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Protective properties of *Terminalia superba* extracts against fungal pathogens in *Drosophila melanogaster* (Diptericin-lacZ II) as a Model

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ABSTRACT

Fungal infections pose significant health challenges, and the identification of natural antifungal agents remains a growing area of research. *Terminalia superba*, known for its bioactive components, was investigated for its protective properties against fungal pathogens using Drosophila melanogaster (Diptericin-lacZ II) as a model organism. The plant materials were air-dried, pulverized, and extracted using solvents (n-hexane, ethyl acetate, methanol, and water) based on solvent polarity. The percentage yield was calculated, and phytochemical screening was carried out using standard methods. Antifungal susceptibility testing was conducted using the agar well diffusion method against *Aspergillus niger* and *Fusarium oxysporum*. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined using broth dilution techniques. Additionally, *D. melanogaster* flies were infected with *A. niger* to evaluate the in vivo antifungal efficacy of the extracts. Acute toxicity testing of the extracts was performed, and survival rates were recorded over seven days. The extraction process revealed that methanol produced the highest percentage yield of bioactive components, with 21.6% from the stem and 12.7% from the leaves. Hexane yielded 9% and 4.3% from the stem and leaves, respectively, while aqueous and ethyl acetate extracts produced lower yields. Phytochemical screening demonstrated the presence of tannins, saponins, flavonoids, steroids, and terpenoids in all extracts, whereas alkaloids were absent. Antifungal activity was highest in the hexane extracts, showing zones of inhibition ranging from 6 ± 1.53 to 18 ± 0.84 mm for stem extracts and from 4 ± 0.00 to 18 ± 0.55 mm for leaf extracts. Methanolic extracts also displayed significant inhibition, whereas ethyl acetate and aqueous extracts exhibited the least activity. The MIC and MFC assays revealed that most extracts had an MIC of 250 mg/ml and an MFC of 500 mg/ml. Probit analysis determined the LD50 values to be 47.18 mg/ml and 53.03 mg/ml for the methanolic and hexane extracts, respectively, while the standard drug, Fluconazole, had an LD50 value of 29.07 mg/ml. In infection studies, 60 mg/ml of the methanolic extract achieved a survival rate of 62.22%, comparable to fluconazole's 75.56%, whereas the hexane extract achieved a survival rate of 53.33%. These findings suggest that *T. superba* extracts, particularly the methanolic and hexane extracts, possess significant antifungal properties and may serve as potential alternative therapeutic agents against fungal pathogens.

Keywords: *Drosophila melanogaster*, antifungal activities, acute toxicity, survival rate.

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INTRODUCTION

Fungal infections remain a major global public health concern because of their increasing prevalence, associated morbidity and mortality, and the emergence of antifungal-resistant strains (Mudenda, 2024). Opportunistic fungal pathogens such as Aspergillus niger, Candida albicans, and Fusarium oxysporum have been implicated in severe infections affecting immunocompromised individuals, agricultural systems,

and food security (Ekwomadu and Mwanza, 2023). The growing resistance of fungal pathogens to conventional antifungal drugs has created an urgent need for the discovery of safer, more effective, and affordable alternative therapies. Plant-derived natural products have gained substantial attention because they contain diverse bioactive compounds capable of targeting fungal cell walls, membranes, mitochondria, and biofilm formation pathways (Mulat et al., 2025). Recent studies have emphasized the importance of medicinal plants as promising sources of novel antifungal agents with reduced toxicity and improved therapeutic potential.

Medicinal plants belonging to the genus *Terminalia* are widely recognized for their pharmacological and ethnomedicinal importance (Zhang et al., 2015). Among these species, *Terminalia superba* has attracted increasing scientific interest because of its rich phytochemical composition and broad-spectrum biological activities. Various parts of the plant, including the stem bark and leaves, contain important secondary metabolites such as flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds, which exhibit antimicrobial, antioxidant, anti-inflammatory, and antifungal activities (N'do et al., 2024; Selvamurugan et al., 2025). These phytochemicals are known to interfere with fungal growth through membrane disruption, inhibition of spore germination, suppression of enzyme activities, and impairment of fungal metabolic pathways. Solvent extraction using polar and non-polar solvents has also been reported to influence the yield and efficacy of these bioactive compounds (Ferreira and Sarraguça, 2024). Consequently, *T. superba* represents a promising candidate for the development of alternative antifungal therapeutics.

The search for reliable experimental models to evaluate antifungal efficacy and host–pathogen interactions has led to the increased use of Drosophila melanogaster. *D. melanogaster* has emerged as an effective model for studying fungal infections because of its well-characterized innate immune system, genetic tractability, short life cycle, and low maintenance cost (Younes et al., 2020; Vidal et al., 2024). The immune response of the fruit fly shares remarkable similarities with mammalian innate immunity, particularly in antimicrobial peptide production and signaling pathways such as the Toll and Imd pathways (Cammarata-Mouchtouris et al., 2022). The Diptericin-lacZII transgenic strain serves as a valuable reporter model for monitoring immune activation and evaluating the protective effects of bioactive compounds against fungal pathogens. Recent investigations have demonstrated the usefulness of *D. melanogaster* in understanding fungal virulence, antifungal drug screening, and immune modulation studies involving medically important fungi (Zhou et al., 2024).

