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Research Article

Phytochemical profiling and evaluation of fermented *Eurycoma longifolia* Jack root extract on blood glucose and sexual behavior in rats

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ABSTRACT

Eurycoma longifolia Jack (Tongkat Ali) is traditionally used to support male reproductive and metabolic health. Fermentation represents a promising bioprocessing approach to alter phytochemical composition and influence the biological properties of herbal materials. This study aimed to standardize and characterize fermented *Eurycoma longifolia* root extract and evaluate its effects on glycemic control and male sexual behavior in streptozotocin-induced hyperglycemic rats. Fermented root simplicia was prepared using *Aspergillus oryzae* and extracted with 70% ethanol. Extract standardization included organoleptic evaluation, extractive values, phytochemical screening, ash values, and heavy metal analysis. Metabolite profiling was performed using UHPLC–HRMS.Thirty-five male Wistar rats were divided into normal, negative, positive, and extract-treated groups (50, 100, and 150 mg/kg BW). Blood glucose consentration and male sexual activity was monitored over 21-days. The fermented extract fulfilled standardization requirements, with water-soluble extractive content of 83.68% w/w, ethanol-soluble extractive content of 37.77% w/w, and total ash value of 3.17% w/w. UHPLC–HRMS analysis detected 194 metabolite peaks, with kojic acid and palmitic acid were recognized as the predominant constituents. The extract significantly improved glycemic control and enhanced male sexual behavior parameters compared with the negative control group ($p < 0.05$). The 100 mg/kg BW dose demonstrated the most pharmacological response. These findings suggest that fermented *Eurycoma longifolia* Jack root extract possesses potential glucose-lowering and male sexual behavior-modulating effects. However, studies using non-fermented controls and validated metabolite confirmation are needed.

Keywords:
Eurycoma longifolia jack; Fermented herbal extract; UHPLC-HRMS; Blood glucose; Male sexual behavior

1. INTRODUCTION

Eurycoma longifolia Jack, a member of the *Simaroubaceae* family, is a medicinal plant indigenous to Southeast Asia. It is commonly referred to as Tongkat Ali in Malaysia and Pasak Bumi in Indonesia and is widely distributed throughout Indonesia, Malaysia, Thailand, Vietnam, and other tropical regions. Traditionally, this plant has been used to promote male health, enhance physical performance, alleviate fatigue, support metabolic function, and improve reproductive well-being. The roots are the most extensively utilized medicinal part due to their rich content of bioactive constituents, including quassinoids, canthin-6-one alkaloids, β-carboline alkaloids, triterpenoids, squalene derivatives, flavonoids, and saponins. Among these compounds, eurycomanone is recognized as the principal quassinoid and is frequently employed as a phytochemical marker

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because of its reported contribution to several biological activities of *Eurycoma longifolia* Jack [1].

Numerous studies have demonstrated that *Eurycoma longifolia* Jack exhibits a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antiproliferative, antihyperglycemic, and male reproductive health-promoting effects. Beyond its traditional use, the plant has attracted considerable interest as a source of phytopharmaceutical and nutraceutical products owing to its diverse secondary metabolites. For example, *Eurycoma longifolia* Jack is one of the major components of the Thai herbal formulation "Sam Rak," which has been reported to exhibit antiproliferative activity against multiple human malignancies, highlighting its therapeutic potential beyond conventional ethnomedicinal applications [2,3].

Among its various pharmacological properties, the effects of *Eurycoma longifolia* Jack on male reproductive health have received substantial scientific attention. A systematic review and meta-analysis demonstrated that supplementation with *Eurycoma longifolia* Jack significantly increased total testosterone levels in both healthy men and individuals with testosterone deficiency. Elevated testosterone concentrations may contribute to improvements in reproductive function, libido, and sexual performance [4]. In addition to endocrine regulation, the biological activity of *Eurycoma longifolia* Jack may involve neurobiological mechanisms. Recent evidence suggests that eurycomanone can modulate dopamine secretion through GABAergic signaling pathways in differentiated human neuroblastoma cells, providing a plausible mechanistic basis for the traditional use of *Eurycoma longifolia* Jack roots in supporting male sexual function [5].

Apart from its reproductive health benefits, *Eurycoma longifolia* Jack has also demonstrated promising metabolic effects. Experimental studies have reported that root extracts of *Eurycoma longifolia* Jack may reduce blood glucose levels, improve insulin sensitivity, and alleviate oxidative stress associated with the progression of diabetes-related complications. These effects are thought to be linked to quassinoids and other bioactive metabolites that influence glucose metabolism and pancreatic function [1]. Consequently, *Eurycoma longifolia* Jack represents a promising phytotherapeutic candidate with potential applications in both metabolic and reproductive health management.

In recent years, fermentation has emerged as a valuable bioprocessing strategy for modifying the characteristics of herbal materials. Microbial metabolic activities during fermentation can alter phytochemical composition through biotransformation processes, leading to the generation of new metabolites or structural modification of existing compounds. *Aspergillus oryzae* is known to produce a variety of hydrolytic enzymes capable of transforming natural products and influencing the chemical properties of plant extracts. Therefore, fermentation has been increasingly explored as an approach to modulate metabolite composition and potentially affect the biological responses of herbal preparations [6].

Comprehensive characterization of fermented *Eurycoma longifolia* Jack requires advanced analytical techniques capable of detecting a wide range of metabolites. Ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC–HRMS) has become a powerful platform for metabolite profiling due to its high sensitivity, selectivity, and analytical resolution when applied to complex herbal matrices. This approach enables extensive metabolite characterization and provides valuable insights into the relationship between phytochemical composition and biological activity [7].

Although the pharmacological properties of *Eurycoma longifolia* Jack have been extensively investigated, information regarding the metabolite profile and biological properties of fermented *Eurycoma longifolia* Jack remains limited. Furthermore, studies integrating extract standardization, UHPLC–HRMS-based metabolite characterization, and biological evaluation of both metabolic and male reproductive parameters within a single experimental framework are still scarce. Therefore, the present study aimed to standardize fermented *Eurycoma longifolia* Jack root extract, characterize its metabolite profile using UHPLC–HRMS, and evaluate its effects on blood glucose levels and male sexual behavior in streptozotocin-induced rats.

2. MATERIALS AND METHODS

2.1. Materials and equipment

2.1.1. Materials

The materials used in this study included *Eurycoma longifolia* Jack root simplicia, distilled water, *Aspergillus oryzae*, filter paper, 70% ethanol; ultrapure water, acetonitrile, and formic acid of LC–HRMS grade; glucose test strips (EasyTouch®), blood lancets, adult male and female white rats (*Rattus norvegicus*), streptozotocin (STZ), citrate buffer (pH 4.5), glibenclamide, sildenafil, and sodium carboxymethyl cellulose (NaCMC). Additional reagents included ashless filter paper, concentrated nitric acid (HNO_3), perchloric acid ($HClO_4$), hydrochloric acid (HCl) 0.2 N and 2 N, 2% NaCl solution, 1% gelatin solution, Dragendorff's reagent, 1% $AlCl_3$ solution, chloroform, concentrated sulfuric acid, standard solutions of Pb and Cd, and 0.5 M HNO_3.

