Eurycoma longifolia Jack (Simarubaceae); Advances in Its Medicinal Potentials

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ABSTRACT

Eurycoma longifolia Jack is a tall slender shrub-tree which is well-reputed among the natives of South East Asia for its potent aphrodisiac effect. Its root extracts have shown important biological activities such as antitumor, antimalarial, antibacterial, anti-diabetic, anti-hypertensive, Osteoprotective, and ergogenic which are mainly attributed to quassinoids. Commercially it is available in the form of drinks (along with other herbs), capsules, or loose powders. Based on available online databases it was realized that in spite of numerous reports on medicinal properties of *E. longifolia*, a review of recent developments regarding phytopharmacology, safety and toxicology, pharmacokinetics, and clinical applications was lacking. Therefore, this concise yet informative piece of work was prepared by pulling together trustworthy information from all the accessible published and unpublished scientific resources to serve as a reliable source of reference for future investigations.

Keywords: Malaysian Ginseng, Eurycomanone, Tongkat Ali, Quassinoids, Ergogenic, Pasak Bumi.

INTRODUCTION

Traditional/Herbal Medicine is an ancient system of health which has gained great popularity in recent years. In Malaysia alone about USD 500mln is being spent annually on this type of health system.[1,2] To date, over 200 herbal supplements with *Eurycoma longifolia* as their principal ingredient are available in the form of beverages, capsules, and loose powders in the market which are produced mainly from its root extracts.[3] *Eurycoma longifolia* Jack (family; *Simarubaceae*) is a slender evergreen tree which grows in wild at an altitude of about 500 meters above sea level in the forests of Malaysia, Borneo, southern Myanmar, Cambodia, Vietnam, Laos, Thailand, Indonesia, and Philippines. It attains a height of up to 10 meters (Max. 15 meters) with few upright branches capped by umbrella-like rosette of leaves. The flowers are hermaphrodite with very fine pubescent whereas the fruits are hard, oblong in shape, yellowish brown when young and brownish red when ripe. In Malaysia, it is commonly known as "Tongkat Ali" and/or "Malaysian Ginseng" which are assigned mainly due to its aphrodisiac properties.[4–6] The plant is usually cultivated by direct sowing of the germinated seedlings in well-drained soil with sufficient organic matter. An annual rainfall of 2000–3000mm and a temperature range of 25–30°C is said to be ideal for the growth. Generally, it is harvested 2 to 3 years following the cultivation but the exact harvesting age in not known.[7,8] Traditionally, *E. longifolia* is used to treat bleeding gums, fever, malaria, oedema, jaundice, arthritis, cachexia, rheumatism, dysentery, flatulence, indigestion, erectile dysfunction, wounds, syphilis, boils, lumbago, high blood pressure, and to relieve gastric ulcer. Moreover, the bark is useful as blood coagulant after childbirth while the leaves are found to be effective in relieving stomach ache.[3–5] Today, many of its medicinal potentials such as antitumor,[9] antimalarial and antipyretic,[10,11] aphrodisiac,[12] antimicrobial,[13] anti-hyperglycaemic,[14] antiinflammatory,[15] anti-schistosomal,[16] antifungal,[17] anti-toxoplasmic,[18] anti-hypertensive,[19] and Osteoprotective[20] have been uncovered through extensive scientific research.

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CHEMICAL CONSTITUENTS

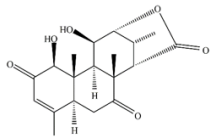
Active constituents

By and large, roots of *E. longifolia* are the powerhouse of this very herb which are home to large amounts of

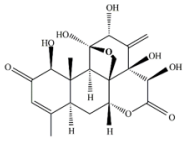
biologically active compounds such as Quassinoids (Scheme 1), Squalene- and Tirucallene-type Triterpenes, Canthin-6-one and *β*-carboline Alkaloids, and Biphenylneolignans, among which quassinoids are well distinguished due to their diverse bioactivity. The term quassinoid itself originates from the word "Quassin" named after a man called "Quassi" who used the barks from Simarubaceae plants to treat fever. Quassinoids are otherwise degraded triterpenes of bitter in nature which are classified based on the number of carbon atoms as C-18, C-19, C-20, C-22, and C-25.[21] Amongst the several quassinoids identified in *E. longifolia* extracts, Eurycomanone (EN) is said to be the major one with strong antiproliferative and steroidogenesis effects.[9–22]