The increasing burden of antifungal resistance has intensified research into natural product-based therapeutics capable of overcoming the limitations associated with conventional antifungal drugs such as Fluconazole and Amphotericin B (Sousa et al., 2020). Many synthetic antifungal agents are associated with toxicity, high cost, limited accessibility, and the rapid emergence of resistant fungal strains (Vanreppelen et al., 2023). Natural bioactive compounds from plants have therefore become important alternatives because they possess multiple mechanisms of antifungal action and may reduce the risk of resistance development. Studies have shown that phytochemicals, including flavonoids, tannins, terpenoids, and phenolics, exhibit fungistatic and fungicidal activities by targeting fungal membrane integrity, ergosterol biosynthesis, oxidative stress pathways, and cell wall synthesis (Elgharbawy et al., 2020). These mechanisms make plant extracts valuable candidates for the management of invasive and opportunistic fungal infections.

Despite the growing interest in medicinal plants and fungal disease management, limited information exists regarding the protective properties of *Terminalia superba* fractions against fungal pathogens using *Drosophila melanogaster* as a model system. Most previous studies have focused primarily on the antibacterial or anti-inflammatory properties of *Terminalia* species, with little emphasis on in vivo antifungal efficacy and immune response evaluation (Courtney and Cock, 2022). Therefore, this study seeks to investigate the protective properties of bioactive components of *Terminalia superba* fractions against selected fungal pathogens in *D. melanogaster* (Diptericin-lacZII). The findings from this research may contribute to the discovery of novel antifungal compounds, provide insights into host–pathogen interactions, and support the development of affordable plant-based antifungal therapies with potential biomedical applications.

MATERIALS AND METHODS

Sample collection and identification

Terminalia superba leaves and stem bark were collected from the Federal College of Forestry Jos, located in Jos North Local Government Area of Plateau State, Nigeria. The plant was identified with voucher number UJH000299, and voucher specimens were prepared and deposited at the Department of Plant Science and Biotechnology, University of Jos. All plant materials were air-dried under shade conditions and pulverized into a fine powder (Ademiluyi et al., 2018).

Extraction of plant materials

Plant extracts were prepared using aqueous (distilled water) and solvent extraction methods involving ethyl

acetate, n-hexane, and methanol. Extraction was performed using the solvent partitioning method. Approximately 200 g of the powdered plant sample was transferred into a large Erlenmeyer flask and soaked in 1500 mL of petroleum ether for 72 h with continuous shaking using a mechanical shaker and intermittent stirring (Nakamura et al., 2017). The plant residue was rinsed three times to ensure exhaustive extraction and complete recovery of metabolites. The extract solution was concentrated to dryness using a rotary evaporator at 40°C, transferred into a beaker, and further dried to powder in a drying cabinet. The weight of each extract was then determined.

The final residue was air-dried and repacked for subsequent extraction with ethyl acetate, dichloromethane, and ethanol. The same procedure was repeated for the stem bark and root powder to obtain the various extracts.

Percentage yield of solvent extracts

After drying, the yield of each extract was measured separately. Extraction efficiency was quantified by determining the weight of each extract, and the percentage yield was calculated using the formula:

$$\text{Percentage Yield} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \times 100$$

(Nakbanpote et al., 2019).

Phytochemical determination

Test for alkaloids

About 0.5 g of each extract was stirred with 3 mL of 1% aqueous hydrochloric acid on a steam bath. One milliliter of the filtrate was treated separately with a few drops of Mayer's reagent, Dragendorff's reagent, and picric acid solution. The formation of precipitates with any of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract (Craker, 2018).

Test for saponins

About 0.5 g of each plant extract was shaken with water in a test tube. Persistent frothing upon warming indicated the presence of saponins (El Aziz et al., 2019).

Test for tannins

About 0.5 g of the plant extract was stirred with 1 mL of distilled water and filtered. Ferric chloride solution was added to the filtrate. The formation of a blue-black, green, or blue-green precipitate indicated the presence of tannins (Sieniawska, 2015).

Test for anthraquinones

Bornträger's test was used for the detection of anthraquinones. About 0.5 g of each extract was placed in a dry test tube, and 5 mL of chloroform was added and shaken for 5 minutes. The extract was filtered, and the filtrate was shaken with an equal volume of 100% ammonia solution. The appearance of a pink, violet, or red coloration in the ammoniacal layer indicated the presence of free anthraquinones (Fouillaud et al., 2018).

Test for cardiac glycosides

About 100 mg of the extract was dissolved in 70% alcohol and filtered. Three drops of lead sub-acetate were added to the filtrate and filtered again. The filtrate was extracted with 10 mL of chloroform using a separating funnel and concentrated to dryness. The resulting residue was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution. This solution was carefully underlaid with 1 mL of concentrated sulfuric acid. The formation of a brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides (Joubert, 2023).

Test for steroids and terpenes

A small quantity of each extract was dissolved in chloroform, and 1 mL of acetic anhydride was added, followed by two drops of concentrated sulfuric acid. The appearance of a pink coloration that gradually changed to bluish-green upon standing indicated the presence of steroids and terpenes (Kolekar et al., 2019).

Test for flavonoids

Five milliliters of dilute ammonia was added to 5 mL of the extract, followed by the addition of 5 mL of concentrated sulfuric acid. The formation of a yellow coloration indicated the presence of flavonoids (Mehmood et al., 2022).