2.1.2. Equipment

The equipment used comprised maceration vessels, a rotary evaporator, a water bath, and laboratory glassware (*Pyrex*®). Subsequent chromatographic profiling was executed on a Thermo Scientific™ Dionex™ Ultimate 3000 RSLCnano UHPLC system coupled with a Thermo Scientific™ Q Exactive™ High-Resolution Mass Spectrometer, equipped with a Phenyl Hexyl column (100 mm × 2.1 mm). Additional instruments included a vortex mixer, micropipettes, autosampler vials, and 0.2 µm membrane filters (*Millex*®). Animal facilities included standard animal cages and a sexual behavior observation cage with dim red lighting. Other instruments included an analytical balance, animal scale, intraperitoneal syringes, oral gavage needles, a glucometer (*EasyTouch*®), stopwatch, and camera. Laboratory instruments also included an oven, furnace, desiccator, hot plate, magnetic stirrer, test tubes, test tube racks, volumetric pipettes, and an atomic absorption spectrophotometer.

2.2. Fermentation of simplicia

Solid-state fermentation was adapted from the method by Moon and Cha (2020) with slight modifications. One kilogram of powdered *Eurycoma longifolia* Jack root simplicia was mixed with 2 L of distilled water (1:2, w/v) and placed in sterile glass jars, followed by steaming for 30 min. After cooling to ambient temperature, the substrate was inoculated with 20 gof *Aspergillus oryzae*. The mixture was then spread evenly on plastic trays, covered with clean paper, and fermented aerobically at room temperature for 72 h. The fermented simplicia was dried at 60 °C for 48 h in a laboratory oven to yield the dried matrix ready for extraction [8].

2.3. Extraction method

The fermented *Eurycoma longifolia* Jack root simplicia underwent extraction via sequential maceration using 70% ethanol as the solvent. The initial maceration was conducted at a ratio of 1:7.5 (w/v) for 5 × 24 h with occasional stirring. Following filtration through standard filter paper, and the resultant filtrate was collected. The residue was then remacerated at a ratio of 1:2.5 (w/v) for 2 × 24 h and filtered. The pooled filtrates were subsequently concentrated under reduced pressure using a rotary evaporator at 60 °C and further evaporated using a water bath at 60 °C until a viscous extract was obtained. The extraction yield was subsequently calculated [9].

2.4. Extract standardization

2.4.1. Extract identity

Plant material identification was conducted for *Eurycoma longifolia* Jack. Botanical authentication was performed at the Research Support and Product Provision Unit of the Ministry of Health, Dr. Sardjito General Hospital, to confirm the taxonomic identity of the plant material used for extraction.

2.4.2. Organoleptic properties

Organoleptic evaluation of the extract included assessment of its physical form, color, odor, and taste. Observations were performed visually and through sensory evaluation.

2.4.3. Water-soluble extractive content

Approximately 2.5 g of the sample was macerated in 100 mL of distilled water under continuous agitation for 1 h, followed by a 18 h quiescent period. A 20 mL aliquot of the resultant filtrate was pre-concentrated using a water bath at 80 °C, and the remaining residue was dried at 105 °C until a constant weight was achieved. The extractive yield was calculated as a percentage of the dry residue mass relative to the initial dry sample weight.

2.4.4. Ethanol-soluble extractive content

To determine the ethanol-soluble extractive content, approximately 2.5 g of the sample was extracted with 100 mL of 95% ethanol, following the exact protocol described for the water-soluble extractives. The resulting filtrate was evaporated to dryness, and the residue was dehydrated at 105 °C until a constant mass was achieved. The final content was expressed as a percentage of the residue weight relative to the initial dry sample mass

2.5. Phytochemical constituents

2.5.1. Alkaloids (Dragendorff's test)

For alkaloid screening, 1 g of the extract was solubilized in 10 mL of distilled water and filtered. A 2 mL aliquot of the aqueous filtrate was subsequently treated with 1 mL of Dragendorff's reagent. The emergence of a distinctive reddish precipitate served as a positive indication for the presence of alkaloids [10].

2.5.2. Flavonoids

To screen for flavonoids, 1 g of the extract was dissolved in 10 mL of distilled water and filtered. The resulting filtrate was treated with a 1% $AlCl_3$ reagent in a test tube. The development of an intense yellow coloration, resulting from the aluminum-flavonoid

complexation, indicated the presence of flavonoid compounds [10].

2.5.3. Terpenoids (Salkowski's test)

Triterpenoid screening was conducted via a modified Salkowski test. Briefly, 1 g of the extract was dissolved in 10 mL of distilled water and filtered. A 1 mL aliquot of the aqueous filtrate was partitioned with chloroform, and the recovered organic phase was subsequently treated with a few drops of concentrated sulfuric acid. The development of a characteristic yellow coloration upon gentle agitation indicated the presence of triterpenoids [10].

2.5.4. Tannins

Tannin screening was performed via the gelatin precipitation assay. Briefly, 1 g of the extract was solubilized in 10 mL of distilled water and filtered. The filtrate was transferred into a test tube, followed by the addition of 1 mL of 2% NaCl solution and 1 mL of 1% gelatin solution. The formation of a distinct precipitate, resulting from protein-tannin complexation, served as a positive indication for the presence of tannins [10].

2.5.5. Saponins

A total of 0.5 g of the extract was homogenized with 10 mL of distilled water and agitated vigorously. The formation of persistent foam with a height of 1–10 cm persisting for at least 10 min and remaining stable after the addition of one drop of 2 N HCl indicated the presence of saponins [10].

2.6. Total ash content.

The total ash content of the fermented *Eurycoma longifolia* Jack root viscous extract was quantified gravimetrically. The viscous extract of fermented *Eurycoma longifolia* Jack root was incinerated in a furnace at 600 °C for 8 h until complete ash formation was achieved. Upon cooling to ambient temperature within a desiccator, the total ash yield was calculated relative to the initial dry matter weight.

2.7. Acid-insoluble ash content

Total ash was refluxed with 25 mL of 0.2 N HCl for 5 min, filtered through ashless filter paper, and the residue was washed with hot water and incinerated to constant weight. Acid-insoluble ash was expressed as the percentage of residue relative to sample dry weight.

2.8. Heavy metal contamination (Pb and Cd)

The levels of lead (Pb) and cadmium (Cd) were determined by atomic absorption spectrophotometry (AAS) after sample preparation using a wet digestion procedure. About 1.0 g of sample was digested with concentrated nitric acid (15 mL) and perchloric acid (5 mL) under heating at approximately 100 °C until a clear solution formed. The cooled digest was filtered, diluted to 10 mL with distilled water, and analyzed by atomic absorption spectrophotometry (AAS). Calibration curves for Pb and Cd were prepared in accordance with SNI 06-6989.16-2009 using working standard solutions within a concentration range of 0.05–1.0 ppm. Absorbance measurements were conducted at a wavelength of 228.8 nm. The relationship between concentration and absorbance was evaluated using linear regression analysis. Heavy metal levels were assessed following the Indonesian National Agency of Drug and Food Control (BPOM RI) Regulation No. 12 of 2014 on quality standards for traditional medicines.

