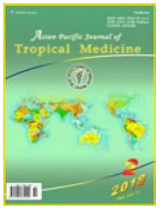

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Exploring dynamic biomedical algorithm of *Eurycoma longifolia* Jack and its bioactive phytochemicals: A review of pharmacokinetic and pharmacodynamic implications and future prospects

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ABSTRACT

Eurycoma longifolia Jack (*E. longifolia*) is a well-recognized traditional herbal medicine that offers a wide dynamic range of biomedical applications including anti-osteoporotic, anticancer, anti-proliferative, anti-malarial, antimicrobial, antioxidant, aphrodisiac, anti-inflammatory, anxiolytic, anti-diabetic, anti-rheumatism and anti-ulcer properties. This review aims to overview the pharmacokinetic and a pharmacodynamic algorithm of *E. longifolia* and its bioactive components. Analysis of pharmacokinetic profile revealed that *E. longifolia* exhibit higher bioavailability, high volume of distribution, slow elimination rate, and does not show inhibitory effects on cytochrome P450 isoenzymes. *E. longifolia* has been used, alone or in combination with other pharmacological agents, in the form of crude extracts, standard extracts, or decoctions of different plant parts (*i.e.*, herbs, shrubs, stem, leaves, and roots) for the treatment of various ailments in animals and humans. Among various bioactive constituents, eurycomanone has been found to be the most remarkable, super-stable, versatile, and most potent phytochemical (isolated or extracted from root extracts) against various types of animals and human diseases. Based on its well-established pharmacokinetic and pharmacodynamic profiles, we suggested that *E. longifolia* can be a well-accepted complementary and alternative medicine for the treatment of different types of human ailments.

1. Introduction

Eurycoma longifolia Jack (*E. longifolia*), a potent medicinal herb in the family of Simaroubaceae, is known locally as tongkat ali in Malaysia, Pasak bumi in Indonesia, Tung saw in Thailand, and cay ba bihn in Vietnam. *E. longifolia* has gained remarkable recognition among various ethnic groups in Malaysia, China and South Africa due to its excellent pharmacological activities. Various parts of *E. longifolia* including roots, stem, and leaves have shown broad range of pharmacological activities including aphrodisiac[1], anti-malarial[2], antibacterial[3], anti-inflammatory[4], anxiolytic[5], anti-diabetic[6], anti-ulcer[7], anti-rheumatic[8], anticancer[9], and anti-osteoporotic activities[10,11].

The pharmacokinetic profile of *E. longifolia* demonstrated that *E. longifolia* and its phytochemicals showed poor absorption after oral administration and low bioavailability. However, the plasma volume distribution of *E. longifolia* and its phytochemicals is greatly high which suggests that they are well-distributed in various tissues.

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The administration of *E. longifolia* and its phytochemicals does not stimulate or down-regulate hepatic enzymes [cytochrome P450 (CYP) isoforms].

Bioactivity guided fractionation of *E. longifolia* and its medicinally active constitutents demonstrated that different parts of plant and its various phytochemicals exhibit varied pharmcodynamic response against various types of diseases. The strongest anti-osteoporotic activity of *E. longifolia* is exhibited by root extracts compared to the other parts of this plant. It is evident from an a wide range of *in vivo* and *in vitro* studies that the antiosteporotic activity of *E. longifolia* is due to its remarkable pro-androgenic effects which in turn improve bone formation and reduce bone resorption. The multi-dimentional pharmacodynamic spectrum of *E. longifolia* has also been evident from its well-recognized traditional use to improve male and female sexual well-being. Recently, Thu *et al*. has written an excellent systematic review in which they have critically analysed the excellent ability of *E. longifolia* and its phytomedicines in improving penile erection, male fertility and sexual libido due to their ability to improve synthesis of testosterone in the body[1].

Similarly, numerous studies evidenced that the aqueous, methanolic, ethanolic or butanolic extract of *E. longifolia* and various bioactive compounds have shown promising anti-proliferative and cytotoxic effects against various human cancer cell lines including liver carcinoma (HepG2), gastric carcinoma (MGC-803 and BGC-823 cells), intestinal cancers (HT-29 and LOVO cells), malignant melanoma (HM3KO), cervical carcinoma (Hela), human ovarian carcinoma (CaOV-3 and A-2780), breast cancers (MCF-7), and lung carcinoma (A-549 cells). Among various phytochemicals, eurycomanone showed highest anticancer efficacy and the mechanism of cytotoxicity is the induction of apoptosis (programmed cell death) via the up-regulation of the expression of p53 (a tumor suppressor protein)[9]. Phytochemical screening of various types of extracts from different parts (roots, stem, and leaves) of *E. longifolia* also displayed a dose-dependent antibacterial, antiprotozoal, and antifungal activities. It has also been explored that among various phytochemicals, eurycomanone from the root extract of *E. longifolia* exhibited strongest antimicrobial and antiplasmodial activities. There is no consensus for the superior efficacy of a specific type of plant part, extract type, and type of phytochemical for the anti-diabetic, anti-inflammatory, analgesic, and anti-ulcer effects. In the present review, we have overviewed a wide dyananic pharmacological spectrum of *E. longifolia* and its phytochemicals as well as their pharmacokinetic profile.