Proteins, minerals and amino acids

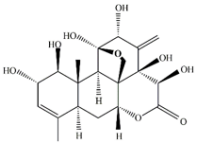
Elemental contents determination of roots of *E. longifolia* by Majid and coworkers[26] revealed the presence of certain elements such as Al, Br, Ca, Ce, Cl, Cs, Cr, Fe, K, La, Mg, Mn, Na, Rb, Sb, Sc, and Zn along with other toxic elements. Subsequent evaluation of aqueous root extracts of *E. longifolia* collected from two climatically and geographically different locations in Malaysia; Perak and Pahang for proteins, minerals, and amino acids showed dissimilarity in the content of these substances. For instance, Tongkat Ali Pahang displayed higher amount of protein matters almost twice as that of Tongkat Ali Perak. Similarly, amino acid



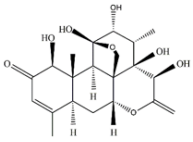
Eurycomalactone[23]



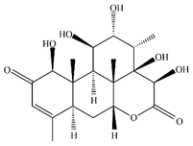
Eurycomanone[24]



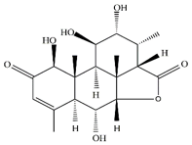
Eurycomanol[24]



13α,21-Dihydroeurycomanone[25]



14,15β-Dihydroxyklaineanone[25]



Longilactone[25]

Scheme 1: Array of some biologically active quassinoids from *Eurycoma longifolia* Jack.

composition determination of the extracts by HPLC unveiled the presence of Lysine and Arginine in Pahang, but not Alanine. Additionally, presence of other nutrient minerals such as Ni, Cu, Co, Ba, Li, In, Sr, Ga, and U detected by Inductively Coupled Plasma Mass Spectrometry were also reported.[27]

BIOLOGICAL ACTIVITIES

Aphrodisiac

E. longifolia has gained tremendous popularity among men in Malaysian peninsula for its potent aphrodisiac action. Based on traditional claims, it has the capacity to augment low testosterone levels in aged men affected with infertility and/or impotence. This was in fact found to be true as the outcomes of recent investigations disclosed its strong steroidogenesis and spermatogenesis in rodents.[28] Elsewhere, its standardized methanol extract reversed the infertility induced by *Andrographis paniculata* through significant boost in testosterone synthesis both in plasma and testes. The resultant testosterone then stimulated spermatogenesis by binding to Androgen Binding Protein (ABP).[29–31] Later, Low and coworkers[12] realized that the action of quassinoids on the generation of testosterone as well as sperm was most likely mediated through hypothalamic-pituitary-gonadal axis.

It was believed that 25mg/kg of F2 fraction obtained from the elution of methanol extract with water, reduced estrogen secretion but rather stimulated the secretion of high amount of LH and FSH. This in turn resulted in enhanced plasma testosterone levels as well as spermatogenesis. Recent advancements suggest the essential role of Eurycomanone (EN) in boosting steroidogenesis process in a dose-dependent manner possibly through inhibiting aromatase and phosphodieasterase enzymes (Figure 1).[22]

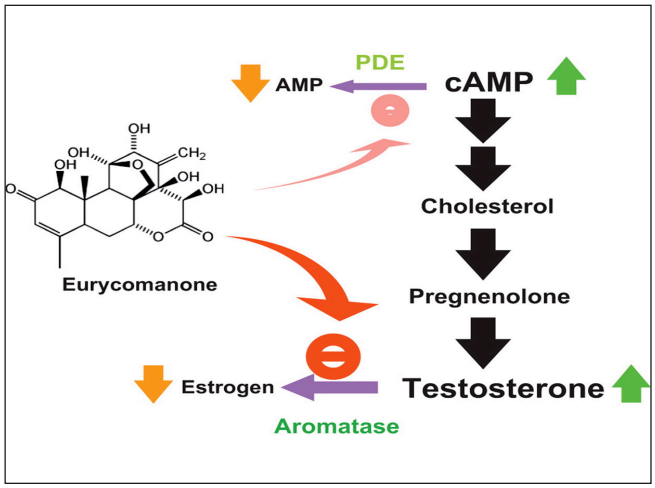


Figure 1: Possible mechanism of action of Eurycomanone (EN) on the stimulation of steroidogenesis.