2.9. Phytochemical profiling by UHPLC–HRMS

Phytochemical profiling was carried out using a Thermo Scientific™ Dionex™ Ultimate 3000 RSLCnano UHPLC system coupled with a Thermo Scientific™ Q Exactive™ high-resolution mass spectrometer. Separation was achieved on a Phenyl-Hexyl column (100 mm × 2.1 mm) using a mobile phase of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), operated at 0.20 mL/min over a 30 min run time with a 5 µL injection volume.

Sample preparation was carried out by dissolving 5 mg of extract in 1 mL of 70% ethanol, followed by vortex mixing for 3 min and subsequent filtration through a 0.2 µm membrane filter. Mass spectrometric analysis was performed using heated electrospray ionization (H-ESI) in both positive and negative ionization modes. Data acquisition was conducted in Full MS mode at a resolution of 70,000 FWHM and data-dependent MS/MS mode at 17,500 FWHM. Compound identification and data processing were performed using Thermo Scientific™ Compound Discoverer software [11].

2.10. Evaluation of blood glucose levels and sexual behavior activities

All animal experimental protocols were reviewed and approved by the Health Research Ethics Committee of Muhammadiyah University of Yogyakarta (Approval No. 022/EC-HC-KEPK FKIK UMY/IX/2025).

2.10.1. Evaluation of blood glucose levels

Dose determination and preparation of test substances were conducted as follows. Streptozotocin

(STZ) was prepared in citrate buffer adjusted to pH 4.5 and administered intraperitoneally at a dose of 40 mg/kg BW. A STZ-induced diabetes model (40 mg/kg BW, i.p.) was used, which predominantly reflects β-cell dysfunction with T1DM-like metabolic characteristics. For comparative purposes, glibenclamide (0.45 mg/kg BW) and sildenafil (4.5 mg/kg BW) were included as reference treatments for antidiabetic and male sexual behavior assessments, respectively. The fermented extract was administered at doses of 50, 100, and 150 mg/kg BW. All test substances were suspended in 0.5% sodium carboxymethyl cellulose (NaCMC) prior to administration.

Thirty-five male rats were randomly distributed into seven groups, each comprising five animals. The normal group (Group A) received 0.5% NaCMC without STZ induction, whereas the negative control group (Group B) was induced with STZ and administered 0.5% NaCMC. Group C served as the positive antidiabetic control and received streptozotocin (STZ) followed by glibenclamide at a dose of 0.45 mg/kg BW. Group D functioned as the positive aphrodisiac control and was treated with STZ and sildenafil at 4.5 mg/kg BW. Groups E to G were induced with STZ and subsequently administered the fermented extract at doses of 50, 100, and 150 mg/kg BW, respectively. Prior to treatment, all animals were acclimatized for 14 days and maintained under standard housing conditions with free access to feed and water.

Diabetes was induced by intraperitoneal administration of streptozotocin (STZ) after a 12-hour fasting period. Seventy-two hours following induction, fasting blood glucose (FBG) levels were measured using tail vein blood samples with a glucometer. Rats exhibiting FBG levels exceeding 250 mg/dL were considered diabetic. Subsequent FBG measurements were conducted on days 0, 7, 14, and 21 throughout the treatment period.

2.10.2. Evaluation of sexual behavior activity

Male sexual behavior was evaluated following the completion of the 21-day treatment period. Each male rat was transferred to an observation chamber and allowed an adaptation period of 5 min under dim red illumination. After acclimatization, a female rat was introduced into the chamber at a 1:1 pairing ratio. Behavioral observations were conducted for 30 min, and all relevant sexual behavior parameters were recorded for subsequent analysis. Male sexual performance was evaluated using several behavioral indices. Mount latency (ML) was defined as the elapsed time (s) between the introduction of the female and the first mounting attempt. Intromission latency (IL) represented the time interval (s) from female introduction to the first successful intromission. Ejaculation latency (EL) was measured as the duration (min) between the initial intromission and the first ejaculation event. Mount frequency (MF), intromission frequency (IF), and ejaculation frequency (EF) corresponded to the total numbers of mounts, intromissions, and ejaculations, respectively, recorded during the 30-min observation period. Post-ejaculatory interval (PEI) was defined as the time required (min) for the male rat to achieve the next intromission following ejaculation. Additional copulatory indices were calculated to further characterize sexual behavior. The intromission ratio (IR) was determined by dividing the number of intromissions by the combined total of mounts and intromissions. Inter-intromission interval (III) was calculated as the ejaculation latency divided by the total number of intromissions. Copulatory rate (CR) was obtained by dividing the sum of mounts and intromissions by the time interval from the first mount to ejaculation, excluding the ejaculation latency period [12].

2.11. Statistical analysis

Statistical evaluation was conducted using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Prior to hypothesis testing, data distribution was examined using the Shapiro–Wilk test, while variance homogeneity was assessed with Levene's test. Variables satisfying both assumptions were subjected to one-way analysis of variance (ANOVA), followed by Duncan's multiple comparison procedure to identify differences among groups. For datasets that failed to meet the requirements of normality and/or homogeneity, non-parametric analyses were applied using the Kruskal–Wallis test, with subsequent pairwise comparisons performed using the Mann–Whitney U test. Differences were considered statistically significant when the probability value was less than 0.05.

3. RESULT

3.1. Yield of fermented *Eurycoma longifolia* Jack root extract

The yield of fermented *Eurycoma longifolia* Jack root extract obtained in this study is presented in Table 1. The extraction yield reached 8.81%, which is markedly higher than yields reported in previous studies using 70% ethanol as the extraction solvent.

Table 1. Yield of fermented *Eurycoma longifolia* Jack root extract.

Solvent	Weight of Simplex (g)	Weight of Extract (g)	Yield (%)
Etanol 70%	918	80.87	8.81

Table 2. Quality standardization of fermented *Eurycoma longifolia* Jack root extract.

No	Parameter	Result
	Specific Parameters	
1	Extract Identification	
	Family	Simaroubaceae
	Latin Name	*Eurycoma longifolia Jack*
	Indonesian Name	Pasak Bumi
	Plant Part Used	Root
2	Organoleptic Test of Extract	
	Form	Thick
	Color	Dark Brown
	Odor	Characteristic
	Taste	Bitter, slightly sour
3	Water-Soluble Extractives	83.68% w/w
4	Ethanol-Soluble Extractives	37.77% w/w
5	Phytochemical Constituents	
	Alkaloids (Dragendorff's Test)	(+)
	Flavonoids	(+)
	Terpenoids (Salkowski's Test)	(+)
	Tannins	(+)
	Saponins	(+)
	Non-Specific Parameters	
1	Total Ash	3.17% w/w
2	Acid-Insoluble Ash	Not Detected (<0.01% w/w)
3	Heavy Metal Contamination	
	Pb	Not Detected (<0.01 ppm)
	Cd	0.155 ppm

3.2. Quality standardization of fermented *Eurycoma longifolia* Jack root extract

The quality standardization results of fermented *Eurycoma longifolia* Jack root extract are summarized in Table 2. The extract was obtained as a dark-brown viscous mass with a characteristic odor and a bitter, slightly acidic taste. The extract yielded water and ethanol-extractable fractions of 83.68% and 37.77% (w/w), respectively. Qualitative phytochemical analysis confirmed the occurrence of several classes of secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, and saponins. Evaluation of non-specific quality parameters indicated a total ash value of 3.17% (w/w), while no acid-insoluble ash was detected (<0.01% w/w). Assessment of heavy metal contamination revealed that lead concentrations were below the analytical detection threshold (<0.01 ppm), whereas cadmium was present at a concentration of 0.155 ppm.

