proven the extract of *E. longifolia* could improve sexual behavior significantly. Sexual performance test showed that *E. longifolia* could improve the initiation of sexual actions like mounting, intromission and ejaculation in inexperienced castrated male rats upon oral administration of *E. longifolia* extract in a dose-dependent manner. *E. longifolia* was also able to stimulate the development of ventral prostate and seminal vesicles in castrated male rats (50). In addition, 12 weeks successive oral administration of different fractions of *E. longifolia* increased the weight of bilateral laevator ani muscle in intact male rats, following the protein anabolic action of androgens which explained the pro-androgenic effect of *E. longifolia* (51).

A study on sexually sluggish male rats showed that the decrease of hesitation time to cross electric grid after administration of *E. longifolia* fractions (water, methanol, butanol and chloroform) at a dose of 0.5 g/kg (52). In addition, the administration of *E. longifolia* fractions caused a transient increase in the percentage (up to more than 50%) of male rats responding to the right choice using the electrical copulation cage (52). Somehow, the researchers were unable to conclude which fraction was more efficient in contributing the aphrodisiac property. Therefore, no conclusive findings on the contributor group of phytochemicals in enhancing aphrodisiac property.

Table 1 Pharmacological Properties of *Eurycoma longifolia* contributed by phytochemicals and its extracts

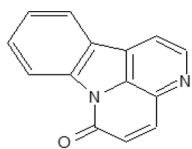
Part of plant	Pharmacological property	Cell line/strain/activity	Compound	References	
Root	Anticancer	Human cancer cell types (breast, colon, fibrosarcoma, lung, melanoma, KB)	9-methoxycanthin-6-one	(3)	
			9-methoxycanthin-6-one-N-oxide		
				9-hydroxycanthin-6-one	
				9-hydroxycanthin-6-one-N-oxide	
				Eurycomanone	(3-5)
			Human breast cancer cell line (MCF-7), human gastric cancer cell line (MGC-803)	5-iso-eurycomadilactone	(6)
				Eurycomanone	
			Human breast cancer cell line (MCF-7), human lung cancer cell line (A549) & human cervical cancer cell (HeLa)	Eurycomalactone	(7)
				Longilactone	
				14,15 β -dihydroxyklaineaneone	
			Human breast cancer cell lines (T47D & MCF-7), human cervical cancer cell (HeLa) and human colon carcinoma cell line (WIDR)	Eurycomanone	(8)
			Human breast cancer cell line (MCF-7) & human lung cancer cell line (A-549)	Eurycomalactone	(9)
				6-dehydroxy-longilactone	(10)
				Longilactone	(11)
				Canthin-6-one	(12)
				9-methoxycanthin-6-one	(12)
				14,15 β -dihydroxyklaineaneone	(11)
				Canthin-6-one 9-O- β -glucopyranoside	(12)
				13 β ,21-epoxyeurycomanone (pasakbumin B)	(4)
				11 β ,20-epoxy-pentahydroxypicras-3-ene-2,16-dione (pasakbumin C)	(4)
			Human breast cancer cell lines (T47D & MCF-7)	Eurycomanone	(13)
			Human breast cancer cell line (MCF-7)	Longilactone	(14)
		Eurycomanone	(15,16)		
	Human breast cancer cell line	Extract	(17)		
	Human leukemia cell lines (HL-60 & Jurkat)	Eurycomanone	(18)		
	Human leukemic cell line (K-562)	Extract	(19)		
	Human lung cancer cell line (A549)	Eurycomanone	(20)		
	Human liver cancer cell (HepG2)	Eurycomanone	(21)		
Stem		Human fibrosarcoma cell line (HT-1080)	Eurycomalactone	(11)	
			13,21-dihydroeurycomanone	(22)	
			14,15-dihydroxyklaineaneone		

Table 1 (continued)

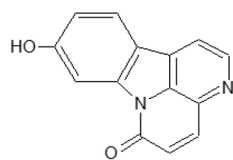
Table 1 (continued)