Test for carbohydrates

About 100 mg of each extract was dissolved in 3 mL of distilled water and mixed with a few drops of Molisch reagent (10% solution of naphthol in alcohol). One milliliter of concentrated sulfuric acid was carefully added down the side of the inclined test tube to form a layer beneath the solution. The appearance of a white ring at

the interface indicated the presence of carbohydrates (Copeland, 2016).

Source of microorganisms

Standard isolates of the fungi *Aspergillus niger* and *Fusarium oxysporum* were obtained from the National Veterinary Research Institute. The organisms were collected in suspension using Sabouraud broth (SB).

Antimicrobial susceptibility testing

Agar well diffusion technique

Antifungal susceptibility testing was carried out using clinical isolates of *Aspergillus niger* and *Fusarium oxysporum* through the agar well diffusion technique as described by Erhonyota et al. (2023). Fungal inocula were prepared from subcultures by suspending spores from three-day-old fungal cultures in broth, and the turbidity was adjusted to 0.5 McFarland standard.

The pour plate method was used for inoculation on Sabouraud Dextrose Agar (SDA). Ten milliliters of broth containing fungal spores was poured into sterile Petri dishes, after which 20 mL of molten SDA was added, mixed thoroughly, and allowed to solidify. Wells of approximately 6 mm in diameter were aseptically bored using a sterile cork borer, with five wells prepared per plate. Each well was filled with 100 µL of different concentrations of the plant extracts.

The plates were allowed to stand for 30 minutes to enable diffusion of the extracts into the agar before incubation at 37°C for 24 h. Zones of inhibition were measured to the nearest millimeter (mm).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The minimum inhibitory concentration (MIC), defined as the lowest concentration showing inhibitory activity, was determined using the broth dilution method. One milliliter of standardized broth culture containing the test organism was introduced into test tubes containing 5 mL of sterile broth. Subsequently, 200 µL of reconstituted extract at different concentrations was added to the test tubes, followed by incubation at 37°C for 24–48 h.

The tubes were examined for visible turbidity as evidence of microbial growth. The lowest concentration showing no detectable growth upon visual inspection was recorded as the MIC. Aliquots from tubes showing no visible growth were further subcultured onto fresh SDA plates to determine the minimum fungicidal concentration (MFC), which was defined as the lowest concentration showing no fungal growth after incubation (Ward et al., 2025).

Drosophila melanogaster fly stock selection

Drosophila melanogaster (Diptericin-lacZII) flies were obtained from the National Species Stock Center, Switzerland. The flies were maintained and reared on cornmeal medium at a temperature of 23 ± 1°C and 60% relative humidity under a 12 h light/dark cycle. All experiments were carried out using the same *D. melanogaster* (Diptericin-lacZII) strain (Zacchaeus et al., 2024).

Acute toxicity testing

Fly food was prepared using gram-per-diet ratios to obtain concentrations of 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 mg/mL. Acute toxicity testing was conducted by introducing 15 unsexed flies into each vial in triplicate, resulting in a total of 45 flies per concentration. Survival of the flies was monitored for seven days, and mortality was recorded (Zacchaeus et al., 2024).

Establishment of infection in flies

Flies were harvested and starved for 8–10 h before being introduced into Yeast Agar Glucose medium containing a three-day-old culture of *A. niger* for 6 h to establish infection. Following exposure, the flies were subjected to various treatments, and survival rates were monitored.

Experimental design

- **Group A:** Normal flies fed with a normal diet
- **Group B:** Normal flies fed with control diet
- **Group C:** Normal flies fed with control diet
- **Group D:** Infected flies treated with standard drug
- **Group E:** Infected flies fed with normal diet
- **Group F:** Infected flies treated with plant extract.

Data analysis

In vitro antifungal assay

Experiments were carried out in triplicate, and the data obtained were subjected to analysis of variance (ANOVA). Statistical significance was considered at $p \leq 0.05$.

In Vivo antifungal assay

Survival data were analyzed using probit analysis and Kaplan–Meier survival analysis with GraphPad Prism version 5.0.

RESULTS

The phytochemical screening of the leaf and stem extracts of *Terminalia superba* showed variations in the presence and abundance of phytochemical constituents across the different solvents used (Table 1). Alkaloids and cardiac glycosides were absent (−) in all the extracts examined. Saponins were absent in the hexane leaf extract but moderately present (+) in the hexane stem and ethyl acetate leaf extracts, while they were highly present (++) in the ethyl acetate stem, methanol leaf, methanol stem, water leaf, and water stem extracts. Tannins were present (+) in the hexane leaf and ethyl acetate leaf extracts, moderately present (++) in the hexane stem extract, and highly present (+++) in the ethyl acetate stem, methanol leaf, methanol stem, water leaf, and water stem extracts. Flavonoids were present (+) in the hexane leaf and hexane stem extracts, highly present (+++) in the ethyl acetate leaf, ethyl acetate stem, methanol leaf, methanol stem, and water stem extracts, while the water leaf extract showed moderate presence (++).

