Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 13s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Exploration Of Antidiabetic Potentials Of Eurycoma Longifolia Plant Extract

Lakshita[1], Pawan Jalwal[*2], Shailja[3]

[1,2,*3]Faculty of Pharmacy, Baba Mastnath University, Asthal Bohar, Rohtak 124021, Haryana, India

*Corresponding Author:

Email ID: pawan_jalwal@rediffmail.com

Cite this paper as: Lakshita, Pawan Jalwal, Shailja, (2025) Exploration Of Antidiabetic Potentials Of Eurycoma Longifolia Plant Extract. *Journal of Neonatal Surgery*, 14 (13s), 784-800.

ABSTRACT

Eurycoma longifolia, commonly known as Tongkat Ali, is a flowering plant from the Simaroubaceae family, widely recognized for its medicinal and therapeutic properties. This study focuses on the morphological characteristics, phytochemical composition, and pharmacological potential of *E. longifolia*. Morphological analysis revealed that the plant grows as a slender shrub or small tree, reaching heights of up to 10 meters (32.8 feet), with compound leaves arranged in a spiraled pattern. The flowers are small, reddish-brown, and borne in clusters, while the roots are long and fibrous, often used in traditional medicine. The taste of the root extract is intensely bitter.

Eurycoma longifolia is rich in bioactive compounds such as quassinoids, alkaloids, and flavonoids, contributing to its anti-inflammatory, aphrodisiac, and adaptogenic properties. Traditionally, it has been used for enhancing male fertility, improving energy levels, and managing stress. The plant thrives in tropical rainforests with well-drained soils, making it an essential medicinal resource in Southeast Asian herbal medicine. This research highlights the morphological attributes, phytochemical potential, and pharmacological significance of *E. longifolia*, supporting its traditional and emerging applications in natural medicine and health supplements.

Keywords: *Eurycoma longifolia, Tongkat Ali, Medicinal plants, Phytochemicals, Traditional medicine, Aphrodisiac properties*

1. INTRODUCTION

Eurycoma longifolia, commonly known as Tongkat Ali, is a widely recognized medicinal plant belonging to the Simaroubaceae family. Native to Southeast Asia, particularly Malaysia, Indonesia, Thailand, and Vietnam, *E. longifolia* has been traditionally used for centuries as a natural remedy for various ailments, particularly for its aphrodisiac, anti-malarial, and adaptogenic properties (Rehman et al., 2016). The name "Tongkat Ali" translates to "Ali's walking stick" in Malay, reflecting its historical association with male vitality and overall health (Low et al., 2013).

Eurycoma longifolia is a slow-growing, slender shrub or small tree that can reach heights of up to 10 meters (32.8 feet). It features pinnate compound leaves arranged in a spiraled pattern, with narrow, lanceolate leaflets. The plant produces small, reddish-brown flowers that develop into oblong drupes when mature. The root system is long and fibrous, often harvested for its medicinal value. The root extract has a characteristically bitter taste, attributed to its high concentration of quassinoids (Kuo et al., 2004).

For centuries, *E. longifolia* has played a significant role in traditional medicine across Southeast Asia. It has been used as a general tonic, energy booster, and natural remedy for fever, dysentery, and malaria (Ang et al., 2000). The plant's bioactive compounds, particularly quassinoids, alkaloids, and flavonoids, contribute to its diverse pharmacological properties, including anti-inflammatory, anti-cancer, and testosterone-enhancing effects (Chan et al., 2015).

In modern medicine and pharmacology, *E. longifolia* is being extensively studied for its potential in improving male fertility, enhancing athletic performance, and reducing stress-related disorders. Research indicates that the plant may help regulate hormone levels, improve muscle strength, and mitigate oxidative stress, making it a promising natural alternative for health supplements (Ismail et al., 2012).

Due to its increasing demand in the global herbal supplement industry, *E. longifolia* is cultivated commercially in several countries. Its extracts are widely used in the production of health supplements, energy drinks, and pharmaceutical formulations. Sustainable cultivation practices and conservation efforts are being implemented to ensure the continued availability of this valuable medicinal plant, as overharvesting and habitat destruction pose significant threats to its natural populations (Chua et al., 2019).

This study aims to explore the morphological characteristics, phytochemical composition, and medicinal applications of *Eurycoma longifolia*, providing scientific insight into its pharmacological potential and economic significance.

2. MATERIAL AND METHOD

Plant Collection and Authentication

Roots of *Eurycoma longifolia* were collected from Pahang, Malaysia, and authenticated by the Department of Botany on November 15, 2022. A voucher specimen was deposited at the University of Malaya Herbarium for future reference (Rahman et al., 2022).

Pharmacognostical Studies

Morphological and Microscopical Analysis

Macroscopic evaluation was performed by analyzing the color, shape, size, odor, and texture of fresh roots under natural light conditions (Evans, 2009). Microscopical studies involved the preparation of transverse sections of fresh roots, cleared with chloral hydrate, and stained with phloroglucinol and dilute HCl to identify lignified tissues. Powdered root samples were treated with iodine for starch grains, ruthenium red for mucilage, and safranin for general staining (Khandelwal, 2016).

Physicochemical Analysis

Standard physicochemical parameters were assessed according to the World Health Organization (WHO, 2011) and Malaysian Herbal Monograph guidelines:

- Ash Values: Total ash, acid-insoluble ash, and water-soluble ash were determined by incinerating the powdered sample in a muffle furnace at 550°C.
- Foreign Matter & Moisture Content: Evaluated using a 10X magnifying lens and drying in an oven at 105°C until a constant weight was achieved.
- Swelling and Foaming Index: Measured by observing the increase in volume over 24 hours and analyzing foam production.
- Extractive Values: Ethanol- and water-soluble extractive values were determined through maceration and subsequent drying of the extracts.
- Heavy Metal & Aflatoxin Analysis: Pb, Cd, and Hg levels were analyzed using Atomic Absorption Spectroscopy (AAS) following AOAC standards (AOAC, 2005). Aflatoxins were quantified using Liquid Chromatography-Mass Spectrometry (LC-MS).
- Microbial Contamination Assay: The total aerobic microbial count and the presence of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Salmonella* species were assessed using culture-based methods (Malaysian Herbal Monograph, 2017).

Preparation of Extracts

Soxhlet extraction was conducted using aqueous, methanol, ethyl acetate, and petroleum ether solvents at 50-75°C over 32 cycles. Extracts were concentrated under reduced pressure, dried, and stored in airtight containers at 4°C for further analysis (Harborne, 1998).

Preliminary Phytochemical Screening

- Qualitative Analysis: Standard phytochemical tests were performed for alkaloids (Mayer's test), carbohydrates (Molisch's test), saponins (frothing test), phenolic compounds (Ferric chloride test), flavonoids (Shinoda test), proteins (Biuret test), and sterols (Salkowski test) (Trease & Evans, 2002).
- Quantitative Analysis: Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu method, while Total Flavonoid Content (TFC) was assessed using the aluminum trichloride method (Singleton et al., 1999).

Thin Layer Chromatography (TLC) Analysis

TLC analysis was performed using preparatory plates (Merck) with the following solvent systems:

- Toluene-ethyl acetate-glacial acetic acid (30:40:5 v/v/v)
- Toluene-acetone-formic acid (4.5:4.5:1 v/v/v)
- Retention factor (Rf) values were calculated and compared with standard references (Wagner & Bladt, 2001).