Part of plant	Pharmacological property	Cell line/strain/activity	Compound	References		
Root	Antimalarial	<i>Plasmodium falciparum</i>	Eurycomanone	(3,23)		
			Eurycomanol	(23)		
			13,18 β -dihydroeurycomanol			
			Eurycomanol-2-O- β -D-glucopyranoside			
			13 β ,21-epoxyeurycomanone (pasakbumin B)	(5)		
			7-methoxy-carboline-1-propionic acid (β -carboline alkaloids)	(3)		
			Eurycomanone	(24)		
			13,21-dihydroeurycomanone			
			Unknown	(25)		
			Root	Anti-leukemic/anti-inflammation	NF- κ B inhibitor (I κ B α phosphorylation and upstream mitogen activated protein kinase signaling)	Eurycomanone
Root	NF- κ B inhibitor	Eurycomalactone	(27)			
		13,21-dihydroeurycomanone				
Root	Antihyperglycaemic	Edema thickness (<i>in vivo</i>)	14,15 β -dihydroklaieanone			
			9,10-dimethoxycanthin-6-one			(28)
			7-methoxy-(9H- β -carbolin-1-yl)-(E)-1-propenoic acid (β -carboline alkaloid)			(29)
			Extract	(30)		
Root	Antihyperglycaemic	Lower blood glucose (<i>in vivo</i>)	Extract	(31)		
			Lower blood glucose and lipid-lowering effects (<i>in vivo</i>)	Extract	(32)	
			Inhibitory activity of advanced glycation end-products (AGEs) formation (excess glycation and thus is believed to cause diabetic complications)	Extract	(33)	
			Lower blood glucose (<i>in vivo</i>)	Extract	(34)	
			Lower blood glucose (<i>in vivo</i>)	Extract	(35)	
			Reduce alpha-2-HSglycoprotein(AHS) in serum	Extract	(36)	
			Unknown	Increase insulin sensitivity through the enhancement of glucose uptake and reduce lipid accumulation in adipose cells	Extract	(37)
Unknown	Anti-hypertriglyceridemic	Decrease high-density lipoprotein (<i>in vivo</i>)	Extract	(30)		
Root	Anti-osteoporosis	High dose (>90 mg/kg) increase bone microarchitecture (<i>in vivo</i>)	Extract	(38)		
Root	Aphrodisiac	Sexual enhancement	Extract	(36)		
		Lower blood pressure	Extract	(39)		
Root	Antiadipogenic (obesity)	Inhibit adipogenesis	Extract	(40)		
		Increase lipolysis	Eurycomanone	(41)		
		13 β ,21-epoxyeurycomanone (pasakbumin B)				
Unknown	Analgesic	Reduce in writhing (<i>in vivo</i>)	Extract	(30)		

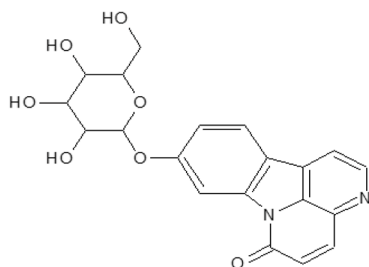
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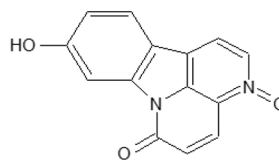
Canthin-6-one
C₁₄H₈N₂O (MW 220)



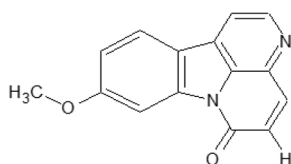
9-hydroxycanthin-6-one
C₁₄H₈N₂O₂ (MW 236)



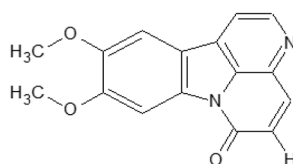
Canthin-6-one 9-O-β-
glucopyranoside
C₂₀H₁₈N₂O₇ (MW 398)



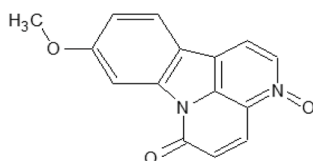
9-hydroxycanthin-6-one-3N-oxide
C₁₄H₈N₂O₃ (MW 252)



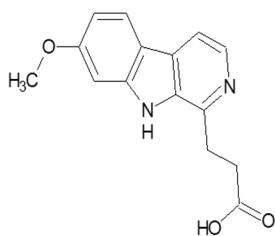
9-methoxycanthin-6-one
C₁₅H₁₀N₂O₂ (MW 250)



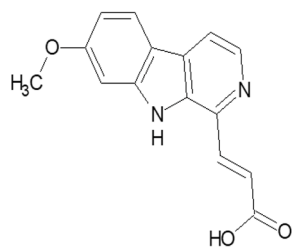
9,10-dimethoxycanthin-6-one
C₁₆H₁₂N₂O₃ (MW 280)



9-methoxycanthin-6-one-3N-oxide
C₁₅H₁₀N₂O₃ (MW 266)



7-methoxy-β-carboline-1-propionic acid
C₁₅H₁₄N₂O₃ (MW 270)



7-methoxy-(9H-β-carboline-1-yl)-(E)-1-propenoic acid
C₁₅H₁₂N₂O₃ (MW 268)