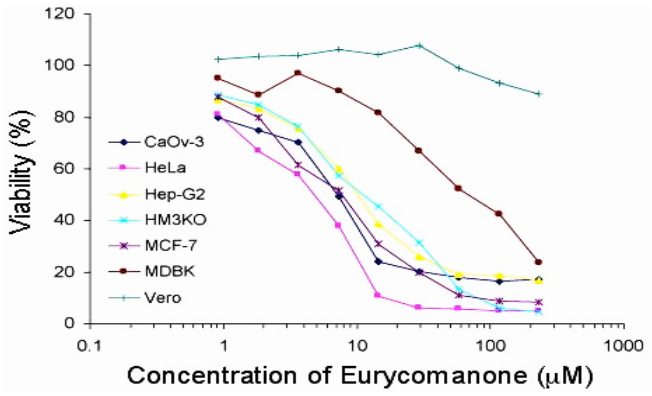


Figure 2: Anti-proliferative effect of Eurycomanone (EN) on normal and cancer cell lines.

Anti-estrogenic activity

Treatment with oestrogen may be associated with deleterious effects on fertility and libido in men.[32] Abdul Wahab *et al.*[33] suggested that the negative impact of estrogen on spermatogenic cells was reversible when treated with *E. longifolia.* Similarly, two quassinoids; 13a, 21-Dihydroeurycomanone (ED) and EN exhibited potent (p<0.001) anti-uterotrophic and antiestrogenic actions in immature and mature female rats by effectively countering the testosterone- and 17a-Ethynyl Estradiol-induced ovarian cystic follicles and irregular oestrous cycles, respectively.[34,35] These actions by *E. longifolia* are probably due to its ability to suppress enzyme aromatase which is responsible for the conversion of testosterone into oestrogen, hence showing anti-oestrogenic effect.

Antitumor

Quassinoids and alkaloids isolated from Simarubaceae plants are known for their robust cytotoxic activity against various cancer cell lines.[36,37] Likewise, antiproliferative activity of *E. longifolia* is attributed to two quassinoids; EN and Longilactone both of which act by inducing apoptosis in tumour cells via diverse pathways. Initially, EN was believed to induce apoptotic cell death via down-regulation of *Bcl-2* protein (anti-apoptotic) and cleavage of caspase-7 and PARP-1.[9] However, later apoptotic cell death induced by EN was found to have been triggered by up-regulation of *p53* and *Bax* (pro-apoptotic) proteins and rather suppression of *Bcl-2* protein in several cancer cell lines (Figure 2).[9] Nevertheless, subsequent evaluations indicated substantial reduction in the expression of certain tumour markers such as Heterogeneous Nuclear Ribonucleoprotein-A2/B1 (hnRNP- A2/B1), p53-tumor suppressor protein and other genes like Endoplasmic Reticulum protein-28 (ERp28), Prohibitin (PHB), and Annexin-1 (ANX-1) in A549 lung cancer cell lines upon administration of EN.[39] Quite the reverse, Longilactone exerted its antitumor action via the activation of caspase-7, caspase-8, and Poly(ADP-ribose) polymerase but not caspase-9, while leaving the levels of both *Bcl-2* and *Bax* proteins unaltered.[40]

Antioxidant & anti-angiogenic

Aging process in human is usually associated with the amount of oxidative stress that is generated by certain endogenous (oxidative burst) and exogenous (cigarette smoking) factors which bring about an imbalance in body's defense mechanism.[41] Human body has developed its own protective mechanism to overcome these stresses. However consumption of natural products with established antioxidant properties could further enhance the process of detoxification in the body. Investigation of standard ethanol root extract of *E. longifolia* (TAF-273) by Purwantiningsih and coworkers[42] for free radical scavenging indicated the presence of 0.253±0.016μg QE/mg d.w. and 17.142±1.102μg GAE/mg d.w. of total flavonoids and of total phenolics, respectively. Moreover, DPPH assay of TAF-273 exhibited an EC_{50} of 754μg/mL (p<0.01).

Dietary supplements containing herbal plants play vital role in the prevention of tumor in human body by means of their antioxidant and anti-angiogenic properties.[43] One good example is *E. longifolia.* Three of its root fractions; TAF-273, F3, and F4 were able to suppress angiogenesis both *in vitro* (rat aortic ring tissues) and *in vivo* (male BALB/c nude mice) 16 days following the treatment (p<0.012).[44]