3.3. HPLC–HRMS profiling of fermented *Eurycoma longifolia* Jack root extract

The UHPLC–HRMS analysis revealed a chemically complex profile consisting of 194 chromatographic peaks detected in positive and negative ionization modes (Figures 1 and 2). The detected features covered a broad retention time range, indicating the presence of structurally diverse metabolites within the fermented extract.

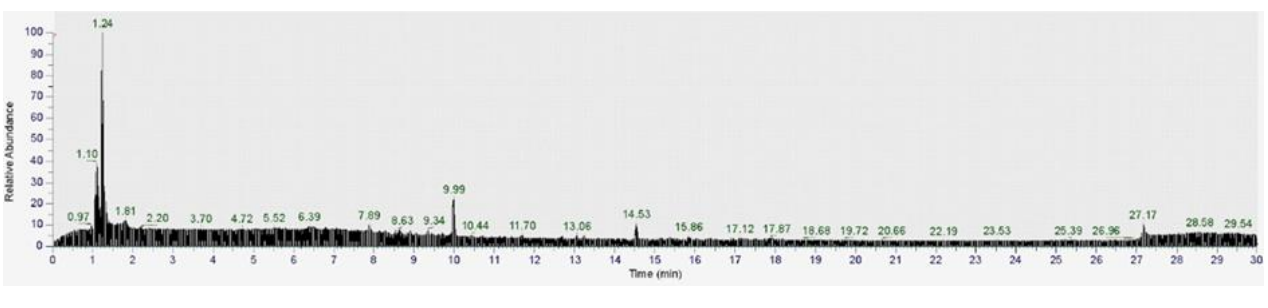


Figure 1. UHPLC–HRMS total ion chromatogram (positive ion mode) of fermented *Eurycoma longifolia* Jack root extract.

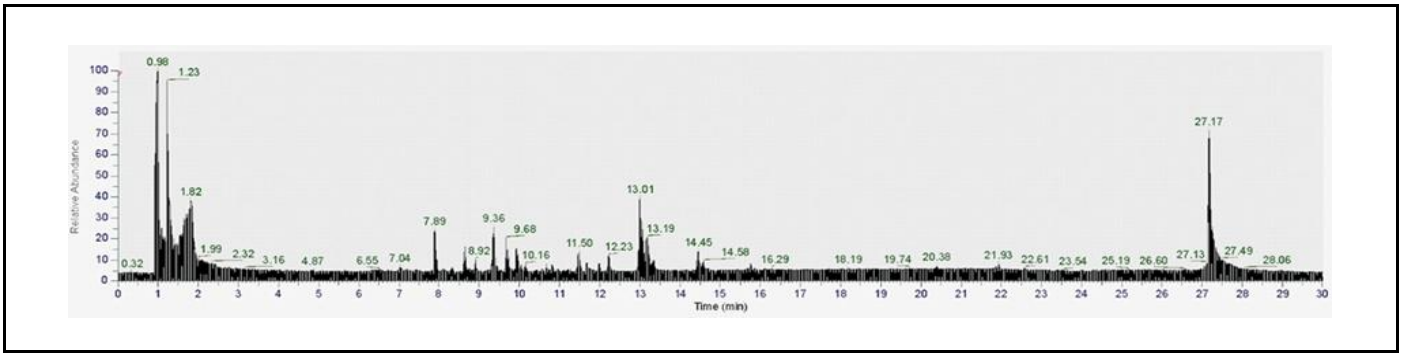


Figure 2. UHPLC–HRMS total ion chromatogram (positive negative mode) of fermented *Eurycoma longifolia* Jack root extract.

Metabolite annotation was performed using accurate mass measurements, isotope distribution patterns, retention time information, and MS/MS spectral matching against the mzCloud database. The annotated metabolites exhibited varying degrees of spectral similarity, with several compounds showing high-confidence matches [11]. The compounds with the largest relative peak areas were kojic acid (52.72%), palmitic acid (12.72%), 3-hydroxy-3-(2-pyridylmethyl)indolin-2-one (5.10%), and 2-amino-1,3,4-octadecanetriol (4.74%) (Table 3).

In addition to these major constituents, the extract contained amino acids, sugar alcohols, carbohydrates, organic acids, and other low-abundance metabolites. Agreement between the observed MS/MS fragmentation data and the proposed molecular structures provided additional validation for the putative metabolite assignments.

3.4. Effects of fermented *Eurycoma longifolia* Jack root extract on blood glucose levels

At baseline (Day 0), blood glucose levels were comparable among all experimental groups, ranging from 99.20 ± 7.40 to 110.60 ± 6.58 mg/dL, with no significant differences observed ($p = 0.504$). Following streptozotocin induction (Day 1), blood glucose levels increased markedly in all induced groups, confirming the successful establishment of hyperglycemia.

A progressive improvement in glycemic status was observed in extract-treated animals during the treatment phase when compared with diabetic controls (Table 4). At the final observation point (Day 21), blood glucose concentrations declined to 233.60 ± 30.68 mg/dL, 201.80 ± 24.96 mg/dL, and 164.40 ± 33.41 mg/dL following administration of 50, 100, and 150 mg/kg BW of the extract, respectively. In contrast, the negative control group maintained elevated blood glucose concentrations throughout the study period (353.40 ± 100.09 mg/dL). Analysis of percentage changes from baseline revealed dose-related reductions of 30.56%, 40.37%, and 49.42% following administration of 50, 100, and 150 mg/kg BW of the extract, respectively. Glibenclamide produced the most pronounced glucose-lowering effect, resulting in a 59.65% reduction, whereas sildenafil treatment was associated with a comparatively small decrease of 9.96%. By the end of the study period, blood glucose concentrations in the untreated diabetic controls remained higher than baseline values.

Non-parametric analysis revealed significant intergroup differences at all post-induction assessment points (Days 1, 7, 14, and 21; $p < 0.05$). Follow-up Mann–Whitney U comparisons further confirmed differences between selected treatment groups, as indicated by the superscript annotations in Table 4. Collectively, the data suggest that administration of fermented *Eurycoma longifolia* Jack root extract contributed to improved glycemic control in streptozotocin-induced rats.

3.5. Influence of fermented *Eurycoma longifolia* Jack root extract on male sexual behavior

The outcomes of sexual behavior assessment following treatment with fermented *Eurycoma longifolia* Jack root extract are summarized in Table 5. Statistical analysis revealed significant variation across all measured behavioral endpoints among the experimental groups ($p < 0.05$).