2. Pharmacokinetic considerations

2.1. Absorption

Low *et al*. studied the pharmacokinetic profile of *E. longifolia* following an oral administration in animals and compared the results with intravenous administration. They demonstrated that *E. longifolia* showed lower absorption rate, C_{max} (maximum plasma concentration), T_{max} (time to achieve maximum plasma concentration), and bioavailability compared to intravenous route. They further demonstrated that following an oral administration, C_{max} and T_{max} values were (0.33±0.03) μg/mL and (4.40±0.98) h, respectively[12].

2.2. Distribution

Low *et al*. has also measured the volume of distribution of active constituents of *E. longifolia*. The volume of distribution of *E. longifolia* was calculated to be (0.68±0.30) L/kg which showed that *E. longifolia* is well-distributed into the extravascular fluids[12].

2.3. Metabolism

There is no consensus regarding the effect of *E. longifolia* and its phytochemcals on various isoforms of hepatic enzymes. Pan *et al*. demonstrated that *E. longifolia* and its phytochemcials did not show inhibitory or stimulatory effects on various CYP isoforms including CYP3A4, CYP2C8, CYP1A2, CYP2A6, CYP2C19, CYP2E1 and CYP2C9[13]. It means that *E. longifolia* and its phytochemical have no drug-drug interactions in the context of metabolism. However, on the other hand, Han and co-workers revealed a dose-dependent inhibitory effects of *E. longifolia* and its phytochemicals on CYP2A6, CYP1A2, and CYP2C19 isozymes of P450 systems[14].

2.4. Excretion

In an animal study, Low *et al*. have estimated the rate of excretion of *E. longifolia* and its active medicial components following an intravenous injection[12]. They measured the mean excretion rate constant and clearance of (0.88±0.19)/h and (0.39±0.08) L/(h•kg), respectively[12].

2.5. Half-life

Low *et al*. have also estimated the half-life of *E. longifolia* and its phytochemcials. They suggested that half-life of *E. longifolia* is slightly longer than its phytochemicals (eurycomanone). They estimated that half-life of *E. longifolia* was measured to be (0.75±0.25) h compared to (0.35±0.04) h for euyrocmanone due to lower elimination rate[12]. The same group of scientists also reported that half-life of eurycomanone was estimated to be (1.00±0.26) h[12].

3. Pharmacodynamic consideration

Phytochemical screening of various types of extracts (methanolic, ethyl acetate, and *n*-butanolic) from different parts (roots, stem, and leaves) of *E. longifolia* have displayed a broad range of pharmacological activities including anti-osteoporotic, aphrodisiac anticancer, antimicrobial, anti-malarial, antioxidant, anti-inflammatory, anxiolytic, anti-diabetic, and anti-ulcer.

3.1. Anti-osteoporotic effects of E. longifolia

Osteoporosis is a bone disease characterised by a decreased bone

density and weaker and porous bones that results in frequent bone fractures. In older males, it is the main cause of morbidity and mortality. It is estimated that approximately 2 million men in the United States suffer from this bone disorder[15]. Worldwide, 1/3 women and 1/5 men over 50 experience osteoporotic fractures.

Numerous studies have shown that *E. longifolia* exhibits promising ability in treating male osteoporosis and reduce bone fractures by up-regulation of bone formation and down-regulation of bone resorption[16]. Shuid *et al*. demonstrated that *E. longifolia* supplementation to orchidectomised rats maintain bone calcium levels and prevent bone mineral loss and therefore has the potential to be used as an alternative treatment for androgen deficient osteoporosis[17]. The anti-osteporotic activity of *E. longifolia* is associated with enhanced biosynthesis of androgen hormones[18]. Due to these pro-androgenic properties of *E. longifolia*, it is able to stimulate osteoblastic proliferation and differentiation, resulting in increased bone formation rate[17]. High levels of testosterone and estrogen may also exert proapoptotic effects on osteoclasts, reducing the bone resorptive activity. This bone health promoting effect of *E. longifolia* appeals for its regular supplementation to avoid bone loss and improve bone health[19].

Oxidative stress is anothoer predator of bones that may stimulate bone loss. An imblance between the synthesis of free radicals (reactive oxygen species) and body scavenging ability may result in bone and extracellular tissue damage[20]. The strong anti-osteoporotic efficacy of *E. longifolia* is due to its storong antioxidant capacity which reduce production of free radicals and thus alleviate oxidative stress[21]. Moreover, according to Tambi and Kamarul[22], *E. longifolia* contains high concentrations of superoxide dismutase, an antioxidantd defense that plays an important role in counteracting oxidative stress.