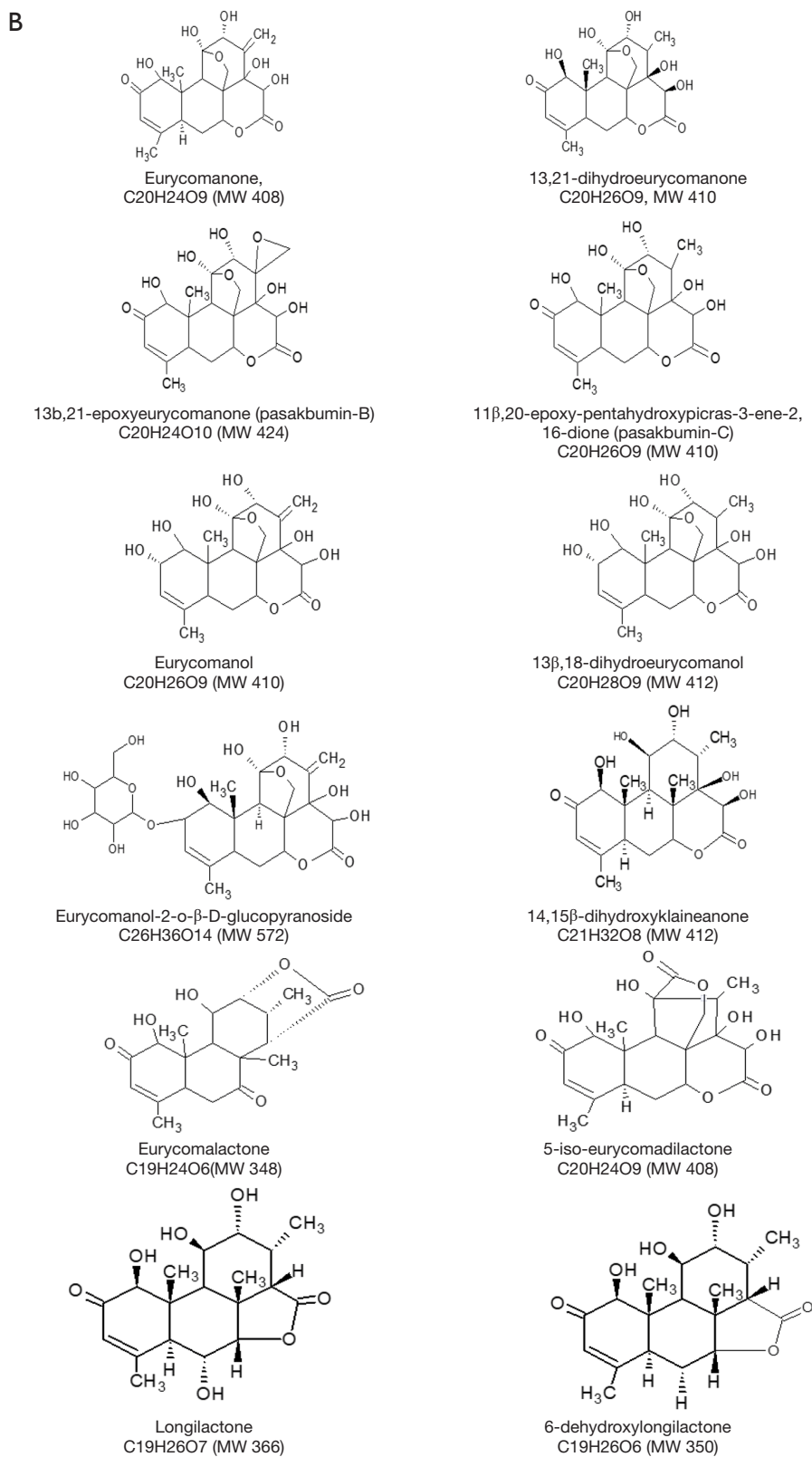


Figure 1 Chemical structures of (A) canthin-6-one and β-carboline alkaloids, and (B) C19-C21 quassinoids. Each compound is given its common name and chemical formula (molecular weight).

In other studies, reported by the same research group that an enhancement of sexual motivation was observed from the increment of adult middle-aged male mice and retired breeders responding to the right choice after the consumption of various extracts (47) and fractions (48) of *E. longifolia*. *E. longifolia* extracts also exhibited the sex drive stimulus such as yawning and stretching by 50% and 16.7%, respectively in sexually sluggish old male rats (49).

Previous studies reported significant increment in serum testosterone level of rats treated with the plant root powder for 6 days, and subsequently resulted in the improvement of copulatory activity (53). The acute administration of the highest dose (1,000 mg/kg) increased the percentage of mounting and ejaculating rats, particularly in sexually sluggish rats (75% and 62.5%, respectively) when compared to the controls (25%). Moreover, the subacute and subchronic treatments increased the percentage of mounting rats from 0% (controls) to 25% and 37.5%, respectively, and the percentage of ejaculating rats from 0% (controls) to 25% in both treated groups of impotent rats. On the other hand, better results related to ejaculation latency and post-ejaculatory interval were obtained after the subacute administration (6 days) in sexually sluggish rats in comparison with the acute and the subchronic (12 days) administrations. However, a single administration of 500 or 1,000 mg/kg of the plant root powder reduced ejaculation latency. This could be due to the excessive doses of administration to the rats. A dose range of 100–200 mg/kg for *in vivo* studies of extracts or 100–200 µg/mL should be assumed as a meaningful upper limit for pharmacological studies (54).