Measures related to copulatory initiation, namely mount latency (ML) and intromission latency (IL), were substantially prolonged in diabetic untreated animals relative to the treated groups. Administration of the fermented extract was associated with shorter ML and IL values compared with those recorded in the untreated diabetic controls. Among the extract-treated groups, the most pronounced reduction in latency measures was observed at 150 mg/kg BW, where ML and IL reached 101.00 ± 15.67 s and 120.60 ± 13.28 s, respectively

Behavioral indicators related to copulatory frequency, namely mount frequency (MF) and intromission frequency (IF), were elevated in animals receiving the fermented extract compared with untreated diabetic controls. Among the treatment groups,

Table 3. UHPLC-HRMS analysis of fermented *Eurycoma longifolia* Jack root extract.

No	Name	Formula	Annot. DeltaMass [ppm]	Calc. MW	RT [min]_ Min	RT [min]_ Max	Total Area (Max)	Area (%)	n_Peaks	mzCloud Best Match	MzCloud Best Match Co.	Precursor Ion m/z	Ion Type	Major MS/MS fragments (m/z)
1	Kojic acid	C6 H6 O4	-7.92	142.03	1.24	1.81	19506562885	52.72	3	99.90	93.40	143.03	[M+H]+1	126.03; 115.03; 97.02
2	Palmitic Acid	C16 H32 O2	66437.45	273.26	13.02	13.33	4707634869	12.72	2	84.20	52.50	274.27	[M+H]+1	256.23; 188.61; 169.38
3	3-hydroxy-3-(2-pyridylmethyl)indolin-2-one	C14 H12 N2 O2	-8.36	240.09	7.90	-	1888068292	5.10	1	75.20	8.80	241.09	[M+H]+1	223.08; 195.09; 181.07
4	2-Amino-1,3,4-octadecanetriol	C18 H39 N O3	-9.16	317.29	13.18	14.67	1752333338	4.74	2	72.20	8.60	318.29	[M+H]+1	256.26; 88.07; 70.06
5	L-Isoleucine	C6 H13 N O2	-6.92	131.09	1.22	1.58	926668883	2.50	2	99.60	10.00	132.1	[M+H]+1	86.09; 70.07; 62.74
6	L-Iditol	C6 H14 O6	-8.72	182.08	1.09	-	744098256	2.01	1	93.20	80.40	183.08	[M+H]+1	160.41; 129.05; 69.03
7	13-methoxy-1,6-diazatetracyclo	C15 H10 N2 O2	-8.63	250.07	12.23	12.76	591528144	1.60	3	99.80	10.00	251.08	[M+H]+1	236.06; 208.06; 179.06
8	D-(+)-Glucose	C6 H12 O6	255486.16	226.07	1.10	-	506716521	1.37	1	69.10	66.80	225.06	[M-H]-1	119.03; 89.02; 59.01
9	(2S,3S,4S,8R,9S,13R,14R,15R,16R)-3,4,8,14,15-pentahydroxy-2,13,16-trimethyl-6-methylidene-10-oxatetracyclo	C19 H28 O7	-48925.22	350.17	6.70	10.82	309088525	0.84	5	85.00	78.80	351.18	[M+H]+1	333.17; 315.16; 305.17
10	Acetyl-β-methylcholine	C8 H17 N O2	-8.03	159.12	1.22	-	290285753	0.78	1	70.20	44.30	160.13	[M+H]+1	101.06; 60.08; 55.05
11	Biotin	C10 H16 N2 O3 S	90044.47	266.07	7.69	11.89	275265435	0.74	7	76.00	8.80	267.07	[M+H]+1	252.05; 183.05; 160.63
12	4-Dodecylbenzenesulfonic acid	C18 H30 O3 S	-6.43	326.19	17.12	17.31	200503322	0.54	2	99.30	10.00	325.18	[M-H]-1	225.06; 201.99; 183.01
13	D-(+)-Arabitol	C5 H12 O5	-10.04	152.07	1.13	-	196509462	0.53	1	64.30	65.90	151.06	[M+H]+1	101.02; 89.02; 71.01
14	D-(+)-Maltose	C12 H22 O11	-6.32	342.11	1.07	1.10	187153130	0.51	3	74.50	67.00	387.11	[M+FA-H]-1	341.12; 221.06; 179.05
15	L(-)-Carnitine	C7 H15 N O3	-7.94	161.10	1.10	-	171722040	0.46	1	99.80	93.40	162.11	[M+H]+1	103.04; 85.03; 60.08
16	Citraconic acid	C5 H6 O4	-13.86	130.02	1.24	1.83	169768778	0.46	3	93.50	9.70	129.02	[M-H]-1	101.02; 85.03; 71.01

Table 3. UHPLC-HRMS analysis of fermented *Eurycoma longifolia* Jack root extract. (Continued).

No	Name	Formula	Annot. DeltaMass [ppm]	Calc. MW	RT [min] _Min	RT [min] _Max	Total Area (Max)	Area (%)	n_Peaks	mzCloud Best Match	MzCloud Best Match Co.	Precursor Ion m/z	Ion Type	Major MS/MS fragments (m/z)
17	3-Hydroxybutyric acid	C4 H8 O3	-16,84	104,05	1,85	-	165375544	0,45	1	75,70	8,80	103,04	[M-H]-1	96,26; 73,03; 57,03
18	DL-Stachydrine	C7 H13 N O2	-7,41	143,09	1,20	-	136367383	0,37	1	98,60	9,90	144,1	[M+H]+1	98,09; 85,03; 70,06
19	NP-020205	C11 H16 O4	-8,43	212,10	12,15	13,24	129789759	0,35	3	82,60	70,20	211,09	[M-H]-1	167,10; 164,93; 136,94
20	L-Tyrosine	C9 H11 N O3	-7,46	181,07	1,22	-	129460350	0,35	1	99,40	98,20	182,08	[M+H]+1	165,05; 147,04; 136,07
21	L-(+)-Lactic acid	C3 H6 O3	-19,27	90,03	1,27	-	121002088	0,33	1	99,20	10,00	89,02	[M-H]-1	87; 71,01; 59,01
22	Muramic acid	C9 H17 N O7	-127551,21	219,07	1,08	-	119353465	0,32	1	62,70	65,80	252,1	[M+H+MeOH]+1	234,09; 216,08; 188,09
23	Dodecyl sulfate	C12 H26 O4 S	-6,36	266,15	15,38	-	106594261	0,29	1	98,90	9,90	265,15	[M-H]-1	112,98; 96,96; 79,95
24	N-Acetyl-α-D-glucosamine	C8 H15 N O6	-7,25	221,09	1,13	-	96074541	0,26	1	98,00	98,40	222,09	[M+H]+1	204,08; 168,06; 138,05
25	L-Glutamic acid	C5 H9 N O4	-7,59	147,05	1,09	-	95025179	0,26	1	99,20	89,80	148,06	[M+H]+1	130,04; 102,05; 84,04
		Area (Max.) Total (194 Peaks)	36997977738,00											

the greatest MF and IF values were recorded in rats administered 150 mg/kg BW of the extract, reaching 15.80 ± 1.48 and 18.60 ± 1.95, respectively.