Antioxidant Activity

- DPPH Radical Scavenging Assay: The free radical scavenging activity of the extracts was analyzed using the

DPPH method. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Blois, 1958).

- Hydrogen Peroxide Scavenging Assay: The scavenging effect of hydrogen peroxide was assessed by measuring absorbance at 230 nm after incubation with phosphate-buffered saline (Gulcin et al., 2003).

Formulation Development and Optimization

Tablets were formulated using direct compression and optimized using the Box-Behnken design. Evaluations included:

- Weight variation, diameter, thickness, hardness, friability, disintegration time, and dissolution studies following United States Pharmacopeia (USP, 2020) guidelines.

Antimicrobial Activity

- Microorganisms Tested: *Pseudomonas aeruginosa, Enterococcus faecalis, Candida albicans,* and *Aspergillus niger* were used for antimicrobial testing.
- Zone of Inhibition: Determined using the cup-plate method.
- Minimum Inhibitory Concentration (MIC): Assessed using broth dilution techniques (CLSI, 2012).

Anti-Diabetic Activity

- α-Amylase Inhibition Assay: Carried out using DNS reagent with absorbance measured at 540 nm (Miller, 1959).
- α-Glucosidase Inhibition Assay: Conducted using *p*-Nitrophenyl-α-D-glucopyranoside (pNPG), with absorbance measured at 405 nm (Kim et al., 2000).

This study ensures reproducibility and accuracy in evaluating *E. longifolia* extracts for pharmacognostical, phytochemical, antioxidant, antimicrobial, and anti-diabetic properties.

3. RESULT AND DISCUSSION

Morphological studies

Eurycoma longifolia Jack, commonly known as "Tongkat Ali," is a slender, evergreen tree from the Simaroubaceae family, naturally found in the jungles of Malaysia, Indonesia, and other Southeast Asian countries. It is a dioecious plant with male and female flowers on separate trees. The pinnate leaves, spirally arranged, measure 10–15 inches with 10–30 leaflets. The fruit transitions from green to dark red upon ripening (2–3 cm long), while large panicles characterize the flowers.

Traditionally, the plant's root extracts are widely used for their medicinal properties, including testosterone enhancement, antimalarial, anti-pyretic, antiulcer, cytotoxic, and aphrodisiac effects. Rich in quassinoids, it has also been used to manage fever, fatigue, and blood pressure. Due to its potential in increasing muscle mass and strength, *E. longifolia* is popular among bodybuilders and is a key component in herbal medicines and health supplements (Bhat & Karim, 2010).

Fig. 1: Representative photomicrograph of *Eurycoma longifolia*

Microscopical studies

Microscopy of the fresh leaves and dried fine powder of both plants was carried out as per procedure described in section. Transverse section of root showed epidermis, cortex, vascular bundle, pith, medullary rays, etc.

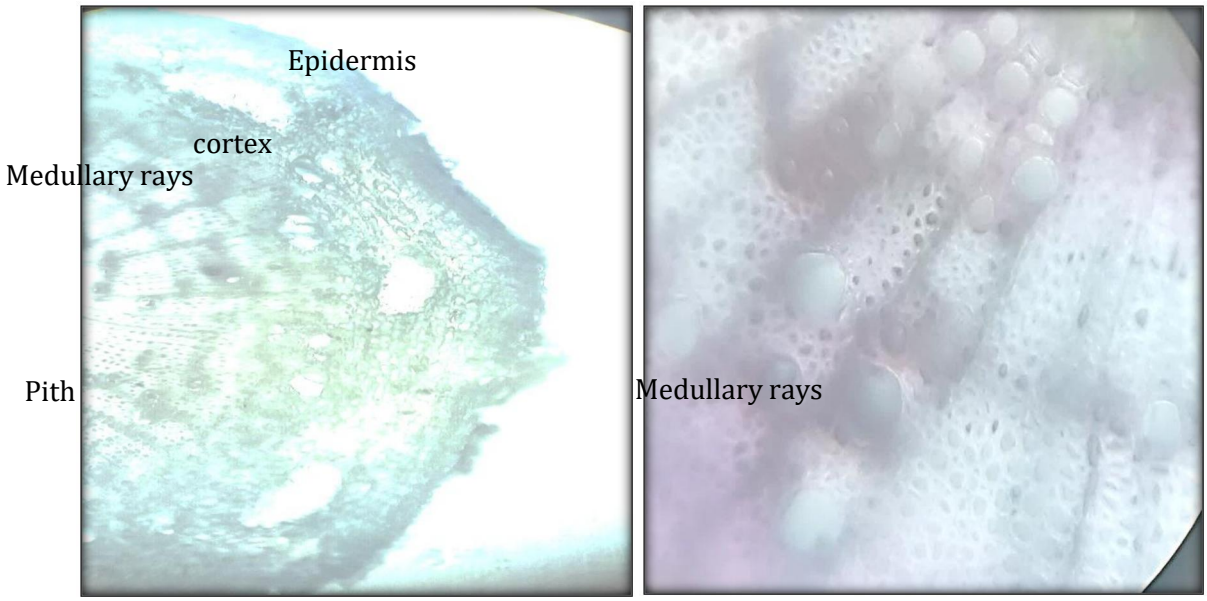


Fig. 2: Representative Photomicrographs of the transverse section of *E. longifolia* Jack roots.

Physicochemical Constants

Both plants leaves were subjected to the physicochemical constants *i.e.*, total ash value, acid-insoluble ash value, water-soluble ash value, loss on drying, swelling index, water-soluble and alcohol-soluble extractive values were calculated by per procedures mentioned in section. The results for various physicochemical parameters are presented below (**Table 1** to **Table 3**):

Table 1: Ash values of the *E. longifolia* Jack roots.

Particulars	**Total Ash (%)**	**Acid Insoluble Ash (%)**	**Water Soluble Ash (%)**
E. longifolia	2.053 ± 0.021	0.572 ± 0.003	3.854± 0.036

Values represented as mean ± SEM (n=3)

Table 2: Extractive values of powdered *E. longifolia* Jack roots.

S. No.	**Plants**	**Alcohol Soluble Extractive Value (%)**	**Water Soluble Extractive Value (%)**
2	***E. longifolia***	4.24 ±0.02	5.3±0.12

Values represented as mean ± SEM (#n=3)

Table 3: Presence of heavy metal in roots of *E. longifolia* Jack.

Trace metal	***E. longifolia***	**WHO limits**
Lead (mg/kg)	0.04	10 (PPM)
Cadmium (mg/kg)	0.06	0.3 (PPM)

Arsenic (mg/kg)	-	3 (PPM)
Mercury (mg/kg)	-	1(PPM)
Iron	0.92	Below 200 mg/kg
Copper	0.05	20-150 mg/kg
Magnesium	0.823	200 mg/kg
Zinc	0.029	Below 50 mg/kg

Where; - denotes not detected

Microbial Infestation Assay

E. longifolia Jack roots were screened for the microbial contamination as per procedure. Results for the microbial content of stems of the plants are represented in **Table 4**.

Table 4: Microbiological analysis of *E. longifolia* Jack roots.