3.2. Efficacy of E. longifolia to promote sexual health

Sexual health significantly affects the quality of life of males and females, regardless of socio-economic status, age, gender, and religion. Sexual activity requires an efficient synchronization and integrity between different biological systems (hormones, muscles, neurological pathways, blood circulation etc.) of the body[23]. Sexual dysfunction (inability to perform pleasant sexual activity) negatively affects the quality of life and can be the leading cause of various physical, psychological, and pathologic distresses[24]. In this chain of events, the most prevalent male sexual dysfunctions include premature ejaculation, erectile dysfunction, low testosterone levels and low libido (reduced sexual desire)[24].

To date, numerous *in vivo* and human clinical studies have explored the therapeutic effectiveness of *E. longifolia* in the management of various male sexual dysfunctions[25-28]. The critical analysis of the literature revealed that excellent clinical efficacy of *E. longifolia* in managing male sexual health is due to its ability to improve hardness and erection of penis, enhance sexual libido, improve male fertility, and escalate testosterone levels[25-28].

In recent years, Udani and co-workers conducted a double-blind and placebo-controlled trial on old males (40–65 years ages) complaining severe erectile dysfunction[26]. The outcomes measured in this study included sexual pleasure, penile erection, erectile hardness, and sexual performance. A remarkable improvement in frequency of sexual intercourse, penile erection and hardness, and overall sexual well-being was observed in males treated with *E. longifolia*. The improved sexual health in males supplemented with *E. longifolia* was also explored by Tambi and co-workers[25]. They observed profound improvement in the sexual well-being performance including penile hardness score, sexual intercourse performance, and erectile function[25].

In another clinical trial, Tambi *et al*. conducted a placebo-controlled trial on 350 young patients having a history of infertility. They prescribed a daily dose of 200 mg of soluble extract of *E. longifolia* to all patients[22]. The therapeutic significance of *E. longifolia* in improving male fertility was evaluated by analyzing concentration of sperms, semen volume, and sperm motility in accordance to World Health Organization (WHO) guidelines (WHO 1999). Male fertility improving efficacy of *E. longifolia* was also demonstrated by Ismail *et al*[29]. They conducted randomized placebo-controlled trial on 109 Malay males (ages of 30–55 years) according to the Good Clinical Practice (ICH-6) guidelines and Declaration of Helsinki. A daily dose of 300 mg of *E. longifolia* was given to all patients. A profound improvement in sexual libido and male fertility was observed[29].

Despite of its promising efficacy of improving erectile dysfunction, sexual libido, and male fertility, supplementation of *E. longifolia* has also shown remarkable proficiency to enhance testosterone levels in the body[30,31]. Talbott *et al*. conducted a clinical trial on 63 subjects by supplementing them with daily dose of 200 mg of standardized hot-water extract of *E. longifolia* for 4 weeks period[5]. As a result, they noticed noticeable improvements in testosterone levels in *E. longifolia*-treated subjects with subsequent enhancement in sexual activities and well-being[5]. These results were later supported by Henkel *et al*[28]. They conducted a clinical trial by supplementing 13 subjects (ages of 57–72 years) 400 mg of *E. longifolia* daily. They observed a significant increase in total free and bound testosterone concentration in all the patients. The increased levels of male sex hormone, in the later phase, produced significant improvement in sexual performance and male sexual libido[28].

3.3. Anticancer and anti-proliferative activities of E. longifolia

Numerous studies evidenced that the aqueous, methanolic, ethanolic or butanolic extract of *E. longifolia* and various bioactive compounds isolated from this medicinal plant have shown promising anti-proliferative and cytotoxic effects against various human cancer cell lines including liver carcinoma (HepG2), gastric carcinoma (MGC-803 and BGC-823 cells), intestinal cancers (HT-29 and LOVO cells), malignant melanoma (HM3KO), cervical carcinoma (Hela), human ovarian carcinoma (CaOV-3 and A-2780), breast cancers (MCF-7), and lung carcinoma (A-549 cells)[9].

Eurycomanone, one of the most bioactive medicinal compounds isolated from the extract of *E. longifolia*, has shown strong anticancer efficacy against various types of human cancers. Wong *et al*. evaluated the anti-proliferative and anticancer efficacy of the purified eurycomanone on the expression of selected genes

of human lung adenocarcinoma (A549 cells) at a concentration range of 5–20 μg/mL[32]. Eurycomanone significantly inhibited the proliferation of human A549 lung adenocarcinoma cells in a dose-dependent manner with lowest cell growth observed at 20 μg/mL[32]. Mahfudh and co-workers evaluated the cytotoxicity of eurycomanone against Hela cells using methylene blue staining assay, Hoechst 33258 nuclear staining, flow cytometry and TUNEL assay[33]. They further investigated the mechanism of cytotoxicity of eurycomanone by examining the protein expression of p53, Bax and Bcl-2 using Western blotting and immunostaining assays[33]. Eurycomanone showed selective cytotoxicity against various cancerous cell lines (MCF-7, HeLa, CaOv-3, HM3KO, and HepG2) but was least toxic against normal human cells (MDBK and Vero cells)[33]. The cytotoxicity of eurycomanone is mainly attributed to its ability to induce apoptosis in cancerous cells by inducing chromatin condensation, appearance of apoptotic bodies, and DNA fragmentation in cancerous cells treated with this potent medicinal compound[33].