Antimalarial, toxoplasmicidal, & anti-schistosomal

Quassinoids along with canthine alkaloids isolated from roots of *E. longifolia* are known to possess potent antimalarial activity against various strains of *Plasmodium falciparum* parasites including those resistant to Chloroquine such as Gombak A.[10,11] It is claimed that a combination of standard antimalarial agent and antimalarial herbal extract is beneficial in combating the infection caused by malaria parasite. For instance, co-administration of TAF-164 and Artemisinin brought about substantial suppression of parasitemia by up to 80% well higher than that displayed by a single drug administration ($p<0.05$).[45] It is anticipated that the antimalarial activity presented by quassinoids is probably due to their ability to hinder protein synthesis in *plasmodium falciparum*.[46] In a separate study, Kavitha *et al.*[18, 47] reported toxoplasmicidal action by *E. longifolia* extracts against *Toxoplasma gondii*. Their findings suggested the inhibition of toxoplasmosis 3 hours following the treatment. This was assumed to be mediated through alterations in the cell wall along with formation of invaginations followed by complete distortion of cells. Elsewhere, Longilactone, 11-dehydroklaineanone,and14,15β-dihydroxyklaineanone showed anti-schistosomal effect by successfully impeding the movement and egg laying capacity of *Schistosoma japonicum*.[16]

Antibacterial & anti-mycobacterial

In spite of accumulating large amounts of active constituents, root extracts of *E. longifolia* have failed to demonstrate desirable antibacterial or antifungal activities.[17] In contrast, leaves and stem extracts have shown relatively strong antibacterial action against both gram positive (*Bacillus subtilis, staphylococcus aureus, Enterococcus faecalis, Micrococcus leteus*) and gram negative bacteria (*Proteus vulgaris, Serretia marcescens*).[13] Moreover, various extracts of *E. longifolia* also displayed significant anti-mycobacterial effect by inhibiting *Mycobacterium smegmatis* growth with MIC values ranging from 800 to 3200μg/ml.[48]

Osteoprotective

Supplementation with testosterone is associated with the augmentation of minerals in the bone during the treatment of osteoporosis especially in men with low serum testosterone concentrations.[49] A similar effect was reported by Shuid *et al.*[20] for *E. longifolia* where 6 weeks administration of its aqueous extract in orchidectomized rats restored the depleted bone calcium levels but failed to show a meaningful rise in serum testosterone concentration. However, in their subsequent study treatment with 15mg/kg of aqueous extract (for 6 weeks) by oral gavage showed marked rise in both testosterone and osteoprotegerin levels in androgen-deficient orchidectomized rats. Meanwhile, C-terminal telopeptide of type I collagen (CTX) and Macrophage-Colony Stimulating Factor (M-CSF) genes expressions were down-regulated. Also, Receptor Activator of Nuclear Factor Kappa-B ligand (RANKL) and osteocalcin levels did not experience any noticeable change. Moreover, bone micro-CT assessment of undecalcified femora of the animals revealed ineffectiveness of aqueous extract of *E. longifolia* in restoring the Trabecular Thickness, Trabecular Volume, Trabecular Number, and Trabecular Separation.[51] However, its co-administration with testosterone in orchidectomized rats exhibited a significant development in the strain parameter in femoral bone.[52]

OTHER BIOLOGICAL ACTIVITIES

Effect on nitric oxide, cAMP, & cGMP

For erection to occur presence of certain physiologic mediators is essential which upon release induce vasodilatation followed by penile erection through increase in blood flow to penile tissues. Certain medicinal plants with known aphrodisiac effect like ginseng are reported to stimulate the release of Nitric Oxide (NO) in animals and bring about a state of relaxation.[53] According to a report by Basir[54] effect of *E. longifolia* extract (10mg/kg; i.p.) on NO levels in rats was unsatisfactory as it failed to cause significant elevation of NO levels following acute and chronic treatments. On the contrary, treatment with aqueous extract of *E. longifolia* markedly enhanced the levels of cGMP and cAMP for 30 and 60 minutes, respectively in rabbit corpus cavernosum (Azimahtol, 2001; unpublished data).

Anti-hypertensive & anti-hyperglycemic

As mentioned above, *E. longifolia* is traditionally used by natives in Malaysia to lower high blood pressure for centuries. Upon intravenous administration of 25–50mg/kg of aqueous extract immediate reduction in blood pressure well below the normal level for more than 30 min was noted in normotensive rats which was assumed to be of peripheral origin.[19] Elsewhere, findings of a preliminary investigation by Husen[14] suggested anti-hyperglycemic activity displayed by two aqueous extracts (TA-a & TA-b) of *E. longifolia* upon oral administration (150mg/kg) which was comparable to that of Glibenclamide at 10mg/kg.