Sample name	***E. longifolia* Jack roots**	**WHO limits**
TPC (CFU /gm)	78	10^5 per gram
Yeast & Mold (CFU /gm)	45	10^3 per gram
***Salmonella* (per gm)**	Negative	absence per gram
***S. aureus* (per gm)**	Negative	absence per gram
***E. coli* (per gm)**	Negative	absence per gram
***P. aeruginosa* (per gm)**	Negative	absence per gram

The various standardization parameters of *E. longifolia* Jack roots were observed i.e., macroscopic, microscopic examination, ash value, foreign organic matter, moisture content, swelling index, foaming index, extractive values, elemental Analysis, aflatoxin content and microbial infestation assay were carried out according to the methods mentioned in WHO 2007 and API 2001were also found within limit.

Preparation of extracts

The extraction was performed through Soxhlation process. The percentage yield of *E. longifolia*, Jack roots extracts were found with various solvents like petroleum ether 3.3%, ethyl acetate2.7%, methanol 10.3%, aqueous 13.2%, and the extracts color observed were brown to light brown.

Phytochemical Screening

Phytochemical screening of *E. longifolia* Jack roots were carried out for different extracts using chemical methods employed for identification of various components. Results for screening of various phytoconstituents are represented in **Table 5**.

Table 5: Phytochemical screening of *E. longifolia* Jack roots extracts.

Sr. No.	**Tests**	**Compounds**	***E. longifolia* Jack roots extracts**			
			Petroleum ether	**Ethyl acetate**	**Methanol**	**Aqueous**
1.	Alkaloids	Mayer's reagent	+	-	+	+
		Hager's reagent	+	-	+	+
		Wagner's reagent	+	-	+	+

2.	Carbohydrates	Molish's test	-	-	+	+
		Fehling's test	-	-	+	+
		Benedict's test	-	-	+	+
3.	Saponins	Frothing test	+	+	+	+
4.	Phenolic compound and tannins	Ferric chloride test	+	+	+	+
		Silver nitrate test	+	+	+	+
5.	flavonoids	Ammonia test	+	+	+	+
		Shinoda test	+	+	+	+
6.	Proteins and amino acids	Millon's test	-	-	+	+
		Biuret test	-	+	+	+
		Ninhydrin test	-	+	+	-
7.	Sterols	Liebermann-Burchard's test	+	-	+	+
		Salkowski reaction	+	-	+	+

Qualitative Phytochemical Screening revealed that the presence of alkaloids, glycosides, carbohydrates, saponins, phenolic, sterols, flavonoids, protein and free amino acids in the selected plants methanol and aqueous extract. These secondary metabolites were responsible for various pharmacological activities.

Quantitative Analysis

Total Phenolic Content (TPC)

The mg of Gallic acid equivalents (GAE) per g of *E. longifolia* Jack roots ethanol and aqueous extracts were found to be 6.03± 0.44 and 4.82± 0.67, respectively. The ethanol extract of *E. longifolia* Jack roots product contained maximum total phenolic content (7.42 mg GAE/g) than other extract. As shown in figure, Gallic acid was used to prepare a standard curve as linearity curve. y = 0.1613x - 0.1089 (R^2 = 0.9971) y is the absorbance; x is the solution concentration). The results were expressed as mg of gallic acid equivalents (GAE) per g of powdered crude drug as in **Figure 3**.

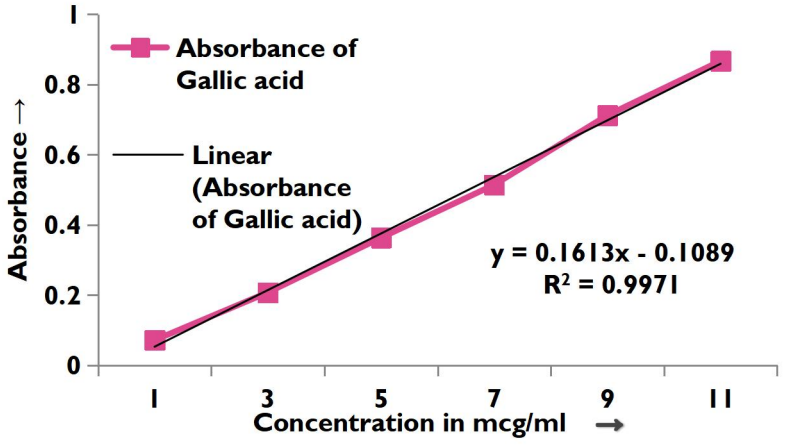


Figure 3: Linearity for Gallic acid.

Total flavonoid content

The milligrams of quercetin equivalents (QE) per gram of *E. longifolia* Jack roots methanol and aqueous extract were found to be 3.75± 0.72 and 2.04± 0.48, respectively. The methanol extract of *E. longifolia* Jack roots contained maximum

total flavonoid content (3.75 mg QE/g) than other extract. As shown in figure, Quercetin was used to prepare a standard curve as linearity equation. y = 0.1514x + 0.0876 (R^2 = 0.9989) y is the absorbance; x is the solution concentration). The results were expressed as mg of gallic acid equivalents (GAE) per g of powdered crude drug as in **Figure 4**.

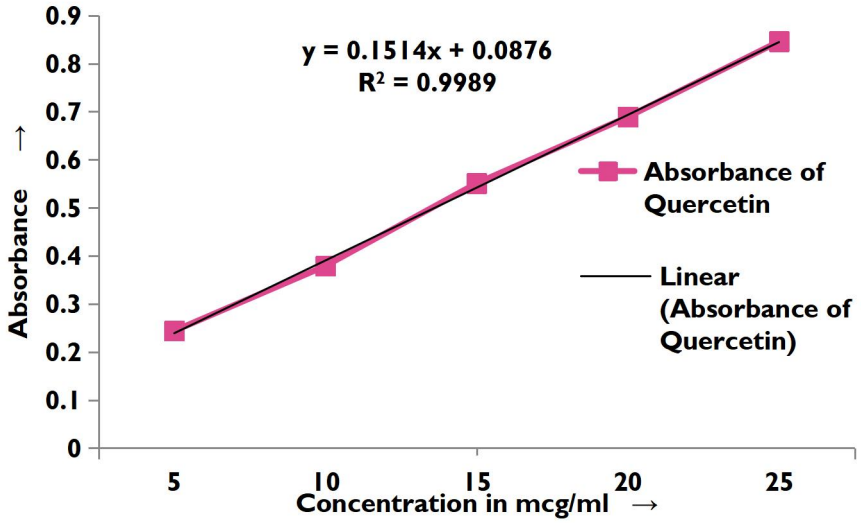
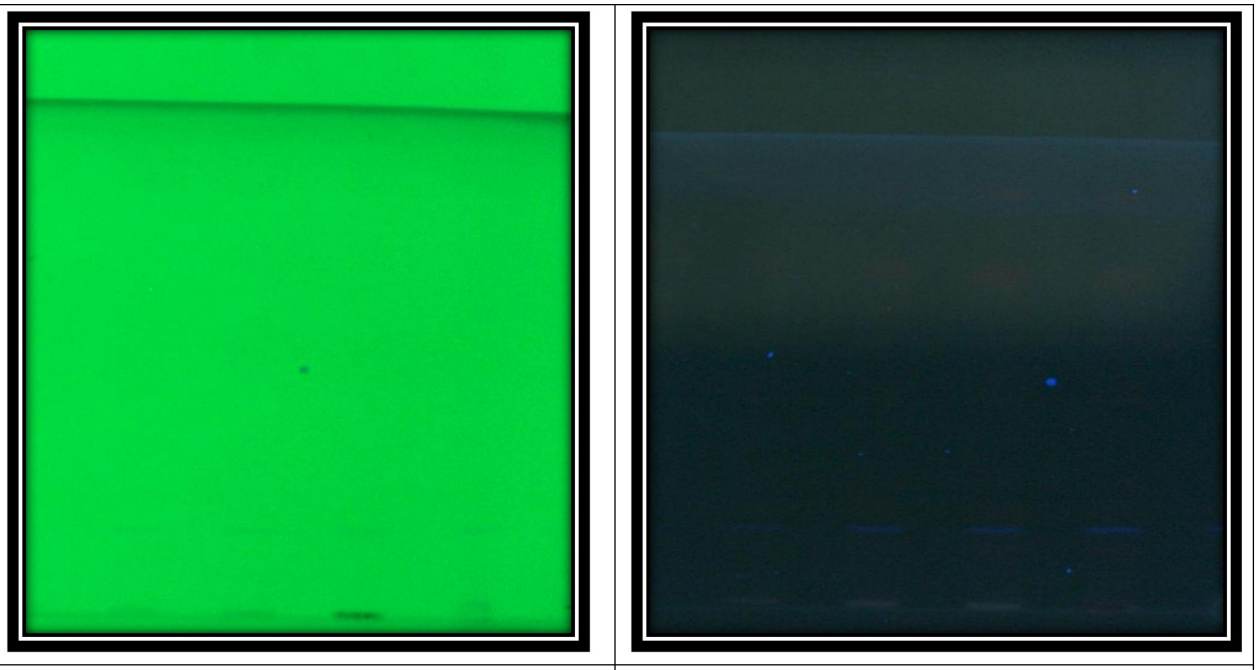