Methanolic extract of *E. longifolia* has also been investigated against human leukemia cells (K-562)[34]. In this study, a wide range of *in vitro* experiments were performed including cell viability, clonogenic assay, annexin V-FITC/PI assay, Hoechst 33342 staining, cell cycle, and RT^2 profiler™ PCR array. The anticancer potential of *E. longifolia* was also evaluated by measuring the tumor volume, inhibition of tumor growth and histological examination using Balb/C nude mice. Their results showed a significant anti-proliferative and growth inhibition activity against K-562 cells treated with various fractions of *E. longifolia*. Potent cytotoxicity and anti-proliferative effects were observed after 48 h of treatment with half inhibitory concentration (IC_{50}) value of 1 963 and 661 mg/mL, respectively[34]. In this study, authors have also observed a dose- and time-dependent cytotoxicity of K-562 cells at different concentrations of *E. longifolia* at various time points. They further explored the anti-proliferative and cytotoxicity mechanisms of *E. longifolia*. By evaluating the biochemical parameters of early apoptosis (Annexin-V positive) and late apoptosis/necrosis (Annexin-V/PI positive), they suggested that treatment of K-562 cells with various fractions of *E. longifolia* caused induction of apoptosis in a dose- and time-dependent manner. Nuclear changes such as chromatin condensation and DNA fragmentation which are the hallmark features of apoptosis were also identified in K-562 cells treated with *E. longifolia* (Hoechst 33342 staining). A promising anti-leukemic potential of *E. longifolia* was also confirmed by an *in vivo* animal study that showed a remarkable decrease in the tumor volume, number of viable tumor cells, increased numbers of apoptotic cells and the necrotic cells in the *E. longifolia* (TAF273)-treated mice compared to the control groups. Further analysis of histological micrographs using Image J software revealed a significantly higher percentage of necrosis in *E. longifolia* (TAF273)-treated group compared to the control group. The mean count of apoptotic cells were 2 566 and 103 611 in the control and TAF273 groups, respectively. These findings suggested that *E. longifolia* exhibits a strong anti-leukemic potential[34]. *E. longifolia* has also been used in combination with *Dipterocarpus obtusifolius* and *Tamilnadia uliginosa* for the treatment of various types of human cancers[35].

The standardized quassinoid extract of *E. longifolia* (SQ40) was also investigated for the treatment of human prostate cancer[36]. SQ40 is an extract that contains 40% (w/w) of quassinoids was tested for anti-proliferative and anticancer activities against the human prostate cancer using a series of *in vitro* and *in vivo* experiments. The cell viability analysis revealed that SQ40 showed a strong dose-dependent cytotoxicity against LNCaP cells (human prostate cancer cell line); however, it showed no cytotoxicity against human normal prostate (RWPE-1) and liver (WRL-68) cells[36]. The concentrations of SQ40 that cause maximal half inhibitory effects (IC50) against RWPE-1, WRL-68 and LNCaP cell lines were 59.26, 27.69, and 5.97 μg/mL, which indicated that SQ40 exhibits potent cytotoxicity against human prostate cancer. The analysis of growth kinetics of LNCaP, RWPE-1 and WRL-68 cells using impedance-based cell sensing measurement system further validated that QS40 showed cytostatic effects at lower concentrations (2.5–10 μg/mL) and cytotoxic response at higher concentrations (20–80 μg/mL). A significant dose-dependent down-regulation of cell cycle regulatory proteins such as CDK4, CDK2, Cyclin D1 and Cyclin D3 and subsequent up-regulation of cell cycle inhibitory protein ($p21^{Waf1/Kip1}$) in SQ40-treated LNCaP cells further validated the anti-proliferative and cytotoxic mechanisms of this quassinoid extract against human prostate cancer[36]. The anticancer activity of SQ40 was also evidenced using the *in vivo* LNCaP tumor xenograft growth in nude mice. An intra-peritoneal administration of SQ40 for a period of 6 weeks in prostate cancer induced nude mice showed a significant dose-dependent decrease in the tumor volume compared to the control groups. These findings evidenced that *E. longifolia* exhibits strong anticancer potential against human prostate cancer[36]. The potency of quassinoid extract of *E. longifolia* has also been demonstrated by other researchers[37]. They suggested that the quassinoids from the leave extract of *E. longifolia* exhibits promising potential against A-549 (human lung cancer cells) and other human cancers[37].

Several other medicinal compounds of *E. longifolia* including eurycomanol, 13-β, 21-dihydroxyeurycomanone, 14-hydroxyglaucarubol, eurycomalactone, eurycomadilactone, 5-iso-eurycomadilactone, 13-epi-eurycomadilactone, longilactone, 6-dehyroxylongilactone, canthin-6-one, 9-methoxycanthin-6-one, canthin-6-one 9-*O*-β-glucopyranoside, 14,15 β-dihydroxyklaineanone, pasakbumin B, and pasakbumin C have also shown strong anti-proliferative and anticancer activities against a wide variety of human cancers *in vitro* and *in vivo*[38,39].