Anxiolytic & Cognitive Enhancer

Along with other biological activities mentioned above, *E. longifolia* is said to alleviate anxiety and also enhance cognition in rodents. Anti-stress tests such as open-field, elevated plus-maze and anti-fighting tests conducted in anxious mice produced interesting outcomes. 300 mg/kg of its extracts (Butanol, Chloroform, Methanol, and Water) brought about major improvement in square crossings as well as reduction in immobility and number of fecal pellets in open field test 5 days following the treatment ($p<0.05$). While, the number of entries and time spent in open and closed arms were markedly increased and decreased, respectively, in elevated plus maze-test, the fighting episodes were substantially reduced in all treated groups as compared to control groups ($p<0.05$).[55] On the other hand, ethanol extract from its roots was able to significantly enhance cognition and glutamic acid levels in male Wistar rats which was perhaps mediated via high testosterone production.[56]

PHARMACOKINETICS

Bioavailability

Bioavailability studies are essential in order to define the appropriate dose and route of administration for the drug of choice. Findings of a study by Low and coworkers[57] suggested poor bioavailability of EN upon oral gavage with C_{max} and T_{max} of 0.33±0.03μg/ml and 4.40±0.98h, respectively. High first pass metabolism and/or low membrane permeability were thought to be the cause though. Subsequently, upon intravenous and oral administration of EN, Eurycomanol, 13α, 21-Epoxyeurycomanone (EP), and ED, only EP and EN displayed relatively longer biological life, better membrane permeability, and lower elimination rate.[58]

Drug-drug interactions

Those suffering from erectile dysfunction (ED) due to diabetes or even hypertension might consider consuming *E. longifolia* supplements in conjunction with conventional drugs for improving their sexual life. This may enhance or reduce the metabolism of either of the drugs due to drug-drug interactions. For instance, concurrent administration of *E. longifolia* extract (TAF-273) and Aminopyrine in normal and diabetic rats caused marked ($p<0.05$) enhancement in the metabolism of Aminopyrine in a dose dependent manner via activation of G-protein, Protein Kinases G, and A in the cAMP pathways and Protein Kinase C.[59,60] Likewise, Hussin[61] showed that co-administration of TAF-273 and Rosiglitazone considerably enhanced formaldehyde formation by inducing phase I metabolism of Rosiglitazone in hepatocytes of diabetic and normal rats.

HUMAN TRIALS

Aphrodisiac

Various preliminary human trials have recognized the effectiveness of aqueous extract from *E. longifolia* roots in improving the quality of life and well-being in men particularly those suffering from infertility.[62] More precisely, administration of twice daily of 100mg capsules containing *E. longifolia* in 75 patients suffering from partial infertility showed significant improvement in sperm concentration, motility, normal morphology, and sexual performance.[63] Next, 76 patients identified with hypogonadism and late hypogonadism displayed reduction in Ageing Male Symptoms ($p<0.0001$) and increase in testosterone concentrations by 46.8% following the treatment with 100mg capsule of *E. longifolia* twice daily for one month.[64] This effect by *E. longifolia* was believed to be mediated via the stimulation of Eurypeptides which activated CYP17 enzyme which in turn enhanced metabolism of pregnenolone and 17-hydroxypregnenolone to ultimately produce more testosterone.[65]

Ergogenic

Herbal beverages/drinks containing *E. longifolia* and other herbs such as tea, ginseng, and coffee are normally consumed by men in Malaysia assuming it can augment physical performance. High testosterone production is generally associated with increase in muscular mass and size.[66] It is also known that long term consumption of *E. longifolia* is associated with boost in testosterone level in the body. Hamzah and coworkers[67] claimed that 5 weeks daily administration of aqueous extract (100mg) in men considerably improved muscular size and strength through enhancing mean arm circumference ($p<0.011$) and lean body mass ($p<0.0012$). This was further reconfirmed by a relatively well-structured study by Henkel and coworkers[68] whose findings suggested that the administration of 400mg (twice daily) of aqueous extract for 5 consecutive weeks was associated with substantial elevation of total and free testosterone concentrations as well as augmentation in muscular strength in physically active senior males and females (52–72 years).