Clinical trials for male infertility

Apart from animal studies, there were also several human clinical trials conducted to investigate the therapeutic feasibility of *E. longifolia* in managing male sexual disorders including erectile dysfunction, male infertility, low libido, and downregulated testosterone levels. Neurological, vascular, and hormonal disorders are major factors that contribute to the sexual dysfunction in male along with bad diet and lifestyle (55). Erectile dysfunction, a frequent and persistent inability of male to attain and/or to retain penile erection during the course of performing sexual activity is one of the primary causes of dissatisfaction in sexual performance (56,57). Physta, a proprietary freeze-dried water extract of *E. longifolia* root notably improved scores for the sexual intercourse attempt diary, erection hardness scale, sexual health inventory of

men, and aging male symptom scale ($P < 0.05$ for all) in a 12-week randomized, double-blind, and placebo-controlled parallel-group study consisted of men aged from 40 to 65 years old. Besides, there was no clinically significant changes in the vital organs of body such as liver and kidney by laboratory evaluation (58).

Erectile dysfunction is remarkably influenced by age and prevalence among men between 70ties and 80ties of age (59). The decreased levels of testosterone and inadequate blood supply flowing to penis induce erectile dysfunction. cGMP-specific phosphodiesterase type 5 inhibitors such as sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra) are the commonly prescribed medications to treat erectile dysfunction. These drugs improve penile erection by regulating blood flow to the penis (58). *E. longifolia* is a potent alternative medicine for erectile dysfunction without any of side effects associated with medicines such as visual disturbances, flushing, muscle pain, and back pain (60).

E. longifolia was found to facilitate conversion of pregnenolone to progesterone, cortisol, 5-dehydroepiandrosterone, and testosterone in rabbit corpus cavernosum tissues (58). Eurypeptides, the bioactive complex polypeptides with a molecular weight of 4.3 kDa exerted its effects on the biosynthesis of various androgens. CYP17 (17 α -hydroxylase/17,20 lyase) is activated by eurypeptides to enhance the metabolism of pregnenolone and 17-OH-pregnenolone to yield dehydroepiandrosterone, 4-androstenedione and testosterone (61). CYP17 is a P450 enzyme that catalyzes the last step of androgen biosynthesis in both testis and adrenals (62). Eurypeptides increase the release rate of free form of testosterone from its binding proteins, sex hormone-binding-globulin. The testosterone balancing effects of this water soluble glycoprotein extract showed to deliver anti-aging and anti-stress benefits (63). A study was conducted to investigate the efficacy of a patented standardized water-soluble extract of *E. longifolia* in the treatment of 76 hypogonadic men suffering from late onset hypogonadism. The results showed a significant improvement ($P < 0.0001$) in the serum testosterone concentration from 5.66 ± 1.52 to 8.31 ± 2.47 nM, which denoted an average increase of 46.8% (61).

Several human clinical studies also depicted the effects of *E. longifolia* in managing male infertility. Men with idiopathic male infertility characterized by low sperm concentration with or without low motility and with normal morphology were treated with 200 mg of patented standardized water-soluble extract of *E. longifolia* root daily for 9 months (64). Subsequent semen analysis was conducted

for every 3 months based on the World Health Organization guidelines (65). The results showed the extract significantly improved ($P < 0.05$) sperm qualities of patients such as improvement in semen volume, sperm concentration, sperm motility and percentage of morphologically normal sperm, allowing for 11 (14.7%) spontaneous pregnancies. In addition, a randomized, double-blind, placebo controlled, parallel group study investigated the aphrodisiac clinical evidence of *E. longifolia* extract in accordance with the Good Clinical Practice (ICH-6) guidelines and Declaration of Helsinki in 109 men between the ages of 30 to 55 years. The results showed higher scores in the overall erectile-function-domain ($P < 0.001$), the sexual libido (14% by week 12), seminal fluid analysis—with sperm motility at 44.4%, and semen-volume at 18.2% following 12-week treatment with 300 mg of freeze-dried water extract of *E. longifolia* daily. The results of the study also demonstrated the positive effect of *E. longifolia* on male fertility with considerable positive effects on seminal parameters such as volume and concentration of sperms in ejaculate and the proportion of sperms having normal motility (59).

Antiproliferation of cancer cells

The potential of *Eurycoma longifolia* as anti-cancer agent has been proven in a number of studies. Previous studies reported the anticancer properties of *E. longifolia* on different kinds of cancerous cell lines and solid tumors including lung, breast and cervical cancers. Extracts from the leaves, stems and roots, as well as isolated bioactive compounds have been thoroughly tested for the anti-tumor effect. 14,15 β -dihydroxyklaineanone isolated from the plant leaves was found to be the most active compound to promote anti-tumor property by inhibiting tumor promoter-induced Epstein-Barr virus activation with the IC_{50} value of 5 μ M (66). The value was comparatively higher than that of quercetin ($IC_{50}=23$ μ M) and β -carotene ($IC_{50}=30$ μ M) which are two commonly known as natural anti-cancer agents (66). Among the 65 compounds, Kuo *et al.* (5) revealed that some compounds (eurycomalactone, 6-dehydroxylongilactone, 9-methoxy-canthin-6-one, canthin-6-one, longilactone, 14 β , 15 β -dihydroxyklaineanone, pasakbumin C and canthin-6-one 9-O- β -glucopyranoside) demonstrated strong cytotoxicity toward human lung cancer (A-549) cell lines, while some compounds (eurycomalactone, 6-dehydroxylongilactone, 9-methoxy-canthin-6-one, 14,15 β -dihydroxyklaineanone, eurycomanone, pasakbumin B and pasakbumin C) exhibited strong cytotoxicity toward