Table 1. Phytochemical screening of the extract of *Terminalia superba.*

Constituents	Hexane leaf	Hexane stem	Ethyle Acetate leaf	Ethyle Acetate stem	Methanol leaf	Methanol stem	Water leaf	Water stem
Alkaloids	-	-	-	-	-	-	-	-
Saponins	-	+	+	++	++	++	++	++
Tannins	+	++	+	+++	+++	+++	+++	+++
Flavonoids	+	+	+++	+++	+++	+++	++	+++
Carbohyrdrates	+	+	+	-	++	+++	++	++
Steroids	-	++	+++	+	-	+	-	-
Anthraquinones	-	+	+	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	-
Terpenoids	+++	-	++	-	+	-	-	-

Were + = Presence; ++= Moderately present; +++= Highly present; - = Absence.

Carbohydrates were present (+) in the hexane leaf, hexane stem, and ethyl acetate leaf extracts; absent (−) in the ethyl acetate stem extract; moderately present (++) in the methanol leaf, water leaf, and water stem extracts; and highly present (+++) in the methanol stem extract. Steroids were absent in the hexane leaf, methanol leaf, water leaf, and water stem extracts; moderately present (++) in the hexane stem extract; highly present (+++) in the ethyl acetate leaf extract; and present (+) in the ethyl acetate stem and methanol stem extracts. Anthraquinones were absent in most extracts but present (+) in the hexane stem and ethyl acetate leaf extracts. Terpenoids were highly present (+++) in the hexane leaf extract, moderately present (++) in the ethyl acetate leaf extract, present (+) in the methanol leaf extract, and absent (−) in all remaining extracts.

The percentage yield of the various extracts of *Terminalia superba* varied according to the solvent used and the plant part extracted (Table 2). For the leaf extracts prepared from 150 g of dry plant powder, the methanol leaf extract produced the highest yield of 19.1 g, corresponding to a percentage yield of 12.73%, followed by the hexane leaf extract, which yielded 13.5 g (9%). The water leaf extract produced 5.7 g with a yield percentage of 3.8%, while the ethyl acetate leaf extract recorded the lowest yield of 0.9 g, corresponding to 0.6%. In the stem extracts prepared from 200 g of dry plant powder, the methanol stem extract also recorded the highest extraction yield of 43.2 g, with a percentage yield of 21.6%. This was followed by the hexane stem extract, which produced 8.6 g with a yield percentage of 4.3%, while the water stem extract yielded 8.2 g, corresponding to 4.1%. The ethyl acetate stem extract produced 3.7 g with a percentage yield of 1.85%.

The antifungal activity of the leaf and stem bark extracts of *Terminalia superba* against Aspergillus niger showed a clear dose-dependent response across all solvent fractions (Table 3). At 62.5 mg/mL, inhibition zones ranged from 1 ± 0.07 mm in ethyl acetate stem bark extract (EaSS) to 5 ± 0.55 mm in both water stem bark extract (WSS) and methanol stem bark extract (MSS). At 125 mg/mL, inhibition increased across all extracts, with values ranging from 1 ± 0.17 mm in ethyl acetate leaf extract (EaLS) to 8 ± 0.82 mm in MSS and hexane stem bark extract (HSS). At 250 mg/mL, further increases were observed, with values ranging from 3 ± 0.15 mm (EaLS) to 11 ± 0.58 mm (HSS). At the highest concentration of 500 mg/mL, inhibition zones were highest overall, ranging from 4 ± 0.75 mm in EaLS to 15 ± 0.55 mm in HSS.

Among the extracts, HSS consistently recorded the highest antifungal activity across all concentrations (5 ± 0.12 mm at 62.5 mg/mL to 15 ± 0.55 mm at 500 mg/mL), followed by WSS, which showed strong activity (5 ± 0.55 mm to 12 ± 0.71 mm). MSS also demonstrated substantial activity (5 ± 0.58 mm to 11 ± 1.41 mm),

Table 2. Percentage yield of the various extracts of *Terminalia superba.*

Solvent	Weight of dry plant powder (gm)	Weight of dry extracts (gm)	Percentage yield (%)
Hexane leaf	150	13.5	9
Hexane stem	200	8.6	4.3
Ethyl acetate leaf	150	0.9	0.6
Ethyl acetate stem	200	3.7	1.85
Methanol leaf	150	19.1	12.73
Methanol stem	200	43.2	21.6
Water leaf	150	5.7	3.8
Water stem	200	8.2	4.1

Table 3. Antifungal activity of the leaves and stem bark of *Terminalia superba* on *Aspergillus niger.*

Extracts	62.5 mg/ml	125 mg/ml	250 mg/ml	500 mg/ml
WLS	2± 0.00	4± 1.52	6± 0.52	7± 0.67
MLS	4±0.51	5± 0.57	7± 0.12	9± 0.55
EaLS	1± 0.14	1± 0.17	3± 0.15	4± 0.75
HLS	2± 0.00	5± 0.41	7± 0.55	11± 0.82
WSS	5± 0.55	7± 0.51	10± 0.41	12± 0.71
MSS	5± 0.58	8± 0.82	10± 0.36	11±1.41
EaSS	1± 0.07	2± 0.00	4± 0.71	7± 4.44
HSS	5± 0.12	8± 0.55	11± 0.58	15± 0.55

whereas the ethyl acetate extracts (EaLS and EaSS) generally showed the lowest inhibition values across all concentrations. Leaf extracts (WLS, MLS, EaLS, and HLS) showed comparatively lower inhibition zones than their corresponding stem bark extracts at most concentrations, although the hexane leaf extract (HLS) showed relatively strong activity, reaching 11 ± 0.82 mm at 500 mg/mL.