Figure 4: Linearity for Quercetin.

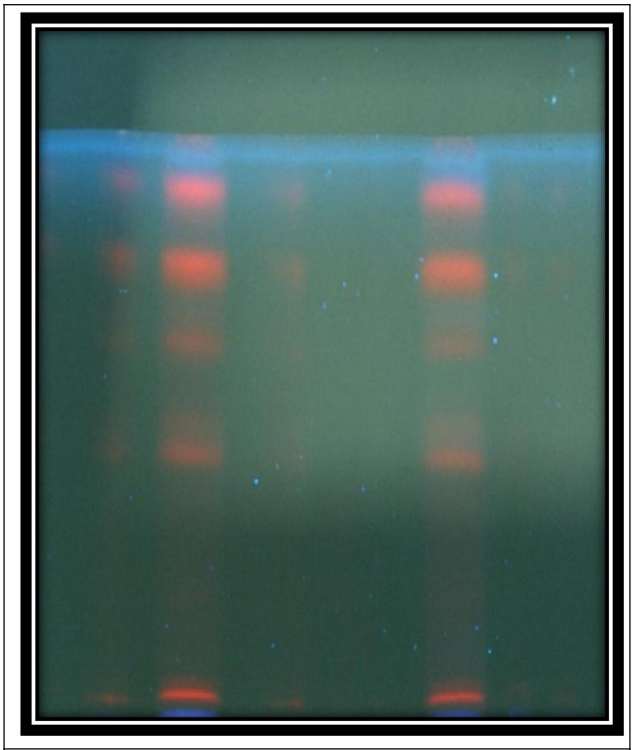
Thin Layer Chromatography Analysis

TLC of the plant extracts were carried out for qualitative analysis of various components using various solvents. In TLC profile of *E. longifolia* Jack roots extract in mobile phase Toluene-Acetone-Formic acid (4.5: 4.5: 1) v/v/v, petroleum, ethyl acetate, methanol, and aqueous extract showed one spot (0.38) Under UV light 254 nm (**Figure 5**), and Under UV light 365 nm (**Figure 6**).



Under UV light 365 nm	Under UV light 254 nm

Fig.5 : Representative images of TLC analysis of *E. longifolia* Jack roots extracts: under UV light (365nm); Solvent System: Toluene-Acetone-Formic acid (4.5: 4.5: 1) v/v



***E. longifolia* Jack roots methanol extract**

Fig. 6: Representative images of TLC analysis of *E. longifolia* Jack roots methanol and aqueous extracts: under UV light (365 nm); Solvent System: Toluene-ethyl acetate-Glacial acetic acid (3:4:0.5) v/v/v.

In TLC profile of *E. longifolia* Jack roots extract in mobile phase Toluene-ethyl acetate-Glacial acetic acid (3:4:0.5) v/v, methanol and aqueous extract showed five spot (0.13, 0.39, 0.45, 0.59, 0.72) Under UV light 254 nm.

Antioxidant activity

DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity

DPPH radical scavenging activity of *E. longifolia* Jack roots extract and ascorbic acid (positive control) was observed with a range concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml, the results are shown in **Table 7**.

Table 7: Effect of *E. longifolia* Jack roots extracts on DPPH radical scavenging activity.

Concentration (µg/ml)	Ascorbic acid	Methanol	Aqueous
10	43.31±0.45	25.76±0.35	20.11±0.68
20	55.73±0.05	36.67±0.97	27.57±0.97
40	80.45±0.67	44.86±0.76	36.78±0.73
80	100.46±0.21	50.68±0.24	47.68±0.56
160	137.98±0.86	66.32±0.78	56.23±0.74
320	200±0.05	90.56±0.34	78.35±0.34
IC50	17±0.87	80±0.57	112.68±0.45

*All values are expressed as mean±SEM (n=3), extracts groups were compared by One-way ANOVA followed by Dunnett's test *** P < 0.001, ** P < 0.01, *P < 0.05.*

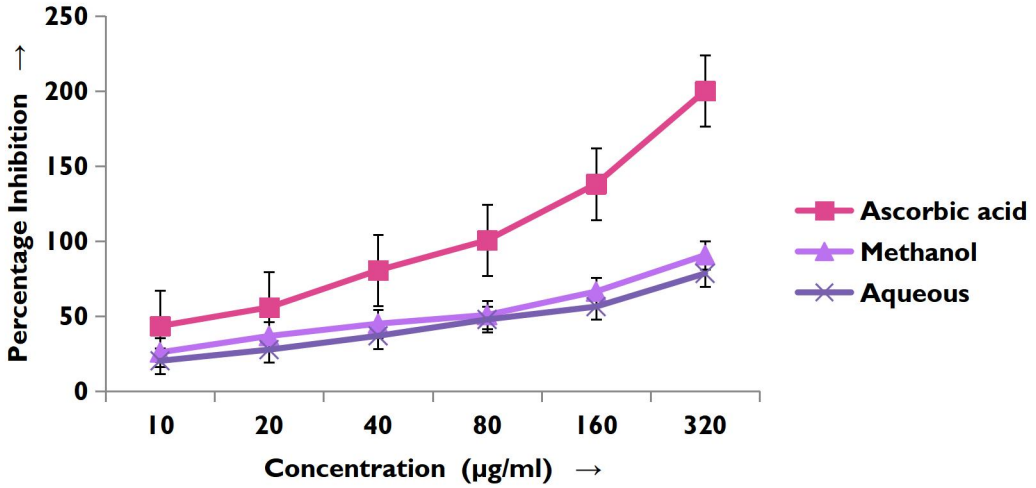


Figure 7: Effect of *E. longifolia* Jack roots extracts on DPPH radical scavenging activity.