human breast cancer (MCF-7) cell lines. Moreover, Chuen and Pihie (67) showed that the semi purified eurycomanone also possessed toxicity towards breast cancer cells, but was relatively non-toxic to non-cancerous breast cells (MCF10A). These are interesting findings to prove the feasibility of phytochemicals in *E. longifolia* extracts as potential leads in drug development.

Delineating cellular mechanism against cancer cells

Several studies were also attempted to investigate the cellular mode of action attributed to phytochemicals in anti-tumor promoting properties. The cytotoxic activities of *E. longifolia* prominently followed the pathway of programmed cell death of apoptosis (16). The methanolic fraction from the plant roots selectively exhibited higher cytotoxic activity towards human breast cancer cell line, MCF-7 with the IC_{50} value of 15.23 ± 0.66 μ g/mL without affecting normal breast cells (MCF-10A). That fraction demonstrated the growth inhibition of MCF-7 cells through apoptotic cell death by maintaining the pro-apoptotic Bax protein levels and decreasing the anti-apoptotic Bcl-2 protein expression (16). A similar mode of action was also noticed by Mahfudh and Pihie (68) who reported that eurycomanone showed anticancer activity against cervical carcinoma HeLa cells. Eurycomanone triggered apoptosis by promoting the up-regulation of p53 tumor suppressor protein which was followed by the increasing of pro-apoptotic Bax protein and the decreasing of anti-apoptotic Bcl-2 (21). They also discovered that eurycomanone considerably reduced the viability and proliferation of cancer cells such as CaOv-3, HeLa, HepG2, HM3KO and MCF-7 in a concentration dependent manner. Park *et al.* (7) concluded that eurycomalactone, eurycomanone, longilactone and 14, 15 β -dihydroxyklaineanone showed to be cytotoxic to MCF-7 among the 15 compounds investigated in their studies. In addition, the methanolic fraction also induced caspase activation to mediate apoptosis in MCF-7 cells. The role of that fraction was to induce the cell death of MCF-7 cell death, and followed by the cleavage and activation of caspase-7 which led to the specific proteolytic cleavage of poly(ADP-ribose) polymerase-1 (69). As reported by Muhamad *et al.* (14), longilactone in *E. longifolia* extract induced apoptosis in MCF-7 via an extrinsic pathway. Meng *et al.* (6) concluded that quassinoids showed the best inhibitory activity on MCF-7 cancer cells among the cancer cell lines such as HT-29, MCF-7, LOVO, BGC-823, MGC-803, HepG2, HeLa, and A549.

Al-Salahi *et al.* (19) conducted a study to assess *in vitro*

and *in vivo* anti-proliferative and apoptotic potentials of *E. longifolia* on K-562 leukemic cell line. Their findings indicated that standardized methanolic extract (TAF-273) and eurycomanone could induce cellular responses in K-562 cells. The anti-proliferative effect of TAF-273 on K-562 cells could be attributed primarily to the induction of cell arrest at the G1 and S phase which mostly targeted at cell division and DNA synthesis. Apart from cell cycle arrest, TAF273 also inhibited cell proliferation by preventing the formation of blood vessels. Cancerous cells that undergo apoptosis are generally characterized by condensed and fragmented nuclei which could be observed after Hoechst staining (16). Most cancerous cells showed the apoptotic features after treated with *E. longifolia* extract (19,68).

A standardized extract containing 40% of total quassinoids (SQ40) from *E. longifolia* inhibited LNCaP human prostate cancer cell growth at the IC₅₀ value of 5.97 µg/ml by suppressing the expression of regulatory proteins involved in G₁-to-S transition of cell cycle such as CDK4, CDK2 and Cyclin D1 (70). SQ40-treated LNCaP cells also demonstrated the up-regulation of expression level of the Cyclin inhibitor protein p21Waf1/Cip1. SQ40 also suppressed the growth of LNCaP cells through the inhibition of androgen receptor activities. The suppression of androgen receptor translocation from cytoplasm into nucleus could prevent the transcription of androgen-responsive genes, thus significantly reducing the secretion of prostate-specific antigen.

It was found that eurycomanone promoted cell apoptosis through the inhibition of NF-κB signaling pathway by inhibiting phosphorylation of IκBα and upstream mitogen activated protein kinase signaling in Jurkat and K562 human leukemia cell models in a low micromolar range (26). The recent findings reported by Tung *et al.* (18) also proved the selectively potential antiproliferation of eurycomanone on leukemia cell lines (HL-60 and Jurkat) and low toxic effects on normal skin fibroblast cell line (NB1RGB). Eurycomanol was unable to have such inhibitory action against leukemia cells, probably due to the lack of α,β-unsaturated ketone in the molecular structure of compound (26). Eurycomalactone, 13,21-dehydroeurycomanone, and 14,15β-dihydroklaeanone are also the quassinoids compounds that exhibited strong inhibition for NF-κB with IC₅₀ values less than 1 µM (0.5, 0.7, and 1.0 µM, respectively) (27).