The antifungal activity of the leaf and stem bark extracts of *Terminalia superba* against Fusarium oxysporum demonstrated a concentration-dependent increase in inhibition zones across all extracts tested (Table 4). At 62.5 mg/mL, inhibition zones ranged from 2 ± 0.00 mm in ethyl acetate stem bark extract (EaSS) and water stem bark extract (WSS) to 7 ± 0.29 mm in methanol leaf extract (MLS), while HSS also showed relatively high activity (6 ± 1.53 mm). At 125 mg/mL, inhibition values increased, ranging from 4 ± 0.58 mm (WSS) to 9 ± 0.58 mm (MLS and HSS), indicating improved antifungal effects with increasing concentration.

At 250 mg/mL, inhibition zones further increased, with values ranging from 5 ± 0.52 mm in EaLS to 13 ± 1.41 mm in HSS, while MLS also showed strong activity (12 ± 0.65 mm). At the highest concentration of 500 mg/mL, the inhibition zones were highest overall, ranging from 9 ± 2.12 mm in EaLS to 18 ± 0.84 mm in HSS. Across all concentrations, HSS consistently recorded the highest antifungal activity (6 ± 1.53 mm to 18 ± 0.84 mm), followed by MLS (7 ± 0.29 mm to 13 ± 0.71 mm) and EaSS (3 ± 0.15 mm to 11 ± 0.12 mm). Water and ethyl acetate extracts generally showed lower inhibition zones compared to hexane and methanol extracts.

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) results for the extracts of *Terminalia superba* against *A. niger* and *F. oxysporum* demonstrated a clear concentration-dependent inhibition pattern across all solvent fractions (Tables 5 and 6). For *A. niger*, all extracts (HLS, HSS, EALS, EASS, MLS, MSS, WLS, and WSS) showed growth (+) at 62.5 mg/mL, indicating no inhibitory effect at this lowest concentration. At 125 mg/mL, MSS was the only extract that still showed growth (+), while all other extracts exhibited no growth (−), indicating inhibitory activity beginning at this concentration for most fractions. At 250 mg/mL, inhibition became more evident, with growth observed only in HLS, EALS, MLS, and WLS, whereas HSS, EASS, MSS, and WSS showed no growth (−). At the highest concentration of 500 mg/mL, all extracts showed no growth (−), indicating complete inhibition of *A. niger* across all fractions.

In contrast, for *F. oxysporum*, all extracts showed growth (+) at 62.5 mg/mL, while at 125 mg/mL, only MSS showed no growth (−), indicating early inhibition at this concentration. At 250 mg/mL, complete inhibition (−) was observed in HLS, HSS, MLS, MSS, WLS, and WSS, while growth persisted only in EALS and EASS. At 500 mg/mL, all extracts showed no growth (−), indicating total inhibition of *F. oxysporum* at the highest concentration.

The probit analysis of the lethal concentration of *Terminalia superba* extracts on Drosophila melanogaster (Diptericin-lacZ II) revealed variation in toxicity levels among the tested treatments (Table 7). The methanolic extract produced a regression equation of $Y = 0.3655x + 3.27$ with a chi-square value of 11.34 ($P > 0.05$),

Table 4. Antifungal activity of the leaves and stem bark of *Terminalia superba* on *Fusarium oxysporum.*

Extracts	62.5 mg/ml	125 mg/ml	250 mg/ml	500 mg/ml
WLS	3± 0.51	6± 0.41	8± 0.45	10± 0.58
MLS	7±0.29	9± 0.58	12± 0.65	13± 0.71
EaLS	2± 0.00	4± 0.71	5± 0.52	9 ± 2.12
HLS	4± 0.00	6± 0.71	9± 2.12	13± 0.55
WSS	2± 0.00	4± 0.58	6± 0.00	9± 0.45
MSS	3± 1.41	5± 0.52	7± 0.52	10±0.67
EaSS	3± 0.15	5± 0.15	8± 0.50	11± 0. 12
HSS	6± 1.53	9± 0.58	13± 1.41	18± 0.84

Table 5. MIC and MFC results for *Aspergillus niger***.**

Concentration	HLS	HSS	EALS	EASS	MLS	MSS	WLS	WSS
500	-	-	-	-	-	-	-	-
250	+	-	+	-	+	-	+	-
125	+	+	+	+	+	-	+	+
62.5	+	+	+	+	+	+	+	+

Were - = absence of growth, + = presence of growth.

Table 6. MIC and MFC results for *Fusarium oxysporum.*

Concentration	HLS	HSS	EALS	EASS	MLS	MSS	WLS	WSS
500	-	-	-	-	-	-	-	-
250	-	-	+	-	-	-	-	-
125	+	+	+	+	+	-	+	-
62.5	+	+	+	+	+	+	+	+

Were - = absence of growth, + = presence of growth.

Table 7. Probit analysis of the lethal concentration of the mortality rate of *D. melanogaster* (Dipterian-lacz II) on the extracts of *Terminalia superba*.