The prime mode of cytotoxicity of *E. longifolia* and its medicinally active compounds in cancerous cells is the induction of apoptosis (programmed cell death) via the up-regulation of the expression of p53 (a tumor suppressor protein) and pro-apoptotic protein (Bax), and the down-regulation of the expression of anti-apoptotic protein (Bcl-2)[33,40]. However, other studies suggested that activation of caspases (apoptotic signaling cascades)[41] and/or inhibition of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) have also been identified as the important molecular targets of *E. longifolia* and its medicinal compounds to provoke anti-proliferative and anticancer activities[42].

3.4. Antibacterial activity of E. longifolia

Being a well-known traditional herbal medicine, plethora of *in vitro*, *in vivo*, and human clinical studies have demonstrated a strong

antibacterial efficacy of *E. longifolia* and its bioactive constituents. Phytochemical screening of various types of extracts (methanolic, ethyl acetate, and *n*-butanolic) from different parts (roots, stem, and leaves) of *E. longifolia* displayed a dose-dependent antibacterial response against a wide spectrum of Gram positive and Gram negative bacteria. Comparative analysis revealed that the root extract of *E. longifolia* exhibited highest antimicrobial efficacy in comparison with the other plant parts.

The antibacterial activity of *E. longifolia* has been evaluated in the form of crude extracts, standardized extracts, and/or various medicinal compounds isolated or extracted from the standardized extracts. Farouk and Benafri evaluated the antibacterial activity of alcoholic and water extracts from the roots, stem, and leaves of *E. longifolia* against four pathogenic gram negative (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus feacalis*, and *Micrococcus leteus*) and four gram positive microorganisms (*Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, and *Serratia marcescens*) using the agar well diffusion method[3]. The antibacterial potency of *E. longifolia* was evaluated in comparison with reference standard antibiotics such as 5 mg/mL tetracycline and 5 mg/mL chloranphenicol. The diameter of inhibition zones were measured after 24 h of incubation. Farouk and Benafri identified that alcoholic root extracts did not show antibacterial activity against all bacterial strains; however, the extracts from the stem and leaves showed significant antibacterial activity against Gram positive bacteria and moderate activity against Gram negative bacteria except *Escherichia coli* and *Salmonella typhi*. The aqueous leaf extracts were active against *Bacillus subtilis* and *Serratia marcescens*. They concluded that comparatively the leaf extracts showed stronger antibacterial activity than the stem extracts[3].

In another study conducted by Khanam *et al*., a dose-dependent antibacterial activity was observed from the root and stem extracts of *E. longifolia*[43]. In this study, various extracts of methanol, ethyl acetate, acetone, chloroform, and petroleum ether extracts from the stem and root parts of the plant were tested. Resulting data identified that the extract from the stem displayed strongest antibacterial activity against all the strains of bacteria than the root extracts. The highest antibacterial activity [zone inhibition of (17.00±6.56) mm] was observed against *Staphylococcus aureus* from methanolic extract of stem at a dose of 200 μg/μL. These results indicated that *E. longifolia* extracts were more active against Gram positive bacteria than the Gram negative bacteria[43]. These results are also in agreement with other studies[44,45]. Choo *et al*. tested *n*-hexan, chloroform, *n*-butanol, and aqueous extracts derived from a methanolic-soluble residue of *E. longifolia* against various pathogenic bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*)[44]. Results showed that *n*-hexan and chloroform extracts showed highest antibacterial activity against *Bacillus cereus* and no activity against Gram negative bacteria. Bioactivity-guided fractionation of chloroform extract resulted in isolation of 9-methoxycanthin-6-one, eurycomalactone, and 14,15 β-dihydroxyklaineanone. Among these three qussinoids, 9-methoxycanthin-6-one displayed strongest antibacterial activity (MIC=9 μg/mL) against *Bacillus cereus*; however, other two quassinoids did not display antibacterial activity against *Bacillus cereus*[44].

Similarly, Łos *et al*. have also identified that *E. longifolia* extracts exhibited higher antibacterial activity against Gram positive microorganisms than the Gram negative bacteria[45]. They investigated the antibacterial efficacy of aqueous and methanolic extracts of *E. longifolia* against various strains of Gram positive (*Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis*, and *Micrococcus luteus*) and Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Proteus mirabilis*). Results showed that methanolic extracts showed higher antibacterial activity compared to the aqueous extracts against Gram positive bacteria compared to the Gram negative bacteria at 1 000–2 000 mg/L[45].