Anti-stress

Effect of various extracts of Tongkat Ali on anxiety and stress has been reported in anxious mice linking it to high Testosterone production. Outcomes of latest human trials conducted in 63 moderately stressed subjects (men and women) were indicative of significant anti-stress effect by *E. longifolia* ($p<0.05$). Treatment with 200mg/day of its standardized hot-water root extract for four weeks exhibited meaningful improvements in parameters such as tension, confusion and anger with no effect on vigor, fatigue and depression. Also, stress hormone profile assessment showed increased Testosterone and reduced Cortisol levels which further highlighted the role of Testosterone in stress reduction.[69]

Drug-drug interactions

As per earlier reports, *E. longifolia* was found to strongly interact with other drugs by affecting their metabolism and absorption in animals. The similar effect was observed in human subjects where the co-administration of Propranolol and aqueous extract of *E. longifolia* in fourteen non-smoker males (19–24 years old) caused significant increase in Propranolol concentration in the blood up to 10hr post-treatment which ultimately reduced its absorption. It was assumed *E. longifolia* triggered this action either; (i) by inducing the efflux transport P-glycoprotein which in turn increased the extrusion of Propranolol from the epithelial cells into the intestinal lumen, or (ii) via the formation of insoluble and non-absorbable chemical complexes with Propranolol which hindered its absorption.[70]

SAFETY AND TOXICOLOGY

In general, aqueous extracts of roots of *E. longifolia* are considered to be relatively safe with no reported toxic effects. In contrast, its alcoholic extracts at higher concentrations have been associated with serious toxicities both *in-vitro* and *in-vivo*.

Acute, subacute and subchronic toxicities

Initial toxicity evaluation of *E.longifolia* extracts by Satayavivad[71] recommended a LD_{50} of 1500–2000mg/kg and >3000mg/kg for 34% alcoholic and aqueous extracts, respectively. Subsequent evaluation of EN toxicity in mice and brine shrimp indicated LD_{50} of 0.05g/kg and 3.5µg/ml, respectively. Presence of unsaturated double bound at C-13 and C-21, an α, ß-unsaturated ketone moiety at C-2 in ring A, and oxymethylene bridge linking C-11 to C-8 were said to have contributed to its toxicity.[72] Likewise, F2 fraction showed a LD50 of 2.71g/kg for acute toxicity in rats whereas a dose of up to 51.2mg/kg was found to be safe with no visible damage to vital organs and testes following its sub-chronic (90 days) and chronic (180 days) toxicities. In general, preclinical studies have established the safety of the aqueous extract of *E. longifolia* roots at as high as 5000mg/kg whereas its organic extracts are reported to be relatively toxic[73] Lately, 4 and 13-weeks sub-acute and sub-chronic toxicity evaluation of *E. longifolia* root powder showed no notable toxic effects. Not to mention that, a dose of up to 1.2g/adult/day was recommended to be safe for consumption in human.[74]

Heavy metals contamination

By and large, plants absorb large amounts of heavy metals from earth which mostly get concentrated in the roots. These may pose threat to human life up on consumption by causing various ailments. Over 200 products containing *E. longifolia* are reported to be present in Malaysia most of which lack appropriate toxicological evaluation.[75] Over 200 products containing *E. longifolia* are reported to be present in Malaysia alone most of which lack appropriate toxicological evaluation. For example, heavy metals contamination in about 100 herbal products with *E. longifolia* as the main ingredient showed that about 36 of these products failed to comply with the limits for heavy metals contamination defined by the Drug Control Authority (DCA) of Malaysia. Equally, a similar assessment of 100 more herbal products indicated the presence of 10.3–20.3ppm of lead and 0.53–2.35ppm of mercury in 17 and 26 products, respectively, well above the permitted levels set by DCA.[76–78]

CONCLUSION

E. longifolia possesses numerous medicinal activities some of which have been investigated in detail to explore the underlying mechanism of actions involved particularly with regard to antitumor and aphrodisiac effects. Its traditional reputation as aphrodisiac has attracted scientists to investigate its effect on sexual arousal in both sexes especially in male. Apparently, aphrodisiac activity displayed by *E. longifolia* is linked to its ability to boost testosterone which is the main mediator of sexual function. In this regard, methanol extract has shown relatively stronger steroidogenesis and spermatogenesis effects than that of aqueous extract probably owing to accumulation of large amounts of bioactive quassinoids particularly EN. In a comparative study methanol extract was reported to be

about ten times more potent than the aqueous extract in generating more testosterone in Rodents.[12] Based on the above literature *E. longifolia* seems to have a lot to offer in managing and/or even treating conditions such as erectile dysfunction, malaria, and cancer. Nonetheless, more detailed investigations are necessary to further assess its effectiveness and safety in human subjects.

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