Extracts	Regression equation	Chi square (P > 0.05)	LC 50	LC90	Lower	Upper
Methanolic	Y = 0.3655*x+3.27	11.34	47.18	219.94	0.99	1.83
n-Haxane	Y = 0.3264*x+2.41	9.19	53.03	269.73	1.366	2.262
Standard drug	Y = 0.3364*x+7.80	20.71	29.07	223.81	1.037	1.855

indicating a statistically acceptable model fit. It recorded an LC_{50} value of 47.18 and an LC_{90} value of 219.94, with lower and upper confidence limits of 0.99 and 1.83, respectively. The n-hexane extract showed a regression equation of $Y = 0.3264x + 2.41$ with a chi-square value of 9.19 ($P > 0.05$), an LC_{50} of 53.03, and an LC_{90} of 269.73, with confidence limits of 1.366 and 2.262.

The standard drug, Fluconazole, exhibited a regression equation of $Y = 0.3364x + 7.80$ and a chi-square value of 20.71 ($P > 0.05$), with the lowest LC_{50} value of 29.07 and an LC_{90} of 223.81, with confidence limits of 1.037 and 1.855. Comparatively, the methanolic extract demonstrated higher toxicity than the n-hexane extract, as indicated by its lower LC_{50} value, whereas the standard drug showed the highest toxicity among all treatments.

The infection studies further showed that 60 mg/mL of the methanolic extract compared favourably with 60 mg/mL of the standard drug, fluconazole, with survival rates of 62.22% and 75.56%, respectively, whereas the n-hexane extract produced a survival rate of 53.33% (Figures 1 and 2).

DISCUSSION

The phytochemical screening of *Terminalia superba* leaf and stem extracts demonstrated a varied distribution of secondary metabolites across different solvent fractions, and these findings are largely consistent with earlier

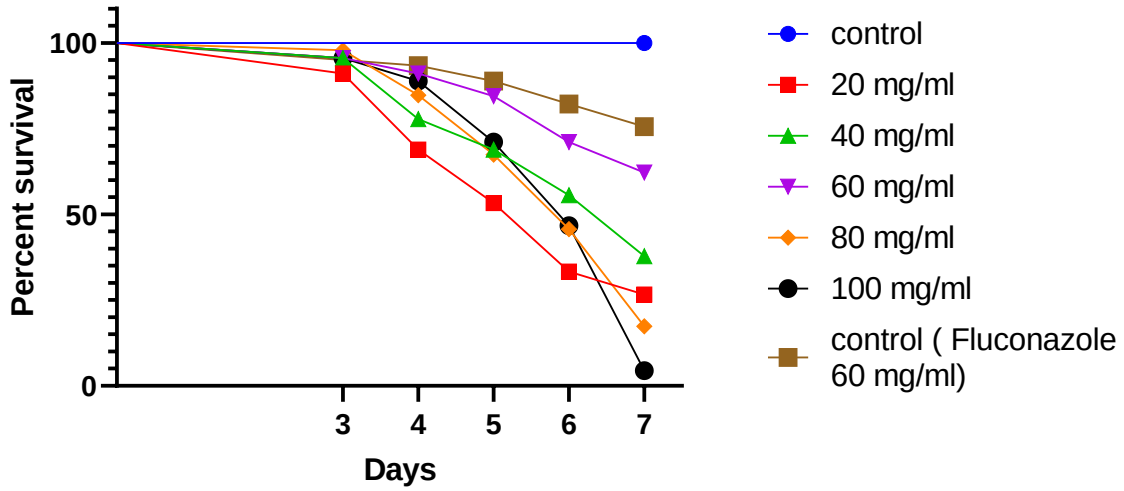

Figure 1. *Drosophila melanogaster* (Diptericin-lacZII) infected with *Aspergillus niger* and treated on methanolic stem extract of *Terminalia superba*.

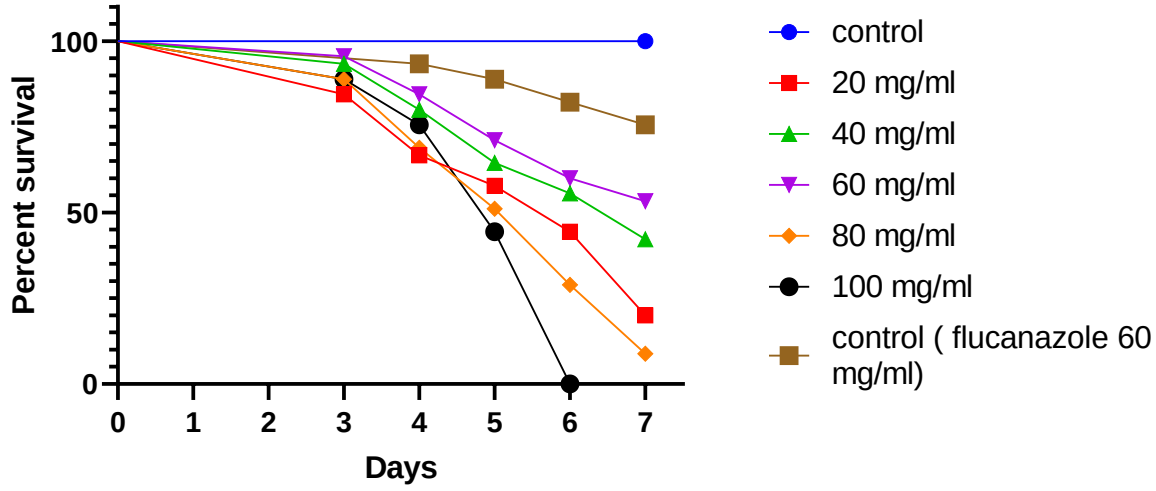

Figure 2. *Drosophila melanogaster* (Diptericin-lacZII) infected with *Aspergillus niger* and treated with hexane stem extract of *Terminalia superba*.