Antimalarial properties of Eurycoma longifolia

Plasmodium falciparum is the most deadly and complicated

form of parasite species which causes malaria and resistant against chloroquine treatment (23). Hence, it is important to find alternatives of drugs against chloroquine-resistant *P. falciparum*. *E. longifolia* or its bioactive compound is likely to be a potential lead. Its decoction has traditionally used to treat malarial fevers. The traditional usage of the plant extract also shows a promising anti-malarial effect against *P. falciparum*. Scientifically, studies have intensively proven the anti-malarial activities of the plant extract and its bioactive compounds against chloroquine-resistant malaria.

The evaluation was conducted on the ethanolic extract of *E. longifolia* dried root from Thailand. The SYBR green I-based assay indicated a great antimalarial activity with the IC₅₀ of 2.16 and 1.79 µg/mL against *P. falciparum* strain 3D7 (chloroquine sensitive) and K1 (chloroquine resistant), respectively (71). Among different extracts of *E. longifolia* from Cambodia, the dichloromethane extract of the plant bark was proved to be the most active extract against multidrug-resistant *P. falciparum* strain (W2) with the IC₅₀ value of 1.7 µg/mL (72). Possibly, eurycomanone and pasakbumin B from the plant attributed to the potent antimalarial activity against two resistant *P. falciparum* clones, W2 (IC₅₀=14.912 and 22.658 ng/mL, respectively) and D6 (IC₅₀=26.094 and 34.001 ng/mL, respectively). While the IC₅₀ values of Chloroquine for W2 and D6 were 75.270 and 2.722 ng/mL, respectively (5,72). However, the inhibitory activity of the standardized *E. longifolia* extract containing three major quassinoids, namely eurycomanone, 13,21-dihydroeurycomanone, and 13α(21)-epoxyeurycomanone against *P. falciparum* schizont maturation was higher than that for individual quassinoids. The observation explains the synergistic effect between the quassinoids, or possibly the presence of other unidentified compounds in the extract (73).

There was also a study to test the *in vitro* anti-malarial activity of quassinoids rich *E. longifolia* extract against six chloroquine-resistant *P. falciparum* isolated from Malaysia (23). The quassinoids rich extract inhibited the growth of parasites in a dose-dependent manner. The result was comparable to that of chloroquine treatment. A complete inhibition was also noticed at the concentration range of 1.25–5.00 µg/mL extract after 3 days of post-treatment (23). Eurycomanone was one of the quassinoids which exhibited high antiplasmodial activity compared to the chloroquine treatment against chloroquine-resistant (3,5,24) or multidrug-resistant (9) *P. falciparum*. The findings revealed that eurycomanone was found to be 8.66 times more potent than chloroquine

diphosphate against the isolate. The presence of an α,β -unsaturated ketone at C-2 in ring A of eurycomanone was reported to confer the strong antiplasmodial activity of eurycomanone (24). The other eurycomanone derivatives such as 13,21-dihydroeurycomanone and 13,21-epoxyeurycomanone were also more potent than chloroquine diphosphate against the Gombak A isolate of *P. falciparum*, whereas eurycomanol was found to be less potent compound in antimalaria.

The antiplasmodial potential of *E. longifolia* could be explained by the inhibitory action of glutathione (GSH) synthesis (74). *Plasmodium* grows inside oxygen-rich erythrocytes by consuming haemoglobin and releasing reactive oxygen species. This leads to the formation of superoxides anion and haem. GSH conjugation in haem polymerization process would detoxify superoxides anion and haem, and thus assisting the growth of parasites. High level of GSH synthesis would protect the drug resistant *Plasmodium* in which chloroquine acts as a competitive inhibitor of the GSH conjugation. The study demonstrated that the methanol extracts of *E. longifolia* (TA164) decreased the GSH content of both infected and healthy erythrocytes (74). The findings indicated that TA164 was able to completely inhibit the growth of *P. falciparum* at concentration 16 $\mu\text{g}/\text{mL}$. The inhibitory capability of TA164 was significantly higher than the GSH synthesis inhibitor, D,L-buthionine-(S,R) sulphoximine which only exhibited 95% inhibition at high concentration, 64 $\mu\text{g}/\text{mL}$.