Some other studies revealed that crude extract of *E. longifolia* exhibited strongest antibacterial activity against Gram negative bacteria than the Gram positive bacteria[46,47]. Faisal and co-workers tested ethanol extracts from the roots of *E. longifolia* against various Gram positive and Gram negative strains of bacteria using agar disc diffusion and broth microdilution test[46]. Though, a dose-dependent increase in antibacterial activity was observed in both Gram negative and Gram positive bacteria; however, greater inhibition zones were observed in *Bacillus cereus* (11.76 mm) and *Salmonella typhi* (14.33 mm) at a dose of 150 mg/mL. However, *Escherichia coli* and *Pseudomonas aeruginosa* did not show any inhibition by the ethanol extracts[46].

Some studies suggested a weaker or no antibacterial activity of *E. longifolia* against Gram positive and Gram negative bacteria[48,49]. Tzar and co-workers investigated the antibacterial potential of aqueous extract of *E. longifolia* against various pathogenic bacteria such as *Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus*, group-1 β lactamase-producing *Pseudomonas aeruginosa*, extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*, *Salmonella typhi*, and multidrug-resistant *Acinetobacter baumanii*) at various doses of 50, 25, 12.5, 6.25, and 3.125 mg/mL[48]. Results indicated that aqueous extract did not showed antibacterial activity at all doses[48]. On the other hand, Jaafar *et al*. tested antibacterial activity of methanolic extract of *E. longifolia*, alone or in combination, with *Alpinia galanga*, and *Syzygium aromaticum* against *Escherichia coli*[49]. Results showed that *E. longifolia* showed weaker antibacterial activity when used alone and moderate activity when used as mixture with *Alpinia galanga* and *Syzygium aromaticum* methanolic extracts. These results clearly evidenced a mixed-type antibacterial efficacy of *E. longifolia*[49].

3.5. Antiprotozoal activity of E. longifolia

A plethora of *in vitro*, *in vivo* and human clinical studies demonstrated an efficient antiprotozoal activity of *E. longifolia* in the form of crude extracts or isolated bioactive medicinal components against a variety of protozoa including *Blastocystis* species, *Plasmodium falciparum* strains, *Plasmodium yoelii*, and *Schistosoma japonicum*[50,51].

Blastocystis species are classified as a Stramenopile and genetically diverse organisms that inhabit the intestinal tract of a large range of host species including humans[52]. Among a range of

chemotherapeutic agents, metronidazole has become the mainstay in treating *Blastocystis* induced diseases. However, potential side effects and susceptibility of developing resistance against these pharmacological agents reasoned to search and explore alternative antiprotozoal therapies. Girish *et al*. tested antiprotozoal activity of aqueous and ethyl acetate extracts of *E. longifolia* against *Blastocystis* species derived from human stool samples at two different concentrations of 0.1 and 1.0 mg/mL[51]. They investigated growth profile and viability of *Blastocystis* species after 72 h of treatment with herbal extracts. Amongst all of the tested herbal extracts, *E. longifolia* displayed the highest inhibition in growth profile and viability which was achieved at 1.0 mg/mL. Moreover, comparative analysis between aqueous and ethyl acetate fractions revealed that ethyl acetate-based extract showed significantly higher (94.9%–95.1%) antiprotozoal activity against *Blastocystis* species at 1.0 mg/mL. The antiprotozoal activity of *E. longifolia* was comparable with metronidazole (95.8%)[51].

Malaria is a mosquito-borne life-threatening blood disease affecting humans and other animals caused by parasitic protozoa (*Plasmodium* genus). The typical symptoms associated with malaria include consistent tiredness, on and off fever, nausea, vomiting, and headaches. Among various pharmacological interventions, artemisinin is one of the commonly employed anti-malarial agents used in the treatment of malaria. It is mainly derived from a medicinal herb, *Artemisia annua*. It has displayed promising efficacy against various strains of protozoa; however, some potential side effects (including neurotoxicity) associated with its monotherapy reduced its clinical applicability as antiprotozoal agents[53]. Mohd Ridzuan *et al*. tested anti-malarial efficacy of standardized root extract (TA164) of *E. longifolia*, alone and in combination with artemisinin[54]. They administered different doses to *Plasmodium yoelii*-infected mice via oral and/or subcutaneous routes. Monotherapy of TA164 resulted in a dose-dependent suppression of parasitemia in *Plasmodium yoelii*-infected mice and highest suppression (approximately 50%) was observed at 60 mg/kg body weight via oral administration. However, combination of TA164 (10 mg/kg body weight) with a fixed dose of artemisinin (1.7 mg/kg body weight) resulted in significantly higher suppression of parasitemia (approximately 63%)[54]. Moreover, an increased dose of TA164 (up to 60 mg mg/kg body weight) in conjunction with artemisinin (1.7 mg/kg body weight) resulted in progressive increase in suppression of parasitemia (approximately 80%). Similarly, subcutaneous administration of a combined therapy of TA164 and artemisinin has also resulted in higher suppression of parasitemia compared to the monotherapy of TA164 in infected mice. These findings suggested that combination of *E. longifolia* with artemisinin can significantly improve antiprotozoal and anti-malarial efficacy of artemisinin[54].

