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Biochemical response in *Drosophila melanogaster* infected with *Aspergillus niger* and treated with the methanolic stem extracts of *Terminalia species*

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ABSTRACT

Aspergillus niger is an opportunistic fungal pathogen capable of inducing oxidative stress, tissue damage, and persistent infection in susceptible hosts, posing significant clinical and public health challenges. *Drosophila melanogaster* is a well-established model for investigating host–pathogen interactions and evaluating therapeutic agents due to its conserved immune and biochemical pathways. Species of the genus *Terminalia* are rich in bioactive phytochemicals and possess antimicrobial and antioxidant properties; however, their antifungal and biochemical modulatory effects against *A. niger* remain poorly characterized. This study evaluated the biochemical and antifungal effects of methanolic stem bark extracts of *Terminalia catappa*, *Terminalia mantaly*, and *Terminalia avicennioides* in *D. melanogaster* infected with *A. niger*. Stem bark samples were extracted by cold maceration in methanol. Phytochemical screening was conducted using standard procedures, while antioxidant activity was assessed in vitro using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Acute toxicity was evaluated by exposing adult flies to diets containing 6.25–100 mg/mL of each extract. Virgin female flies were infected by ingestion of *A. niger* and treated with sub-lethal (LD_{50}-based) doses of the extracts. Data were analyzed using one-way ANOVA at $p \leq 0.05$. Phytochemical screening revealed abundant tannins, flavonoids, phenols, and saponins in all extracts, with variable alkaloids and steroids. *T. avicennioides* showed the highest antioxidant activity (41.95 ± 0.07% to 80.50 ± 0.33%), comparable to ascorbic acid. Acute toxicity tests indicated that *T. catappa* was the most toxic (LD_{50} = 62.47 mg/mL), while *T. avicennioides* was the least toxic (LD_{50} = 134.62 mg/mL). All extracts significantly improved survival of infected flies, with *T. avicennioides* producing the highest survival rate (77.77%). Biochemical analyses showed elevated malondialdehyde in *T. mantaly*, increased superoxide dismutase in *T. catappa*, and enhanced glutathione levels in *T. avicennioides*. Overall, methanolic extracts of *Terminalia* species, particularly *T. avicennioides*, demonstrated strong antifungal and antioxidant effects in *D. melanogaster*, supporting their potential as natural agents against fungal-induced oxidative stress.

Keywords: Antioxidant activity*, Aspergillus niger, Drosophila melanogaster***,** phytochemical modulation**,** *Terminalia species*.

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INTRODUCTION

Drosophila melanogaster, commonly known as the fruit fly, has long been used as a model organism in genetics and molecular biology because of its well-characterized genome, short life cycle, and ease of maintenance (Giansanti et al., 2025). Over the years, *Drosophila* has also emerged as a valuable model for studying host–pathogen interactions and immune responses, as approximately 75% of human disease-related genes have functional homologs in *Drosophila* (Younes et al., 2020). Infections by fungal pathogens such as *Aspergillus niger*, which cause aspergillosis-like infections in fruit flies, mimic many aspects of fungal diseases in higher organisms, making *Drosophila* an excellent system for investigating fungal pathogenesis and immune

modulation (Mpamhanga and Kounatidis, 2024). Fungal infections in *Drosophila* trigger a cascade of immune responses, primarily mediated by the Toll and Imd signaling pathways, and result in biochemical alterations that reflect the insect's defense mechanisms (Kleino and Silverman, 2014).

Aspergillus niger is an opportunistic fungal pathogen known for causing infections, particularly in immunocompromised hosts (Paulussen et al., 2017). Its ability to produce a wide range of extracellular enzymes and mycotoxins makes it a potent pathogen capable of inducing tissue damage and strong immune responses in infected organisms (Brown et al., 2021). In *Drosophila*, infection with *A. niger* is characterized by oxidative stress, tissue damage, and modulation of antioxidant defenses. The biochemical response involves changes in key enzymatic activities, including superoxide dismutase (SOD), catalase, and glutathione S-transferase (GST), which play critical roles in mitigating oxidative stress and restoring cellular homeostasis (Hanson and Lemaitre, 2020).

Species of the genus *Terminalia*, such as *T. catappa*, *T. mantaly*, and *T. avicennioides*, are well known for their rich phytochemical composition and broad therapeutic applications (Zhang et al., 2019). Most available evidence highlights their antioxidant, anti-inflammatory, and antimicrobial properties, which are largely attributed to flavonoids, tannins, and saponins (Amou et al., 2025; Kováč et al., 2022; Bourais et al., 2023). Methanolic extracts of these species have demonstrated inhibitory effects against fungi such as *A. niger* and the ability to influence immune-related processes in biological systems (Zacchaeus et al., 2024). However, in vivo evidence linking these phytochemicals to biochemical modulation during active fungal infection remains limited. Therefore, the use of *D. melanogaster* experimentally infected with *A. niger* provides a relevant model for addressing this gap by enabling direct assessment of *Terminalia* induced biochemical responses and their potential therapeutic value (Lopez-Ortiz et al., 2023).