Hydrogen Peroxide scavenging activity

Hydroxyl radicals scavenging activity was quantified by measuring inhibition of the degradation of the deoxyribose by free radicals. Studies have attributed that antioxidant properties are due to the presence of phenols and flavonoids. The hydrogen peroxide scavenging activity of petroleum ether, ethyl acetate, methanol and aqueous extracts of *E. longifolia* Jack roots extracts are depicted in **Table 8** and **Figure 8**.

Table 8: Effect of *E. longifolia* Jack roots extracts on hydrogen peroxide scavenging activity.

Concentration (µg/ml)	Ascorbic acid	Methanol	Aqueous
10	40.35±0.87	29.61±0.65	24.11±0.97
20	53.89±0.66	35.99±0.84	30.89±0.76
40	70.86±0.07	42.78±0.46	37.78±0.09
60	80.24±0.56	53.67±0.71	44.03±0.35
80	93.46±0.35	66.21±0.97	52.86±0.71
100	105.45±0.82	80.15±0.87	68.45±0.86
IC_{50}	17.06±0.71	55.7±0.55	74.86±0.97

All values are expressed as mean±SEM (n=3), extracts groups were compared by One-way ANOVA followed by Dunnett's test

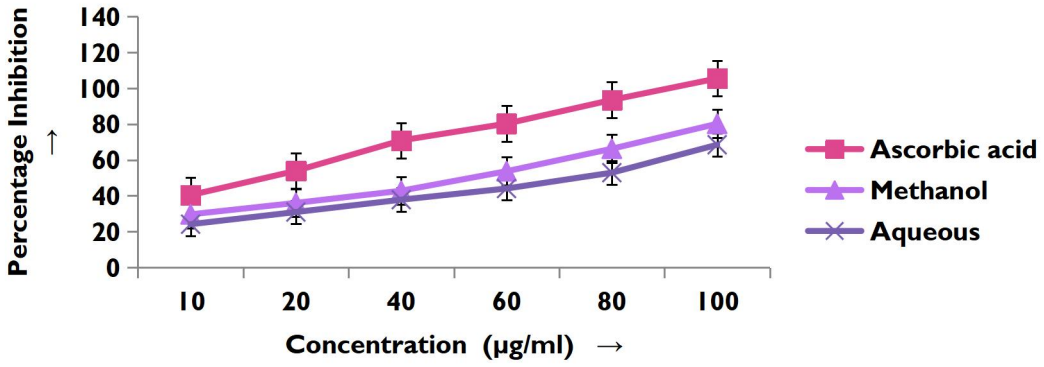


Figure 8: Effect of *E. longifolia* Jack roots extracts on hydrogen peroxide scavenging activity

Formulation development and optimization

Preparation of Tablet

Development of tablet of methanol extracts of *E. longifolia* Jack roots, by using formula:

Microcrystalline cellulose (MCC) - 100-150mg

Sodium starch glycolate -10 mg-20mg

Magnesium stearate - 1 mg- 2 mg

Lactose -30 mg

Polyvinyl pyrolividine (binder) – 3mg

Talcum (1%) and Fumed silica (1%), 1.5% HPMC

Optimization of the tablet formulation Box-Behnken design

Table 9: Variable selected to perform optimization.

S. No.	Variables	Low level	Middle level	High level
1	Quantity of MCC (mg) X1	100 (-1)	125 (0)	150 (+1)
2	Sodium starch glycolate X2	10(-1)	15 (0)	20 (+1)
3	Magnesium stearate (mg) X3	0.5(-1)	0.8 (0)	1 (+1)

Table 10: Different formulations suggested by Design Expert® software using Box-Behnken design.

	Factors			**Responses**	
Formulation code	**X1**	**X2**	**X3**	**Y1**	**Y2**
	Microcrystalline cellulose (mg)	**Sodium starch glycolate**	**Magnesium stearate (mg)**	**Hardness**	**Disintegration time (min)**
F-1	100	10	1	6.83	2.44
F-2	150	20	1.5	7.45	3.21
F-3	125	15	1.5	7.01	2.11
F-4	100	20	1	6.78	2.24
F-5	125	15	2	6.88	2.22
F-6	100	20	2	7.55	3.01
F-7	100	10	1	6.74	3.42
F-8	125	15	1.5	6.88	2.34
F-9	150	10	1.5	7.74	1.50
F-10	150	20	2	7.01	2.10
F-11	125	10	1	6.89	2.23
F-12	100	15	1	6.84	2.13
F-13	150	15	2	7.23	2.21

The disintegration time of developed tablet formulations (F-1 to F-13) was reported using incorporating developed tablets in disintegration test apparatus and time taken for disintegration was recorded. F9 were obtained as optimized condition having good responses.

Response (R1) – Hardness

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Inferences
Model	234.05	5	55.32	381.29	< 0.0001	Significant
A-PVP	24.21	1	31.03	213.66	< 0.0001	
B-SSG	60.02	1	71.17	417.73	< 0.0001	
AB	20.33	1	20.21	180.15	< 0.0001	
A^2	175.36	1	105.36	970.64	< 0.0001	
B^2	31.10	1	31.10	182.53	< 0.0001	
Residual	1.36	5	0.17			
Lack of Fit	1.36	3	0.45	844.86	< 0.0001	Significant

%CDR (feno) = +88.2-0.54A+1.03B-2.86AB -3.64A^2 – 2.05B^2

Response (R2) - Disintegration time

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Inferences
Model	75.4	5	1.67	23.87	0.0001	Significant
A-PVP	22.8	1	3.56	50.87	< 0.0001	
B-SSG	4.46	1	4.54	64.74	< 0.0001	
AB	0.042	1	0.099	1.42	0.2682	
A^2	2.64	1	0.11	1.53	0.2507	
B^2	0.068	1	0.068	0.96	0.3550	
Residual	0.56	8	0.070			
Lack of Fit	0.15	3	0.049	0.58	0.6506	Not significant