A pronounced reduction in PEI was observed across the extract-treated groups when compared with the untreated diabetic group. Likewise, CR values were consistently higher among treated animals. Taken together, the results indicate a beneficial effect of fermented *Eurycoma longifolia* root extract on several behavioral measures of male sexual activity.

4. DISCUSSION

4.1. Yield of the fermented *Eurycoma longifolia* Jack root extract

Extraction of fermented *Eurycoma longifolia* Jack roots using 70% ethanol resulted in a crude extract yield of 8.81%. This yield exceeded the value reported by Chua et al. (2019), who obtained approximately 3.5% crude extract from *Eurycoma longifolia* Jack roots using a reflux extraction procedure with the same solvent concentration [13]. Nevertheless, comparisons across studies should be approached cautiously because extraction outcomes are highly dependent on differences in raw material characteristics, processing methods, extraction parameters, and solvent systems.

Microbial fermentation has been widely investigated as a pre-extraction treatment capable of modifying the physicochemical characteristics of medicinal plants. Throughout fermentation, microbial hydrolytic enzymes such as cellulases, hemicellulases, and β-glucosidases may modify cell wall integrity, increasing access to metabolites located within plant cells. Fermentation using Aspergillus oryzae has been hypothesized to promote partial hydrolysis of plant cell wall constituents, thereby enhancing solvent penetration and increasing the accessibility of bioactive compounds during extraction. Such enzymatic biotransformation processes have been reported to alter phytochemical composition and may influence the recovery of bioactive constituents from herbal materials [6,14].

The comparatively high extract yield obtained in the present study may be related to structural and chemical modifications occurring during fermentation, which could improve the accessibility of extractable compounds. However, extraction efficiency is determined by numerous interacting variables, including botanical source, particle size distribution, moisture content, extraction technique, and solvent characteristics. Therefore, the observed yield cannot be attributed solely to the fermentation process.

The study design did not incorporate a non-fermented *Eurycoma longifolia* Jack extract prepared according to the same methodology, limiting direct assessment of the impact of fermentation. Consequently, the extent to which fermentation contributed to the extraction yield remains uncertain. Further investigations employing direct comparisons between fermented and non-fermented materials are necessary to establish the influence of fermentation on extract recovery and phytochemical availability.

4.2. Quality standardization of fermented *Eurycoma longifolia* Jack root extract

Herbal products require appropriate standardization procedures to support quality assurance, consistency, safety, and reproducible outcomes. The organoleptic characteristics observed in the present study, namely a dark-brown viscous extract with a characteristic odor and a bitter, slightly acidic taste, are

Table 4. Effects of fermented *Eurycoma longifolia* Jack root extract on blood glucose levels.

Day-	Mean and SD of Blood Glucose Levels (mg/dL) (n=5)							
	Normal Control	**Negative Control**	**Positive Control (Glibenklamid)**	**Positive Control (Sildenafil)**	**Extract Dose 50 mg/kgBW**	**Extract Dose 100 mg/kgBW**	**Extract Dose 150 mg/kgBW**	**P**
0	99.20 ±7.40	104.00 ±11.34	110.60 ±6.58	100.60 ±7.20	106.00 ±6.89	101.20 ±11.78	101.40 ±12.97	0.504
1	92.40 ±9.84^{a}	300.80 ±85.77^{b}	354.40 ±65.93^{b}	337.40 ±39.58^{b}	336.40 ±37.92^{b}	338.40 ±37.11^{b}	325.00 ±27.12^{b}	0.029
7	101.00 ±8.43^{a}	308.00 ±96.20^{b}	176.60 ±32.55^{d}	327.80 ±36.23^{b}	274.60 ±46.85^{b}	227.20 ±34.21^{bc}	182.60 ±21.93^{cd}	0.000
14	110.40 ±6.11^{a}	339.80 ±94.96^{bc}	159.00 ±32.41^{ef}	321.20 ±37.03^{b}	247.00 ±34.71^{cd}	218.40 ±28.59^{de}	172.00 ±25.14^{f}	0.000
21	108.80 ±6.83^{a}	353.40 ±100.09^{b}	143.00 ±30.59^{c}	303.80 ±35.41^{b}	233.60 ±30.68^{e}	201.80 ±24.96^{de}	164.40 ±33.41^{cd}	0.000
%	+17.75	+17.49	-59.65	-9.96	-30.56	-40.37	-49.42	

Note: Data are presented as mean ± SD (n = 5). Different superscript letters within the same row indicate significant differences among groups based on the Kruskal–Wallis test followed by the Mann–Whitney U test ($p < 0.05$).

Table 5. Effects of fermented *Eurycoma longifolia* Jack root extract on male sexual behavior parameters (Mean ± SD) (n=5).

Parameter	Control Groups				Fermented *Eurycoma longifolia* Jack Root Extract-Treated Groups			p-value
	Normal	Negative	+ Glibenklamid	+ Sildenafil	50 mg/kg BW	100 mg/kg BW	150 mg/kg BW	
(y)ML (Sec)	119.60±7.16^{a}	419.60±66.40^{c}	140.20±20.80^{b}	60.60±10.06^{d}	181.20±14.15^{e}	129.20±7.82^{ab}	101.00±15.67^{a}	0.000
(y)IL (Sec)	150.40±6.58^{a}	473.00±66.48^{b}	159.20±19.78^{a}	79.80±11.28^{c}	212.80±16.30^{d}	162.00±11.68^{a}	120.60±13.28^{e}	0.000
(y)EL (Min)	9.79±0.44^{a}	19.60±1.09^{b}	11.99±0.28^{c}	14.85±0.39^{d}	12.01±0.25^{c}	14.04±0.19^{e}	15.03±0.15^{d}	0.000
(z)MF	14.20±1.30^{cd}	6.80±2.28^{a}	11.20±1.30^{b}	17.40±1.14^{e}	13.00±1.00^{bc}	14.60±1.14^{cd}	15.80±1.48^{de}	0.000
(z)IF	15.60±1.14^{c}	4.80±2.28^{a}	12.20±0.84^{b}	21.80±1.30^{e}	14.40±1.67^{bc}	16.60±2.19^{cd}	18.60±1.95^{d}	0.000
(y)EF	2.00±0.00^{a}	1.00±0.00^{b}	2.00±0.00^{a}	2.60±0.55^{a}	2.00±0.00^{a}	2.00±0.00^{a}	2.60±0.55^{a}	0.000
(y)PEI (Min)	4.32±0.24^{a}	30.00±0.00^{b}	4.12±0.26^{a}	2.68±0.40^{c}	4.04±0.11^{a}	3.56±0.23^{d}	2.82±0.29^{c}	0.000
(z)IR	0.52±0.04^{a}	0.39±0.08^{b}	0.52±0.04^{a}	0.56±0.03^{a}	0.52±0.04^{a}	0.53±0.02^{a}	0.54±0.02^{a}	0.000
(y)III	0.63±0.06^{a}	6.87±7.66^{b}	0.99±0.07^{c}	0.68±0.06^{a}	0.84±0.09^{c}	0.86±0.11^{cd}	0.82±0.09^{c}	0.000
(z)CR	2.43±0.13e	0.43±0.19a	1.60±0.12b	2.43±0.13e	1.76±0.10bc	1.86±0.19cd	2.02±0.21d	0.000

Note: Data are presented as mean ± SD (n = 5). Parameters marked with (y) were analyzed using the Kruskal–Wallis test followed by the Mann–Whitney U test, whereas parameters marked with (z) were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT). Different superscript letters within the same row indicate statistically significant differences among groups ($p < 0.05$)

consistent with the typical properties of *Eurycoma longifolia* Jack root preparations. The characteristic bitter taste is generally attributed to quassinoid compounds, a group of metabolites considered among the principal bioactive components of *Eurycoma longifolia* Jack [15].