In vivo antimalarial activity was conducted using a standardized *E. longifolia* root extract enriched with four major bioactive quassinoids such as eurycomanone (18.5%), 13 α (21)-Epoxyeurycomanone (4.0%), 13 α ,21-dihydroeurycomanone (0.7%) and eurycomanol (9.5%) (75). 13 α (21)-Epoxyeurycomanone and eurycomanone showed a gradual increase in plasma concentration upon oral administration of the extract at 200 mg/kg to Sprague-Dawley rats. Despite the concentration of 13 α (21)-Epoxyeurycomanone was 4.6-fold lower than eurycomanone, 13 α (21)-Epoxyeurycomanone possessed higher absolute bioavailability, 24.7% than that of eurycomanone which was only 2.6%. In contrast to the chemical degradation of 13 α ,21-dihydroeurycomanone and eurycomanol, 13 α (21)-Epoxyeurycomanone and eurycomanone displayed strong pH stability towards acidic environment in gastrointestinal tract. However, eurycomanone could impart poor membrane permeability of absorption due to its highly polar nature. Meanwhile, the 13 α (21)-epoxy analogue of 13 α (21)-Epoxyeurycomanone

possesses high lipid solubility (hydrophobicity) for absorption in gastrointestinal tract. In spite of lower bioavailability, eurycomanone showed a 1.9-fold higher antimalarial activity than 13 α (21)-Epoxyeurycomanone. Therefore, the antimalarial activity was solely attributed to the synergistic effect between 13 α (21)-Epoxyeurycomanone and eurycomanone.

Antiosteoporotic potentials of Eurycoma longifolia

E. longifolia has been reported for its potential as an alternative antiosteoporotic agent in the treatment of male osteoporosis caused by androgen deficiency called hypogonadism (76). In hypogonadism, the disruption of one or more levels of hypothalamic-pituitary-testicular axis caused failure to the testis to produce physiological levels of androgen, especially testosterone (62). Most bone cells have androgen receptors which play a vital role in bone formation. Androgens may protect men against osteoporosis by maintaining cancellous bone mass and expansion of cortical bone. Interestingly, *E. longifolia* may increase the testosterone levels and prevent bone calcium loss to preserve the bone calcium content. Testosterone was able to improve the trabecular bone volume of orchidectomised rats (62). Based on the findings reported by previous researchers, *E. longifolia* can possibly be used as an alternative treatment for testosterone replacement therapy to prevent and treat male osteoporosis induced by androgen deficiency without causing any side effects. Proandrogenic effects of *E. longifolia* could enhance testosterone level to stimulate osteoblast proliferation and osteoclast apoptosis. This maintains bone remodelling activity, and thus reducing bone loss (76).

An *in vitro* study using MC3T3-E1 cells as osteoblastic model was conducted to investigate the molecular mechanism of *E. longifolia* for the prevention and treatment of male osteoporosis (77). The results found that a dose of 25 mg/mL *E. longifolia* extract could stimulate bone formation through significant up-regulating the expression of various mitogenic proteins such as bone morphogenic protein-2, runt-related transcription factor-, osteocalcin, type I collagen, transforming growth factor- β 1, androgen receptor, alkaline phosphatase activity and down-regulating the expression of osteopontin, a negative regulator of bone formation.

Another *in vitro* study conducted by Thu *et al.* (78) in the following year. They reported the use of RAW 264.7 cells as an osteoclastic model to investigate the effect

of the plant extract on the translational mechanism of osteoclastogenesis. Cell cultures treated with standardized aqueous root extract showed a significant down-regulation of RANKL-induced tartrate-resistant acid phosphatase activity and expression of osteoclast-related protein biomarkers such as matrix metalloproteinase-9, cathepsin-K, tartrate-resistant acid phosphatase, nuclear factor of activated T-cells cytoplasmic 1. Overall, *E. longifolia* suppressed the generation of superoxide free radicals by enhancing superoxide dismutase activity and inhibiting the function of osteoclasts. Osteoclasts play a vital role in bone loss.

Antidiabetic effects of *Eurycoma longifolia*

Diabetes mellitus (DM) is a metabolic disorder of glucose metabolism which is the major chronic disease in many countries in South East Asia. DM can be categorized into two types; Type I DM is known as insulin-dependent DM which occurs due to the destruction of insulin-secreting beta-cells in the pancreatic islets, and thus leading to insufficient insulin production in the body, and Type II DM is known as non-insulin-dependent DM in which muscles, liver, and adipose tissues are insulin resistant and insensitive to insulin (32).

It is worthy to note that *E. longifolia* could exhibit tremendous antidiabetic potentials. Hendra *et al.* (30) reported that *E. longifolia* root extract could reduce triglyceride level compared with the control group of rats treated with diet with high glucose and fructose components. Often, high triglyceride level is associated with prediabetes or type II DM. The plant was proven to be capable of modulating glucose and lipid metabolism by increasing glucose uptake and suppressing lipid production in 3T3-L1 adipocytes simultaneously (37). Therefore, *E. longifolia* extract, particularly quassinoids enriched fraction may prevent the onset of obesity-associated T2DM (40). In addition to reduce intracellular lipid droplets and triglycerides, that fraction could suppress body weight gain, decrease epididymal and perirenal fat pad mass and size. Although fed with high-fat diet, C57BL/6J mice did not show elevated total cholesterol and triglyceride levels, and fat accumulation liver.