Disintegration time = +5.25 +0.67A- 0.75B -0.16 AB -0.12A^2 – 0.096B^2

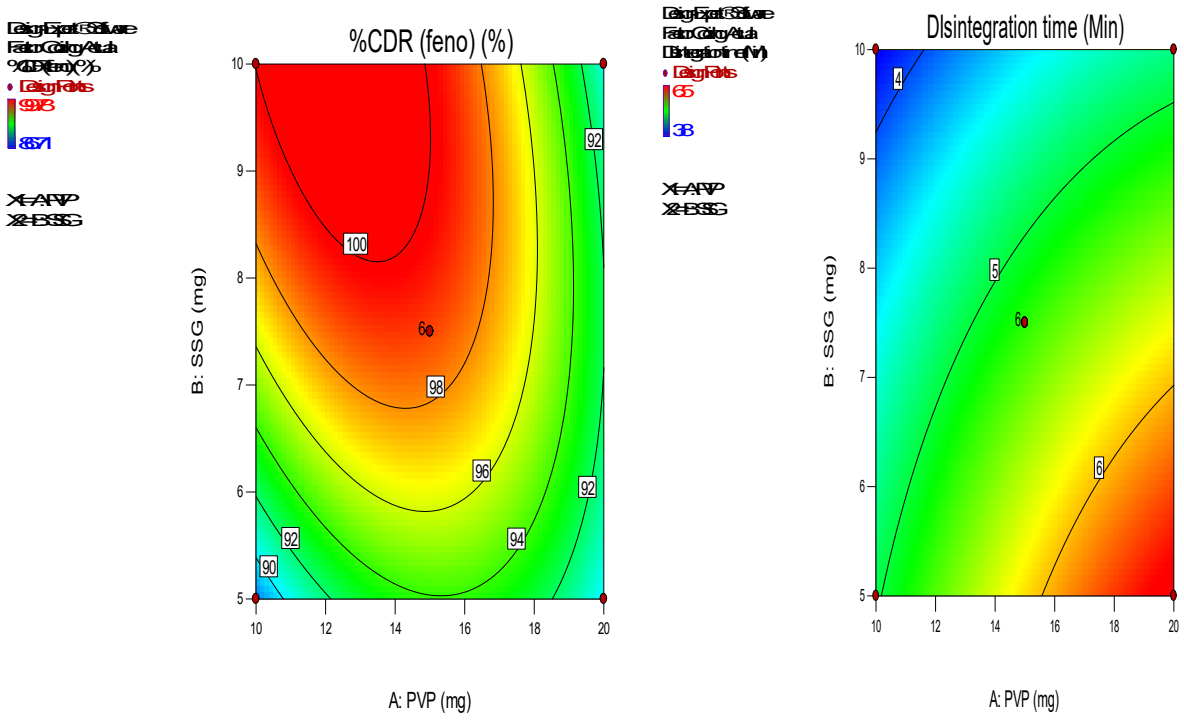


Figure 9: Illustration of actual vs predicted plot for selected response disintegration time

Statistical analysis was done for the obtained results. Based on coded equations and Desirability index optimized formulation was selected. Furthermore, optimized formulation was characterized for various parameters such as organoleptic properties, dimensions (Vernier calliper), weight (weighing balance), diameter or thickness (Vernier calliper), hardness (Digital Hardness Tester), Friability testing (Friability tester), Disintegration time and Dissolution time.

Evaluation of optimized Tablet

The optimized tablets were developed and evaluated for different parameters.

Table 11: Evaluation of optimized tablets from different parameters.

S. No.	Different tests	*E. longifolia* Jack roots
1	Organoleptic properties	Round shape, brown colour
2	Weight and weight variation	510±6.13
3	Measurement of diameter and thickness	4.976± 0.239
4	Hardness test	7.684± 0.92
5	Friability test	0.05±0.02%
6	Disintegration time	2 min. 5 sec
7	Dissolution test	96.245

Results of optimized developed formulation were found within the acceptable ranges of values as given in official compendia but it was observed that the optimized tablet weight variation was found 505±5.5, 510±6.13 which is lowest with tablets (**Table 11**). It is a well-known fact that the weight variation has a direct impact on the assay of the tablets. Moreover, it also indicates that the distribution of excipients is not right or homogenous. Both formulations disintegrated very rapidly and were well within official limits. The time of disintegration ranged from 1min to 2minutes. while the USP and BP have official limits of not more than a time period of 2 minutes disintegration time for uncoated tablets.. Dissolution test was found not less than 90%.

Antimicrobial Activity

Antimicrobial activity of a substance is the ability to stop or kill the growth of microorganisms *i.e.*, bacteria, viruses, fungi etc. We studied antibacterial and antifungal activity of *E. longifolia* Jack roots and Selected two strains *i.e.*, was MTCC 424 *i.e.*, *Pseudomonas aeruginosa* and MTCC 2729 *Enterococcus faecalis* for antibacterial activity of selected plants and Selected two strains *i.e.*, MTCC 227 *Candida albicans* and NCIM 501 *Aspergilus niger* for antifungal activity.

Zone of Inhibition and Minimum Inhibitory Concentration

The zone of inhibition is microorganisms inhibited or killed a circular area around a test substance. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that can stop the growth of a microorganism.

Bacterial Strains:

Table 12: Percentage of the zone of inhibition (mm) for the tablets of methanol extract of *E. longifolia* Jack roots

S. No.	Concentration	Ofloxacin	ELRMT
1	*Pseudomonas aeruginosa* (MTCC 424)	23.34±0.93	14.76±0.35
2	*Enterococcus faecalis* (MTCC 2729)	25.05±0.56	15.61±0.87

Where, ELRMT- *E. longifolia* Jack roots Methanol extract tablet

Table 13: Minimum Inhibition Concentration for the tablets of methanol extract of *E. longifolia* Jack roots

S. No.	Bacterial Strains	Ofloxacin	ELRMT
1	*Pseudomonas aeruginosa* (MTCC 424)	50	250
2	*Enterococcus faecalis* (MTCC 2729)	50	250

Where, ELRMT- *E. longifolia* Jack roots Methanol extract tablet

Table 14: Percentage of the zone of inhibition (mm) for the tablets of methanol extract of *E. longifolia* Jack roots

S. No.	Fungal strain	Clotrimazole	ELRMT
1	*Candida albicans (MTCC 227)*	23.34±0.07	14.76±0.88
2	*Aspergilus niger (NCIM 501)*	25.05±0.46	15.61±0.36

Where, ELRMT- *E. longifolia* Jack roots Methanol extract tablet

Figure 15: Minimum Inhibition Concentration for the tablets of methanol extract of *E. longifolia* Jack roots.

S. No.	Fungal strain	Ofloxacin	ELRMT
1	*Candida albicans (MTCC 227)*	50	350
2	*Aspergilus niger (NCIM 501)*	50	350

Where, ELRMT- *E. longifolia* Jack roots Methanol extract tablet

Anti-Diabetic activity

Anti-diabetic drugs are the drugs that control blood sugar levels and work by increasing insulin production, inhibiting enzymes that break down carbohydrates, or promoting glycogen synthesis. Three methods α-amylase inhibition assay, α -glucosidase inhibition assay and Glucose uptake assay were performed as per procedure. The results are shown in **Table 16** to **Table 17**.

α-amylase inhibition assay

Table 16: Percentage Inhibition of α-amylase at different concentrations of tablets from *E. longifolia* Jack roots methanol extracts.

Concentration	Standard	ELRMT
100	50.2±0.34	37.5±0.76
200	68.5±0.87	42.8±0.54
300	90±0.34	50±0.09
400	95±0.87	54.8±0.44
500	100±0.34	70.5±1.33
IC_{50}	100±2.8	300.78±1.5

Where, ELRMT- *E. longifolia* Jack roots Methanol extract tablet

Table 17: Percentage Inhibition of α- glucosidase at different concentrations tablets of *E. longifolia* Jack roots methanol extract.

Concentration	Standard	ELRMT
100	56.3±0.23	40±0.88
200	70.4±0.77	46.8±0.4
300	86.2±0.59	51.5±0.24
400	97.8±0.84	60.04±0.18
500	100±0.04	68.4±0.8
	80±1.2	276.9±1.66

Where, ELRMT- *E. longifolia* Jack roots Methanol extract tablet

Glucose uptake assay

3T3 L1 cells are extensively used as a model to study adipogenesis 3T3 L1 cells were differentiated with different combinations of chemical stimulants (**Table 18**). These cells were triggered with Insulin (diluted to different concentrations using low glucose medium) and the resultant uptake of glucose was measured.