reports on the genus Terminalia. The absence of alkaloids and cardiac glycosides in all extracts aligns with Ani et al. (2023), who reported that these compounds are either absent or present in negligible amounts in *T. superba*, suggesting that they are not major phytochemical constituents of the species. The widespread presence of saponins, particularly their higher abundance in polar extracts, corresponds with Chitra et al. (2024), who noted that saponins in *Terminalia* species are more efficiently extracted using polar solvents because of their amphipathic nature. Similarly, the high levels of tannins and flavonoids observed across most extracts agree with Zai et al. (2024) and Sasikumar et al. (2024), who reported that these polyphenolic compounds are characteristic of *Terminalia* species and are effectively extracted using medium- to high-polarity solvents.

The moderate to high occurrence of carbohydrates across several fractions is also consistent with Long and Li (2024), who highlighted that their extraction is influenced by solvent polarity and plant matrix composition. The selective distribution of steroids, anthraquinones, and terpenoids across different solvent fractions further supports the findings of Chitra et al. (2024) and Zai et al. (2024), who emphasized that semi-polar and non-polar solvents preferentially extract these classes of compounds.

The variation in percentage yield of the extracts of *Terminalia superba* reflects the influence of solvent polarity and plant matrix characteristics on extraction

efficiency. Higher yields observed in polar solvent extracts compared to semi-polar and non-polar solvents suggest that polar solvents are more effective in solubilizing a wider range of phytoconstituents, including phenolics, flavonoids, saponins, and other polar secondary metabolites commonly reported in *Terminalia* species (Chitra et al., 2024; Ani et al., 2023). This pattern is consistent with previous studies on medicinal plants within the genus, where methanol and other highly polar solvents have been reported to produce greater extractable mass because of their strong penetration ability and broad-spectrum solvation capacity (Sasikumar et al., 2024).

In contrast, lower yields associated with less polar solvents indicate a more selective extraction of lipophilic compounds such as waxes and terpenoids, which are typically present in smaller quantities in plant tissues (Zai et al., 2024). Differences between leaf and stem extracts further reflect variations in tissue composition, with woody stem bark often containing more extractable secondary metabolites bound within lignified structures compared to leaves. Overall, these findings align with established phytochemical extraction principles and previous reports on *Terminalia* species, where extraction efficiency is strongly governed by solvent polarity, plant part specificity, and the distribution of secondary metabolites within plant tissues (Long & Li, 2024).

The antifungal activity of *Terminalia superba* leaf and stem bark extracts against *Aspergillus niger* exhibited a clear concentration-dependent increase in inhibition across all solvent fractions, which is consistent with the general antimicrobial behaviour of plant-derived extracts reported in previous studies. This progressive increase in activity with rising concentration aligns with Long and Li (2024), who noted that fungal growth inhibition by plant extracts typically intensifies as bioactive compound availability increases at higher doses. The comparatively stronger activity observed in stem bark extracts relative to leaf extracts is in agreement with Ani et al. (2023), who reported that the stem bark of *Terminalia* species often contains higher concentrations of bioactive secondary metabolites than leaves because of its structural and defensive role in plants.

Among the solvents, the relatively higher performance of hexane and methanol extracts corresponds with Zai et al. (2024) and Sasikumar et al. (2024), who emphasized that non-polar and polar solvents, respectively, are effective in extracting different classes of antifungal compounds such as terpenoids, flavonoids, and tannins that contribute to fungal inhibition. The moderate activity observed in water extracts also supports findings by Chitra et al. (2024), who reported that aqueous extracts of *Terminalia* species often exhibit antimicrobial effects because of the presence of water-soluble phenolic and saponin constituents, although they are typically less potent than organic solvent fractions. Conversely, the relatively weak inhibition recorded in ethyl acetate extracts is consistent with reports by Zai et al. (2024), which suggest that semi-polar solvents may extract lower quantities of antifungal metabolites in some medicinal plant matrices.

The antifungal activity of *Terminalia superba* leaf and stem bark extracts against *Fusarium oxysporum* showed a consistent concentration-dependent increase in inhibition zones across all solvent fractions, which is in agreement with established reports on plant-derived antifungal agents. This dose-responsive pattern aligns with Long and Li (2024), who observed that the antifungal effectiveness of phytochemical-rich extracts increases proportionally with concentration because of the greater availability of active constituents at higher doses. The generally stronger activity observed in stem bark extracts compared to leaf extracts is consistent with Ani et al. (2023), who reported that the stem bark of *Terminalia* species typically contains higher concentrations of defensive secondary metabolites responsible for antimicrobial activity.