The markedly higher water-extractable fraction relative to the ethanol-extractable fraction (83.68% vs. 37.77% w/w) suggests that polar phytochemicals were predominant in the fermented extract. Extractive values are widely used as indicators of the amount of active constituents that can be extracted by solvents of different polarities and serve as important parameters for assessing the quality and consistency of herbal materials. The detection of multiple phytochemical classes, including alkaloids, flavonoids, terpenoids, tannins, and saponins, demonstrates the chemical complexity of the extract and corresponds with previously documented phytochemical profiles of *Eurycoma longifolia* Jack roots [1,16].

Ash values are important physicochemical parameters used to evaluate the purity of herbal materials and to detect contamination by inorganic matter. Total ash reflects the overall mineral content originating from both physiological and non-physiological sources, whereas acid-insoluble ash primarily indicates contamination by siliceous materials such as sand and soil. In the present study, the total ash content was relatively low (3.17% w/w), and acid-insoluble ash was not detected. These findings suggest minimal contamination by extraneous inorganic materials and indicate that the fermented extract was prepared from raw materials of acceptable quality. Similar observations have been reported in studies emphasizing the importance of ash values as quality-control parameters for herbal products [17].

Heavy metal contamination remains a major safety concern in herbal medicines because toxic metals can accumulate during cultivation, harvesting, processing, or storage. Extended exposure to potentially toxic metals, particularly lead and cadmium, is known to pose significant health risks, including renal injury, neurological abnormalities, and other systemic adverse effects. In the present study, lead was not detected (<0.01 ppm), while cadmium was detected at a low concentration (0.155 ppm), both of which were within the permissible limits established for herbal products. These results indicate that the fermented extract met recommended safety requirements with respect to heavy metal contamination. Similar safety considerations have been highlighted in recent assessments of herbal medicines, which emphasize the importance of routine monitoring of toxic metals as part of quality assurance programs [18].

4.3. UHPLC-HRMS analysis of fermented *Eurycoma longifolia* Jack root extract

The detection of 194 chromatographic peaks demonstrates the substantial chemical complexity of the fermented *Eurycoma longifolia* Jack root extract. Previous metabolomic investigations have similarly reported that *Eurycoma longifolia* Jack roots contain diverse classes of metabolites, including quassinoids, alkaloids, fatty acids, phenolic compounds, and various primary metabolites, reflecting the rich phytochemical composition of this medicinal plant [16,19].

Among the annotated metabolites, kojic acid represented the largest relative peak area. Kojic acid is a well-known secondary metabolite produced by several *Aspergillus* species, including *Aspergillus oryzae*,

through carbohydrate metabolism. Its occurrence in the fermented extract is therefore consistent with the microbial activity associated with the fermentation process [20]. Palmitic acid was identified as the second most abundant metabolite and is commonly found in both plant and microbial matrices. The detection of amino acids, carbohydrates, and various organic acids further highlights the chemical diversity of the extract.

Identification of putative metabolites was achieved through the integration of high-resolution mass accuracy data and MS/MS fragmentation pattern matching using the mzCloud reference database. Several compounds exhibited high spectral similarity scores and characteristic fragmentation patterns corresponding to their proposed structures. Nevertheless, because identification was not confirmed using authentic reference standards, the reported metabolites should be regarded as putatively annotated compounds rather than unequivocally confirmed structures.

Interpretation of the findings is limited by the absence of a non-fermented *Eurycoma longifolia* Jack comparator analyzed using the same experimental protocol. Consequently, although the metabolite profile of the fermented extract was successfully characterized, the specific contribution of fermentation to changes in metabolite composition could not be quantitatively determined. Future studies incorporating comparative metabolomic analysis between fermented and non-fermented extracts are warranted to elucidate fermentation-induced metabolic transformations**.**

4.4. Antidiabetic effects of fermented *Eurycoma longifolia* Jack root extract

A progressive reduction in blood glucose concentrations was observed over the 21-day treatment period in rats receiving fermented *Eurycoma longifolia* Jack root extract. At the conclusion of the experiment, glucose levels in all extract-treated groups were lower than those recorded in the untreated diabetic controls, indicating an association between extract administration and improved glycemic status in streptozotocin-induced rats.

The reduction in blood glucose levels tended to increase with dose, with the 150 mg/kg BW group exhibiting the greatest decline, followed by the 100 mg/kg BW and 50 mg/kg BW groups. While the response remained less pronounced than that observed with glibenclamide treatment, the highest dose of the extract was associated with considerably lower glucose concentrations than those recorded in the untreated diabetic control group.

Findings from earlier studies suggest a possible association between *Eurycoma longifolia* Jack root extracts and improvements in glucose regulation as well as metabolic function. Several bioactive constituents, particularly quassinoids, have been proposed to contribute to these effects through multiple biological pathways [21]. However, the present study did not investigate the underlying mechanisms responsible for the observed reduction in blood glucose levels. Therefore, the physiological processes involved remain to be elucidated through further biochemical and molecular investigations.

UHPLC–HRMS analysis revealed the presence of numerous metabolites within the fermented extract, including organic acids, fatty acids, amino acids, carbohydrates, and other secondary metabolites. While numerous metabolites were identified, the study was not structured to establish the specific contribution of individual constituents to the observed pharmacological responses. Consequently, no direct relationship can be established between specific metabolites and the reduction in blood glucose levels observed in this study.

The lack of a non-fermented extract comparator limits direct assessment of the role of fermentation in modifying biological activity. Therefore, while the fermented extract was associated with improvements in glycemic parameters, the extent to which these effects can be attributed to fermentation-related changes remains unclear. Future studies incorporating comparative analyses between fermented and non-fermented extracts are warranted to clarify the role of fermentation in modifying both chemical composition and biological activity.

4.5. Fermented *Eurycoma longifolia* Jack root extract on male sexual behavior

4.5.1 Latency parameters (ML, IL, and EL)

Mount latency (ML) and intromission latency (IL) are widely recognized behavioral measures reflecting sexual motivation and the ability to initiate mating activity. Statistical analysis revealed significant variation in both parameters across the experimental groups ($p < 0.05$). The untreated diabetic control animals displayed the highest ML and IL values (419.60 ± 66.40 s and 473.00 ± 66.48 s, respectively). In contrast, rats receiving the fermented *Eurycoma longifolia* Jack root extract showed markedly shorter latency periods. Among the extract-treated groups, the 150 mg/kg BW dose produced the lowest ML and IL values, reaching 101.00 ± 15.67 s and 120.60 ± 13.28 s, respectively. Multiple comparison testing further indicated that these values differed significantly from those recorded in the negative control group and were closer to the levels observed in sildenafil-treated animals.