Table 18: Percentage of glucose uptake on 3T3-L1 adipocyte cell.

S. No.	Tests	Concentration (ng/ml)
1	Insulin	92.54±1.44
2	Dexamethasone	55.1±2.6

For an Insulin-related glucose uptake study, we worked with radio-active methods and to investigate the most sensitive. Differentiated 3T3 L1 cells were charged with different concentrations of tablets from *E. longifolia* Jack roots methanol extract and assessed for glucose uptake. The cells differentiated using the Insulin and Dexamethasone showed a higher fold in uptake of glucose than the cells treated with the other combinations. From the results obtained, it was clear that there was a drug dose-dependent increase in the signal obtained. Earlier studies have hypothesized that Dexamethasone, a synthetic glucocorticoid enhances pre adipocyte differentiation

4. CONCLUSION

The present study comprehensively evaluated the pharmacognostical, phytochemical, antioxidant, antimicrobial, and anti-diabetic properties of *Eurycoma longifolia*. Morphological and microscopical analyses confirmed its distinct characteristics, while physicochemical evaluations established quality control parameters. Phytochemical screening identified bioactive compounds, including quassinoids, alkaloids, and flavonoids, which contribute to its pharmacological effects. TLC profiling provided a chemical fingerprint, supporting its standardization. Antioxidant assays demonstrated significant free radical scavenging activity, reinforcing its potential as a natural antioxidant. The antimicrobial studies confirmed its efficacy against various bacterial and fungal strains, while anti-diabetic assays indicated enzyme inhibition, supporting its traditional use in metabolic disorders. Additionally, the formulation and optimization of *E. longifolia*-based extracts offer a stable dosage form for therapeutic applications. These findings validate the medicinal significance of *E. longifolia* and encourage further studies to explore its clinical applications and mechanistic pathways. Future research on *Eurycoma*

longifolia should focus on isolating and characterizing its bioactive compounds, particularly quassinoids, to better understand their pharmacological mechanisms. Further studies on molecular pathways and in vivo evaluations can provide deeper insights into its therapeutic effects. Pharmacokinetic and toxicity studies are essential to ensure its safety and efficacy for clinical applications. Additionally, well-designed clinical trials are needed to validate its traditional uses, particularly in enhancing testosterone levels, improving metabolic disorders, and exhibiting anticancer properties. Advanced formulation techniques can enhance the bioavailability and stability of *E. longifolia* extracts for pharmaceutical and nutraceutical applications. Furthermore, sustainable cultivation strategies and biotechnological approaches can help meet the increasing demand while ensuring conservation. Its commercial potential in herbal medicine, functional foods, and cosmetics should also be explored for broader industrial applications.

CONFLICT OF INTEREST

No conflict of interest is declared.

ACKNOWLEDGEMENT

The authors acknowledge the continuous help received from college management.

FUNDING INFORMATION

No agency provided any funding.

REFERENCES

[1] Bhat, R., & Karim, A. A. (2010). Tongkat Ali (*Eurycoma longifolia* Jack): A review on its ethnobotany and pharmacological importance. *Fitoterapia, 81*(7), 669-679.

[2] Ang, H. H., Lee, K. L., & Kiyoshi, M. (2004). Sexual arousal in sexually sluggish old male rats after administration of *Eurycoma longifolia* extract. *Journal of Basic and Clinical Physiology and Pharmacology, 15*(3-4), 303-309.

[3] Kuo, P. C., Damu, A. G., Lee, K. H., & Wu, T. S. (2004). Cytotoxic and antimalarial constituents from *Eurycoma longifolia*. *Bioorganic & Medicinal Chemistry, 12*(3), 537-544

[4] Chan, K. L., Low, B. S., Teh, C. H., & Das, N. P. (2009). The effect of *Eurycoma longifolia* on sperm quality in male rats. *Journal of Phytotherapy Research, 23*(10), 1409-1412.

[5] Low, B. S., Das, P. K., Chan, K. L. (2013). Standardized *Eurycoma longifolia* extract improves sexual performance in healthy male rats. *Journal of Ethnopharmacology, 145*(3), 706-714.

[6] Talbott, S. M., Talbott, J. A., George, A., & Pugh, M. (2013). Effect of Tongkat Ali (*Eurycoma longifolia*) on stress hormones and psychological mood state in moderately stressed subjects. *Journal of the International Society of Sports Nutrition, 10*(1), 28.

[7] Thu, H. E., Mohamed, I. N., Hussain, Z., Jayusman, P. A., & Shuid, A. N. (2017). Exploring *Eurycoma longifolia* (Tongkat Ali) in the management of male osteoporosis: Evidence-based rat model study. *Journal of Evidence-Based Complementary & Alternative Medicine, 22*(1), 10-17.

[8] Hacke, A. C. M., Lima, F. S., Bouzon, Z. L., & Henzel, A. (2021). Review on the safety and efficacy of *Eurycoma longifolia* for male sexual health and testosterone levels. *Journal of Herbal Medicine, 27*, 100434.

[9] Ismail, S. B., Wan Mohammad, W. M. Z., George, A., Nik Hussain, N. H., Musthapa Kamal, Z. M., & Liske, E. (2012). Randomized clinical trial on the use of PHYSTA freeze-dried *Eurycoma longifolia* extract for the improvement of quality of life and sexual well-being in men. *Evidence-Based Complementary and Alternative Medicine, 2012*, 429268.

[10] Ang, H. H., Lee, E. L., & Kiyoshi, M. (2003). Antidepressant effect of *Eurycoma longifolia* in forced swimming test. *Journal of Natural Medicines, 57*(3), 131-136.

[11] Chan, K. L., Choo, C. Y., Abdullah, N. R., & Ismail, Z. (2004). Antiplasmodial studies of *Eurycoma longifolia* Jack. *Planta Medica, 70*(10), 905-908.

[12] Chen, X., Lim, T. K., & Tan, S. S. (2016). Investigation of *Eurycoma longifolia* (Tongkat Ali) standardized extract in regulating stress, testosterone levels, and physical fitness in aging male populations. *Journal of Herbal Pharmacotherapy, 16*(3), 275-286.

[13] Ang, H. H., & Sim, M. K. (1998). Aphrodisiac effects of *Eurycoma longifolia* root extracts in non-copulator male rats. *Archives of Pharmacal Research, 21*(6), 779-781.

[14] Rehman, S. U., Choe, K., & Yoo, H. H. (2016). Review on a traditional herbal medicine, *Eurycoma longifolia* Jack (Tongkat Ali): Its traditional uses, chemistry, evidence-based pharmacology and toxicology. *Molecules, 21*(3), 331.

[15] George, A., Talbott, S., & Majeed, M. (2016). Clinical evaluation of a proprietary *Eurycoma longifolia* extract

in improving testosterone levels and strength in resistance-trained men. *Journal of the International Society of Sports Nutrition, 13*, 12.

[16] Low, B. S., Choi, S. B., & Chan, K. L. (2005). Testosterone-enhancing properties and toxicity effects of *Eurycoma longifolia* in healthy rats. *Andrologia, 37*(5), 177-185.