Antihyperglycemic potentials

The antidiabetic effect of *E. longifolia* roots was found to be more effective using polar fraction obtained from the solvent mixture of ethyl acetate-ethanol-water in a

ratio of 80:30:1. The effectiveness was determined based on the sample capacity to reduce blood glucose level in streptozotocin induced diabetic rats at the fifty-effective dose (ED₅₀) of 1.71 mg/dL. The other sample with lesser polarity that obtained from the same solvent mixture with different ratios, 100:30:1 was recorded to have the ED₅₀ of 67.48 mg/dL. The observation suggested that phytochemicals and their concentration might contribute to the difference of antidiabetic potentials (34).

An *in vivo* study on the efficacy of powdered *E. longifolia* roots to exhibit antihyperglycemic effect was conducted by Tsai *et al.* (32) recently. They reported the significant reduction from 37.7% to 64% of fasting blood glucose after one to eight weeks of treatment (100 mg/kg powdered extract) in the db/db mice (32). *E. longifolia* root extract could improve islet performance by increasing β -cell number, and pancreatic and duodenal homeobox 1 expression. There was no hypoglycemic response was found in the normoglycemic C57BL/6J mice, even treated with high dose, 200 mg/kg extract. The function of *E. longifolia* extract in antidiabetic property can also be rationalized by the reduction of alpha-2-HS glycoprotein expression in the serum protein of treated rats. Chen *et al.* (36) reported the decreased expression of alpha-2-HS glycoprotein in rats after treated with standardized *E. longifolia* extract at a dose of 50 mg/kg for consecutive 28 days.

However, it is noted that the antidiabetic property of *E. longifolia* was only displayed at high dose of administration. For instance, the high dosage, 150 mg/kg body weight of *E. longifolia* aqueous extract was able to significantly reduce the blood glucose level for about 46.8% in hyperglycaemic rats (31). In another study performed by Fransisca *et al.* (35), who reported that the methanol extract of *E. longifolia* roots demonstrated blood glucose-lowering effect at the doses of 210 and 420 mg/kg body weight.

Toxicity of *Eurycoma longifolia*

Generally, herbal medicines comparatively exhibit fewer side effects than allopathic medicines. Despite the various pharmacological benefits, the safety and toxicity of *E. longifolia* are of great importance to avoid any adverse effects upon consumption. Previously, a subacute and subchronic administration of *E. longifolia* extracts (0, 0.6, 1.2, and 2 g/kg body weight/day) for 4 and 13 weeks produced neither mutagenic nor clastogenic effects in rats. The acute oral of 50% lethal dose (LD₅₀) was more than 6 g/kg body weight (79). Moreover, phytochemicals in the *E. longifolia*

water extract did not also exhibit any adverse effects related to body weight, hematology, serum biochemistry, urinalysis, macropathology, or histopathology. However, the treatment significantly reduced prothrombin time, partial thromboplastin time, blood urea nitrogen, creatinine, aspartate aminotransferase, creatine phosphate kinase, lactate dehydrogenase, and cholesterol levels in male rats ($P < 0.05$) which regarded as beneficiary effects.

In contrast, another acute toxicity study showed that the n-butanol extract of the plant was found to be the most toxic with a LD_{50} of 1.36 g/kg (80). The strong toxicity was attributable to the presence of higher concentration of eurycomanone. The compound was found to be the most toxic compound among other chemical constituents. It possessed an oral LD_{50} of 0.05 g/kg. A structure activity relationship showed the presence of unsaturated double bond at C-13 and C-21, an α, β -unsaturated ketone at C-2 in ring A, and the oxymethylene bridge linking C-11 to C-8 are contribute to the strong toxicity of eurycomanone. On the other hand, the water fractions of *E. longifolia* were considered to be safe as it demonstrated significantly less toxicity with the LD_{50} value of 5.27 g/kg than the extracts prepared by organic solvents. Even though it was previously reported to be toxic compound, recent data revealed that 0.137 $\mu\text{g/g}$ (35% LD_{50}) was considered to be safe for consumption based on the toxicological evaluation using catfishes (81).

Conclusions

The pharmacological importance of *E. longifolia* is mainly contributed by its phytochemicals, particularly quassinoids which are the major compounds in the plant roots. However, the significance of the biological activities is strongly depended upon types of quassinoids and their derivatives with different functional groups, as well as the synergistic effects in the plant extracts or fractions. Mostly, eurycomanone was reported to be the dominant contributor of pharmacological properties, particularly anticancer, antimalarial and antiinflammation of *E. longifolia*. Detail comparison is difficult to achieve conclusive information because many studies did not report the chemical characteristics of plant extract. Therefore, the plant root extract shall be well characterized for its phytochemical profile in order to have an insightful understanding of this traditional herb and its pharmacological relevance. Plant extract in any pharmacological studies must at least obtain from standardized extraction conditions to ensure quality

consistency and data validity. The utmost importance is authenticating plant species for its botanical identity prior to any experimental works.

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