The reduction in ML and IL suggests that the extract enhanced sexual motivation and reduced the time required to initiate successful copulatory behavior. These results are consistent with previous evidence

supporting the role of *Eurycoma longifolia* Jack in promoting male reproductive function and sexual performance. In a systematic review and meta-analysis, Leisegang et al. (2022) reported that *Eurycoma longifolia* Jack supplementation contributed to improvements in reproductive health parameters and testosterone levels, especially in men with low initial testosterone status [4].

Ejaculation latency (EL) varied significantly among treatment groups ($p < 0.05$). Compared with the negative control group, which exhibited an EL of 19.60 min, administration of the fermented extract was associated with shorter EL values ranging from 12.01 to 15.03 min. Although EL is frequently used as an indicator of copulatory performance, interpretation should be made cautiously because ejaculation latency is influenced by multiple physiological and behavioral factors. Therefore, EL should be considered together with other copulatory parameters when evaluating sexual performance.

4.5.2 Frequency parameters (MF, IF, and EF)

Mount frequency (MF) and intromission frequency (IF) are indicators of mating activity and copulatory performance. One-way ANOVA demonstrated significant differences among groups for both parameters ($p < 0.05$). The extract-treated groups exhibited substantially higher MF and IF values than the negative control group. The highest values were observed at 150 mg/kg BW, with MF and IF reaching 15.80 ± 1.48 and 18.60 ± 1.95, respectively. Duncan's post hoc analysis indicated that these values were significantly greater than those of the negative control group.

The increase in MF and IF indicates more frequent mating attempts and successful intromissions during the observation period. Similar findings have been reported in animal studies evaluating *Eurycoma longifolia* Jack, in which administration of root extracts enhanced several behavioral indicators of sexual performance. These observations support the traditional use of *Eurycoma longifolia* Jack as a medicinal plant associated with male reproductive health.

Ejaculation frequency (EF) also differed significantly among groups ($p < 0.05$). The highest EF values were observed in the sildenafil-treated group and the 150 mg/kg BW extract group. However, the numerical differences among treatment groups were relatively small, suggesting that EF may be less sensitive than other behavioral parameters for detecting treatment-related changes under the present experimental conditions.

4.5.3 Derived copulatory parameters (PEI, IR, III, and CR)

Post-ejaculatory interval (PEI) represents the recovery period required before a subsequent copulatory event can occur. Results of the Kruskal–Wallis test confirmed the presence of significant differences between treatment groups ($p < 0.05$). Among all experimental groups, the negative control group displayed the longest post-ejaculatory interval, reaching 30.00 ± 0.00 min, whereas all extract-treated groups showed markedly shorter intervals ranging from 2.82 to 4.04 min. Mann–Whitney U pairwise comparisons further showed that the extract-treated groups were significantly different from the untreated diabetic controls.

The reduction in PEI suggests a shorter recovery period following ejaculation and may indicate improved copulatory capacity. Similar reductions in PEI have been reported in previous studies investigating botanical products used to support male reproductive function.

Intromission rate (IR) also differed significantly among groups ($p < 0.05$). IR values remained largely unchanged across extract-treated groups and fell within the range observed for the normal control and sildenafil-treated animals. These findings suggest that administration of the fermented extract did not adversely affect copulatory efficiency.

Significant variations in both inter-intromission interval (III) and copulatory rate (CR) were observed across the experimental groups ($p < 0.05$). A less favorable pattern was observed in the negative control group, whereas extract-treated animals exhibited lower III values together with higher CR values. Increased CR values indicate improved overall mating performance and greater copulatory efficiency.

4.5.4 Dose–response relationship

A dose-dependent trend was observed for several behavioral parameters. The highest dose tested (150 mg/kg BW) was generally associated with reduced latency measures and enhanced frequency-related behavioral parameters. Nevertheless, treatment at 100 mg/kg BW produced a more balanced pattern of improvement across the evaluated parameters while preserving favorable copulatory performance. This pattern suggests that both doses were biologically active, although the optimal dose cannot be definitively established based solely on the current behavioral observations.

Furthermore, the present study did not include a non-fermented *Eurycoma longifolia* Jack extract control group. Consequently, although the fermented extract demonstrated measurable effects on male sexual behavior parameters, the contribution of the fermentation process itself cannot be determined. Future studies incorporating comparative evaluations between fermented and non-fermented extracts, together with hormonal, neurochemical, and molecular analyses, are necessary to clarify the biological significance of fermentation-induced phytochemical modifications.

4.6. Considerations regarding dose selection

The present study demonstrated that fermented *Eurycoma longifolia* Jack root extract produced measurable effects on both blood glucose regulation and male sexual behavior parameters in streptozotocin-induced rats. The 100 mg/kg BW dose was associated with a favorable balance of responses across both metabolic and behavioral assessments. This dose exhibited favorable effects on both metabolic and behavioral outcomes, characterized by lower blood glucose concentrations and improvements in several measures of male sexual behavior.

Although the highest dose (150 mg/kg BW) was associated with the greatest reduction in blood glucose concentrations, improvements in male sexual behavior parameters were not consistently greater than those observed at lower dose levels. Compared with the other tested doses, the 100 mg/kg BW treatment produced a balanced pattern of responses across metabolic and behavioral outcomes, warranting further evaluation in subsequent studies.

4. CONCLUSIONS

The present study showed that fermented *Eurycoma longifolia* Jack root extract met the required standardization criteria and demonstrated a diverse metabolite profile as revealed by UHPLC–HRMS analysis. Treatment with the fermented extract significantly lowered blood glucose concentrations and improved multiple parameters of male sexual behavior in streptozotocin-induced rats. Of the doses evaluated, 100 mg/kg BW elicited the most favorable overall biological response.

The findings suggest that fermented *Eurycoma longifolia* Jack root extract may possess glucose-lowering and male sexual behavior-modulating properties. However, the lack of a non-fermented extract control group precludes a direct assessment of the specific contribution of fermentation to the observed biological effects. Furthermore, metabolite identification was based on HRMS-assisted annotation and therefore requires additional confirmation using authentic reference standards and targeted analytical approaches.

Future studies should include non-fermented controls and employ validated metabolite identification approaches to better clarify the relationship between fermentation-induced alterations in metabolite profiles and the pharmacological properties of *Eurycoma longifolia* Jack root extract.

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Author contribution

Kiki Rizki Handayani: Conceptualization and study design; literature review; experimental studies; processing and analysis; manuscript drafting; Corresponding Author
Farah Widya Kautsari : Ethical clearance; experimental studies; literature research; manuscript writing.
Ayu Shabrina: Manuscript editing; manuscript review
SA: Manuscript editing; manuscript review
S: Experimental studies, manuscript editing

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Conflict of interest (If any)

None to declare.

Ethics approval

This study received ethical clearance from the Health Research Ethics Committee, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, with approval number 022/EC-HC-KEPK FKIK UMY/IX/2025.

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