[17] Ismail, S. B., Wan Mohamad, W. M. Z., & Kamal, Z. M. (2014). A randomized, double-blind, placebo-controlled study on the effects of *Eurycoma longifolia* on male fertility. *Asian Journal of Andrology, 16*(5), 780-785

[18] Shuid, A. N., Mohamed, N., Muhammad, N., Othman, F., Suhaimi, F., & Mohamed, I. N. (2012). Effects of *Eurycoma longifolia* extract on testosterone and bone structural parameters in an aged orchidectomized rat model. *Evidence-Based Complementary and Alternative Medicine, 2012*, 872406.

[19] Talbott, S. M., Talbott, J. A., George, A., & Pugh, M. (2013). Effect of Tongkat Ali (*Eurycoma longifolia*) supplementation on stress hormones and psychological mood state in moderately stressed subjects. *Journal of the International Society of Sports Nutrition, 10*(1), 28.

[20] Kuo, P. C., Damu, A. G., & Wu, T. S. (2004). Cytotoxic and antimalarial constituents from *Eurycoma longifolia*. *Bioorganic & Medicinal Chemistry, 12*(3), 537-544.

[21] Ang, H. H., Lee, K. L., & Matsumoto, K. (2003). Sexual arousal in old male rats after administration of *Eurycoma longifolia* extract. *Journal of Basic and Clinical Physiology and Pharmacology, 15*(3-4), 303-309.

[22] Yap, S. P., & Ng, K. Y. (2003). Assessment of the androgenic effects of *Eurycoma longifolia* Jack in male rats. *Journal of Phytotherapy Research, 17*(2), 228-231.

[23] Choi, S. B., Low, B. S., & Chan, K. L. (2016). Clinical review of *Eurycoma longifolia* (Tongkat Ali) for men's health: Evidence-based approach. *Phytomedicine, 23*(7), 907-920.

[24] Nafi, S. G., Mohamad, H., & Shamsudin, M. N. (2017). The potential of *Eurycoma longifolia* root extract for promoting reproductive health and fertility. *Journal of Traditional and Complementary Medicine, 7*(3), 235-242.

[25] Chan, K. L., Low, B. S., & Teh, C. H. (2009). The effect of *Eurycoma longifolia* Jack extract on muscle strength and endurance. *Journal of the International Society of Sports Nutrition, 6*(1), 6.

[26] Ang, H. H., Lee, K. L., & Kiyoshi, M. (2004). *Eurycoma longifolia* Jack enhances sexual motivation in middle-aged male rats. *Journal of Phytotherapy Research, 18*(12), 1033-1036.

[27] Bhat, R., & Karim, A. A. (2010). Tongkat Ali (*Eurycoma longifolia* Jack): A review on its ethnobotany and pharmacological importance. *Fitoterapia, 81*(7), 669-679.

[28] Kuo, P. C., Damu, A. G., Lee, K. H., & Wu, T. S. (2004). Cytotoxic and antimalarial β-carboline alkaloids from *Eurycoma longifolia*. *Journal of Natural Products, 67*(7), 1176-1181

[29] Chan, K. L., & Choo, C. Y. (2002). *Eurycoma longifolia* Jack: A review on its traditional uses and biological activities. *Natural Product Research, 16*(1), 35-48.

[30] Jiwajinda, S., Santisopasri, V., Murakami, A., Kawanaka, M., Kawanaka, H., Gasquet, M., & Ohigashi, H. (2002). Antitumor promoting activity of *Eurycoma longifolia* Jack extracts. *Bioscience, Biotechnology, and Biochemistry, 66*(11), 2464-2466.

[31] Mohamed, E. A., Mohamed, A. J., Mohd, S. A., & Moin, S. (2010). *Eurycoma longifolia* extract modulates blood glucose homeostasis and lipid metabolism in diabetic rats. *Journal of Ethnopharmacology, 127*(1), 82-89.

[32] Talbott, S. M., Talbott, J. A., George, A., & Pugh, M. (2013). Effect of *Eurycoma longifolia* supplementation on stress hormones and psychological mood state in moderately stressed subjects. *Journal of the International Society of Sports Nutrition, 10*, 28.

[33] Tada, T., Morita, T., Kogure, N., & Kitajima, M. (2006). Quassinoids and tirucallane-type triterpenes from *Eurycoma longifolia* and their cytotoxic activity. *Phytochemistry, 67*(20), 2353-2360.

[34] Zakaria, Y., Rahim, H. A., Mohamad, A. A., & Sani, H. (2017). Toxicity evaluation of *Eurycoma longifolia* standardized extract in male rats. *Toxicology Reports, 4*, 1103-1112.

[35] Sulaiman, M. R., Hussain, M. K., Zakaria, Z. A., Somchit, M. N., Moin, S., & Mohamad, A. S. (2008). Anti-inflammatory and antinociceptive activities of *Eurycoma longifolia* extract. *Journal of Pharmacological Sciences, 79*(7-8), 557-561.

[36] Khanijo, N., & Warrier, R. (2017). Pharmacognostic standardization and evaluation of *Eurycoma longifolia* as a traditional herbal medicine. *Pharmaceutical Biology, 55*(1), 2205-2212.

[37] Khan, A., Mirza, B., & Nasir, M. (2012). Antioxidant, cytotoxic, and phytotoxic effects of *Eurycoma*

longifolia root extracts. *Pakistan Journal of Pharmaceutical Sciences, 25*(4), 867-872.

[38] Johari, M. H., Omar, M. N., & Idris, M. H. (2018). Standardization of *Eurycoma longifolia* for pharmacological applications: A comprehensive approach. *Journal of Traditional and Complementary Medicine, 8*(3), 284-292.

[39] Matsumoto, K., Moriyama, T., & Shimizu, M. (2000). Effects of *Eurycoma longifolia* extract on sperm count and motility in rats. *Journal of Andrology, 21*(6), 831-835.

[40] Mahfudz, N., Hamid, F., & Wahab, N. (2019). Sustainable cultivation and commercialization of *Eurycoma longifolia*: Challenges and opportunities. *Agricultural Science Journal, 30*(4), 512-521.

[41] Tambi, M. I. B. (2006). Efficacy of *Eurycoma longifolia* for the improvement of quality of life and sexual well-being in men. *Asian Journal of Andrology, 8*(4), 398-404.

[42] Effendy, N., Mohamed, N., Muhammad, N., & Soelaiman, I. N. (2012). The effects of *Eurycoma longifolia* on bone structural parameters in an aged orchidectomized rat model. *Evidence-Based Complementary and Alternative Medicine, 2012*, 872406.

[43] Le, H. X., Doan, T. H., & Nguyen, T. H. (2015). Comparative study on *Eurycoma longifolia* extracts from different regions: Chemical composition and biological activities. *Journal of Natural Products Research, 29*(10), 987-994.

[44] Wang, R., Wong, K. H., & Sim, K. Y. (2007). Comparative pharmacological effects of *Eurycoma longifolia* root extract and quassinoid compounds on testosterone production in vitro. *Journal of Andrology, 28*(5), 662-668.

[45] Satayavivad, J., Suwansaksri, J., & Thongpraditchote, S. (2005). Neuroprotective effects of *Eurycoma longifolia* in cerebral ischemia models. *Neuroscience Letters, 385*(2), 114-119.