Bioactivity-guided fractionation revealed that not only crude extract but various isolated medicinal compounds have also shown moderate-to-potent antiprotozoal activity[55]. Successive fractionation of dried and powdered roots of *E. longifolia* with methanol, chloroform and *n*–butanol resulted in isolation of various medicinal compounds which were tested against *Plasmodium falciparum*. Results showed that some of the compounds exhibited moderate antiprotozoal activity; however, others showed strong anti-malarial activity. In another study, Kuo *et al*. demonstrated that amongst various medicinal compounds isolated from root extracts of *E. longifolia*, eurycomanone and pasakbumin B showed most potent anti-malarial activity; however, eurycomalactone, 14,15 β -dihydroxyklaineanone, and pasakbumin C exhibited moderate efficacy against *Plasmodium falciparum* strains[56].

Eurycomanone, one of the most bioactive medicinal compounds isolated from the root extract of *E. longifolia*, exhibited outstanding antiplasmodial activity. Chan and co-workers isolated four quassinoids including eurycomanone, 13,21-dihydroeurycomanone, 13α (21)-epoxyeurycomanone, eurycomalactone, and an alkaloid, 9-methoxycanthin-6-one from the root extracts of *E. longifolia*[57]. The antiprotozoal activity of these compounds was tested against chloroquine-resistant Gombak A isolate of *Plasmodium falciparum*. Results showed that *n*–butanol root extracts of *E. longifolia* exhibited strongest anti-malarial activity (0.34 μg/mL) than its diethyl ether extract (1.50 μg/mL) and both of these extracts were more potent than chloroquine diphosphate (2.50 μg/mL). Bioactivity guided fractionation of *n*–butanol extract revealed that amongst three of the isolated quassinoids, eurycomanone displayed strongest anti-malarial activity that was approximately 8.66 times more potent than chloroquine diphosphate against the Gombak A isolate compared to 13,21-dihydroeurycomanone (6.83 times potent), and 13,21-epoxyeurycomanone (4.58 times potent)[57].

These results were further validated by Chan *et al*.[58] in which they demonstrated that amongst several quassinoids isolated from root extract of *E. longifolia*, eurycomanone displayed strongest inhibition of chloroquine-resistant Gombak A isolate of *Plasmodium falciparum*. They also suggested that the anti-malarial efficacy of eurycomanone can be modulated by undergoing acylation reaction. Comparative analysis revealed that monoacylated eurycomanones showed comparable activity to eurycomanone; however, diacylated eurycomanones displayed lower antiplasmodial activity against the Gombak A isolates[58].

3.6. Antifungal activity of E. longifolia

Though, some studies provided substantial evidence regarding the antifungal activity of the crude extract or medicinal compounds isolated from various parts of *E. longifolia*; however, there is no consensus that which part(s) and type(s) of extract showed the strongest antifungal activity.

Khanam *et al*. conducted a phytochemical screening of various extracts of methanol, ethyl acetate, acetone, chloroform, and petroleum ether extracts from the stem and root parts of *E. longifolia* against *Aspergillus niger*[43]. Comparative analysis revealed that amongst various parts of *E. longifolia* and a range of extracts, only the ethyl acetate extract from the stem part of *E. longifolia* exhibited antifungal activity. They identified a dose-dependent antifungal activity of ethyl acetate stem extract with highest zone inhibition [(11.00±1.73) mm] was achieved at 200 μg/μL than a slightly lower antifungal activity [(9.0±1.0) mm] at 100 μg/μL[43]. Another study conducted by Choo *et al*. suggested that amongst various extracts of *E. longifolia*, hexane and chloroform extracts exhibited moderate antifungal activity against *Candida albicans*[44].

A phytochemical screening of aqueous extracts from the roots of *E. longifolia* was also conducted by Tzar and co-investigators against *Candida albican*, *Candida glabrata*, and *Candida krusei* at various concentrations (10, 5, 2.5, 1.25 and 0.625 mg/mL)[48]. However, the resulting data indicated no activity of aqueous extract against *Candida albicans* species at any concentration which suggested that *E. longifolia* did not show antifungal activity at 50 mg/mL or lower concentrations. These results were also validated by Jaafar *et al*. that *E. longifolia* extract did not show antifungal activity against *Saccharomyces cerevisiae* when used alone[49]. However, its combination with *Alpinia galanga* and *Syzygium aromaticum* at various ratios of 1:1:1 or 1:2:2 showed promising antifungal activity of (22.4±3.43) mm and (22.6±3.65) mm zone of inhibition, respectively. These findings indicated that ethyl acetate, hexane and chloroform extract of *E. longifolia* exhibited moderate antifungal activity which evidenced that some parts of *E. longifolia* should be further screened for antifungal activity[49].