Among the solvent systems, the superior performance of hexane and methanol extracts corresponds with findings by Zai et al. (2024) and Sasikumar et al. (2024), who highlighted that non-polar and polar solvents effectively extract terpenoids, flavonoids, and tannins that contribute significantly to antifungal activity. The moderate activity exhibited by aqueous extracts supports Chitra et al. (2024), who noted that water-based extracts often contain bioactive saponins and phenolics, although generally at lower potency compared to organic solvent extracts. Conversely, the relatively weaker inhibition observed in ethyl acetate fractions is in line with Zai et al. (2024), who reported that semi-polar solvents may yield lower concentrations of antifungal phytochemicals depending on plant matrix composition.

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) results of *Terminalia superba* extracts against *A. niger* and *F. oxysporum* demonstrated a progressive, concentration-dependent suppression of fungal growth across all solvent fractions, consistent with the established antifungal behaviour of plant-derived bioactive compounds. The absence of inhibitory activity at the lowest concentration for all extracts against *A. niger* aligns with Long and Li (2024), who reported that sub-inhibitory concentrations of plant extracts are often insufficient to disrupt fungal growth because of inadequate accumulation of active phytochemicals. The emergence of inhibitory effects at intermediate concentrations and the eventual complete suppression of growth at the highest concentration are consistent with findings by Sasikumar et al. (2024), who noted that the antifungal efficacy of *Terminalia*-derived compounds increases significantly with concentration as membrane disruption, enzyme inhibition, and oxidative stress effects become more pronounced.

The comparatively higher susceptibility of *F. oxysporum*

at earlier inhibitory thresholds in some extracts aligns with Zai et al. (2024), who observed species-dependent variation in fungal sensitivity to phytochemical agents, often linked to differences in cell wall composition and adaptive resistance mechanisms. The persistence of growth in some fractions at intermediate concentrations, particularly in semi-polar extracts, supports Chitra et al. (2024), who reported that solvent polarity strongly influences the spectrum and potency of extracted antifungal constituents, with polar and non-polar fractions often showing stronger bioactivity than semi-polar ones. The eventual complete inhibition of both fungal species at the highest concentration across all extracts is consistent with Ani et al. (2023), who highlighted that *Terminalia* species possess broad-spectrum antifungal compounds capable of exerting fungistatic and fungicidal effects at sufficient doses.

The probit analysis of the lethal concentration of *Terminalia superba* extracts on *Drosophila melanogaster* (Diptericin-lacZ II) revealed variation in toxicity levels across treatments, reflecting a clear dose–response relationship consistent with previous toxicological evaluations of plant-derived extracts. The methanolic extract demonstrated a lower LC_{50} compared to the n-hexane extract, indicating relatively higher toxicity, which agrees with Sasikumar et al. (2024), who reported that methanol extracts often exhibit stronger biological activity because of their higher capacity to solubilize polar bioactive compounds such as phenolics and flavonoids.

In contrast, the higher LC_{50} observed for the n-hexane extract is consistent with Zai et al. (2024), who noted that non-polar fractions may show reduced or variable toxicity depending on the presence of lipophilic active constituents. The standard drug recorded the lowest LC_{50} value, indicating the highest toxicity among all treatments, a trend consistent with Ani et al. (2023), who emphasized that synthetic reference compounds typically exert stronger biological effects than crude plant extracts. The acceptable chi-square values across treatments indicate a reliable model fit, while the LC_{90} values further confirm a graded increase in mortality with rising concentration, as previously described by Long and Li (2024) in similar dose-dependent toxicity studies.

The results of the in vivo infection studies demonstrated that the methanolic extract of *Terminalia superba* exhibited notable protective activity in *Drosophila melanogaster*, with survival outcomes that compared favourably to the standard antifungal drug, fluconazole, although the latter still produced higher survival rates. The relatively strong performance of the methanolic extract aligns with Sasikumar et al. (2024), who reported that methanol-derived plant fractions often show enhanced biological efficacy because of their ability to extract a wide spectrum of polar bioactive compounds such as flavonoids, tannins, and phenolics, which contribute to antimicrobial action.

The lower survival observed in the n-hexane extract is consistent with Zai et al. (2024), who noted that non-polar extracts may demonstrate reduced in vivo efficacy because of limited solubility and lower bioavailability of active constituents in biological systems. The superior activity of fluconazole compared to the plant extracts agrees with Ani et al. (2023), who emphasized that synthetic antifungal agents typically exert more rapid and targeted mechanisms of action than crude botanical preparations. Nevertheless, the observed in vivo protective effect of *T. superba* supports the findings of Chitra et al. (2024), who reported that species within the *Terminalia* genus possess significant antimicrobial properties attributable to their rich phytochemical composition. Similarly, Long and Li (2024) highlighted that plant-based extracts can demonstrate both in vitro and in vivo antimicrobial efficacy, reinforcing their potential role in alternative therapeutic development.

CONCLUSION

In the course of this study, all the different extracts of Terminalia superba leaves and stem bark exhibited antifungal properties both in vitro and in vivo, as they inhibited the growth of Aspergillus niger and Fusarium oxysporum isolates. These findings support the traditional medicinal use of the plant for the treatment of various ailments. Therefore, further studies aimed at isolating and characterizing the active compounds responsible for the observed antifungal activity are essential. Also, more investigations into the mechanism of action, pharmacological safety, and clinical applicability of the extracts are recommended to support the development of novel plant-based antifungal therapeutics.

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