3.7. Anti-inflammatory activity of E. longifolia

The anti-inflammatory potential of *E. longifolia* and its bioactive phytochemicals has also been established by many researchers[4,8]. Tran and co-workers explored the anti-inflammatory mechanism of *E. longifolia* and its medicinally active components by bioactivity guided fractionation[8]. They suggested that among various medicinal compounds extracted/isolated from the root extract of *E. longifolia*, eurycomalactone, 13,21-dehydroeurycomanone, and 14,15 β-dihydroklaieanone showed the most potent anti-inflammatory activity. The anti-inflammatory activity of these phytochemicals is due to their strong efficacy to inhibit NF-κB[8]. The anti-inflammatory and antioxidant activity of crude extract of *E. longifolia* was also demonstrated by Varghese *et al*[4]. They suggested that *E. longifolia* exhibits a dose-dependent anti-inflammatory and antioxidant activities.

3.8. Anxiolytic effects of E. longifolia

Though, a paucity of data is available for the anxiolytic effects of *E. longifolia*; however, some studies have explored the anti-anxiety efficacy of various fractions of *E. longifolia*. Ang and Cheang conducted various behavioral tests in animals supplemented with *E. longifolia* and the results were compared with control groups[59]. They demonstrated that *E. longifolia* supplementation significantly reduced the anxiolytic behavior of animals and the results are consistent with diazepam treated animals[59]. Consistently, Talbott *et al*. conducted a human clinical trial by supplementing 63 subjects with aqueous extract of *E. longifolia* and screened them for stress hormones and mood states[5]. They noticed a significant improvement in stress hormone and mood state profiles in individuals supplemented with *E. longifolia*[5].

3.9. Anti-diabetic effects of E. longifolia

The prevalence of type-2 diabetes is continuously escalating due to increasing insulin resistance. Lowering of lipids accumulation in adipocytes is one of the important ways to improve insulin sensitivity as well as enhance glucose uptake. Recently, Lahrita *et al*. explored the anti-diabetic efficacy of root extract of *E. longifolia*[6]. They explored that the anti-diabetic efficacy of *E. longifolia* was attributed to increased insulin sensitivity and lowered accumulation of lipids in adipocytes. The supplementation of *E. longifolia* resulted in increased glucose uptake by more than 200% and significant suppression of lipid accumulation at a dose of 50 μg/mL[6]. Moreover, they have also observed no adverse effects of *E. longifolia* on adipocytes at different doses.

3.10. Miscellaneous effects of E. longifolia

Researchers have also recognized various miscellaneous effects of *E. longifolia* including ergogenic effects[60], hormonal effects[61], muscular effects[62], and anti-ulcer effects[7]. The supplementation of standardized extract of *E. longifolia* is associated with profound anti-estrogenic effects, normalizing irregular estrous cycles, and reduced follicular damage associated with chronic testosterone administration[60,61].

4. Conclusions

E. longifolia and its phytochemicals exhibit a wide dynamic range of biomedical applications including anti-osteoporotic, anticancer, anti-proliferative, anti-malarial, antimicrobial, antioxidant, aphrodisiac, anti-inflammatory, anxiolytic, anti-diabetic, anti-rheumatism and anti-ulcer properties. A critical analysis of the reviewed literature revealed that the promising anti-osteoporotic and bone resorption reducing efficacy of *E. longifolia* is attributed to its excellent ability to up-regulate the male and female sex hormones (testosterone, androgens, and estrogens). A number of animal and human clinical studies revealed a remarkable potential of *E. longifolia* and its constituents in improving male and female sexual, which is also attributed to higher testosterone levels following administration of *E. longifolia*. The anticancer efficacy of *E. longifolia* is due to the induction of apoptosis (programmed cell death) via the up-regulation of the expression of p53 (tumor suppressor protein) and pro-apoptotic protein (Bax) and down-regulation of the expression of anti-apoptotic protein (Bcl-2). Comparative analysis revealed that the root extract of *E. longifolia* exhibited highest antimicrobial efficacy in comparison with the other plant parts. Bioactivity-guided fractionation identified that among all of the medicinal compounds isolated/extracted from different parts of *E. longifolia*, eurycomanone displayed strongest antibacterial, antiprotozoal and antifungal activities.

5. Future prospects

Though plentiful reports have explored the pharmacological significance and therapeutic viability of *E. longifolia* and its bioactive compounds for the treatment of a variety of diseases, much has yet to be executed and learned. To further explore its biomedical applications, we have noticed substantial gaps in research which include but not limited to; 1) lack of integration of medicinal chemistry, biology, pharmacology and toxicology which could be a promising way to further explore the anticancer specificity of *E.*

longifolia and its compounds against each type of disease, 2) lack of sufficient attention on pharmacologically active constituents and their determination, identification, standardization and structural manipulation for future developments of new structural and functional analogs, 3) lack of research on individual translational mechanism of most active medicinal compounds against various types of diseases, 4) lack of comparative biomedical potential of various active constituents of *E. longifolia*, 5) lack of sufficient *in vivo* and human clinical studies to further explore demographic specificity and variations, and 6) lack of sufficient safety profile and toxicity data to conduct human clinical trials.

Conflict of interest statement

The authors declare they have no conflict of interest.

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