The biochemical response of *Drosophila* to fungal infection under phytotherapeutic intervention typically involves alterations in antioxidant defenses, detoxification pathways, and stress-related metabolism. Previous studies have shown that plant-derived extracts can enhance detoxification enzyme activity, reduce lipid peroxidation, and modulate stress-responsive gene expression (Pratomo et al., 2022). These responses are essential for maintaining physiological stability during infection and suggest that methanolic extracts of *Terminalia* species may improve survival by strengthening antioxidant defenses and reducing fungal-induced biochemical stress. Consequently, this study aims to evaluate antioxidant activity, survival outcomes, and biochemical modulation in *D. melanogaster* infected with *A. niger* and treated with methanolic extracts of *Terminalia* species. It is hypothesized that treated flies will exhibit improved redox balance and altered biochemical markers compared with untreated infected controls.

MATERIALS AND METHODS

Study area

The study was conducted between January and September 2025 at the Drosophila Laboratory (Fungal Pathogens and Plant Bioactive Compounds), Department of Plant Science and Biotechnology, University of Jos, Nigeria.

Collection and identification of plant samples

Stem bark of the plant species investigated in this study was collected from Naraguta Village, Jos North Local Government Area, Plateau State, Nigeria, between March and April 2025 to ensure a high concentration of bioactive constituents. The plants were identified, assigned the voucher number JUHN25000448PSB, and voucher specimens were deposited at the Department of Plant Science and Biotechnology, University of Jos. All plant materials were air-dried under shade and pulverized into a fine powder.

Extraction of plant materials

Extraction was performed using the cold maceration method. Fifty grams (50 g) of each powdered stem bark sample was macerated in 500 mL of methanol for 72 h. The extracts were filtered using Whatman No. 1 filter paper and allowed to evaporate to dryness at room temperature (Nerlekar et al., 2024).

Phytochemical screening

The extracts were screened for phytochemical constituents, including alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids, anthraquinones, cardiac glycosides, and terpenoids, using standard qualitative phytochemical procedures as described by Onuh et al. (2021).

In vitro antioxidant activity assay (DPPH)

The free-radical scavenging activity of the extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined using the method described by Ochu et al. (2024). A 0.135 mM DPPH solution was prepared in methanol, and 1.0 mL of this solution was mixed with 1.0 mL of different extract concentrations (100, 50, 25, 12.5,

and 6.25 µg/mL). The mixtures were incubated in the dark at 27 °C for 30 min, after which absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as the reference standard.

The percentage scavenging activity was calculated as:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of samples}}{\text{Absorbance of blank}} \times 100$$

Drosophila melanogaster fly stock

The *D. melanogaster* (ISO) strain was obtained from the National Species Stock Center, Switzerland. Flies were maintained on cornmeal medium at 23 ± 1 °C and 60% relative humidity under a 12-h light/dark cycle. All experiments were conducted using the same strain (Wuyep et al., 2020).

Acute toxicity testing

Acute toxicity was evaluated following the method described by Wuyep et al. (2020). Fly food was prepared to contain extract concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL. Fifteen unsexed flies (≤24 h old) were placed in each vial in triplicate, giving a total of 45 flies per concentration. Fly survival was monitored daily for seven days, and mortality was recorded.

Source of test fungus

A clinical isolate of *Aspergillus niger* was obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria. The isolate was subcultured on Sabouraud Dextrose Agar (SDA) and identified based on macroscopic colony morphology and microscopic characteristics.

Establishment of fungal virulence

Frozen glycerol stock of *A. niger* was streaked onto Yeast and Glucose Agar (YAG) plates and incubated at 37 °C for 24 h. A single colony was transferred onto fresh YAG plates and incubated at 37 °C for 72 h to obtain a uniform lawn. Sterile distilled water (0.5 mL) was added to harvest the conidia. Virulence was confirmed based on the ability of the fungus to grow at 37 °C. Subsequently, 100 µL of the conidial suspension was spread onto YAG fly-food vials and incubated for 72 h at 37 °C to obtain a uniform conidial layer (Lionakis and Kontoyiannis, 2010).

Sexing and selection of flies

Flies were anesthetized on ice and sexed based on genitalia, body size, abdominal pigmentation, and foreleg bristles. Virgin female flies were identified by the presence of a dark ventral abdominal mark, an embryonic residue excreted 8–12 h after eclosion (Romeo and Lemaitre, 2008). Virgin females were used because they exhibit lower mortality following *Aspergillus* infection, and the use of a single sex minimizes sex-dependent variability in infection susceptibility.

Establishment of infection and treatment

Virgin female flies were starved for 8–10 h and then transferred to Yeast Glucose Agar containing a three-day-old culture of *A. niger* for 6 h to allow infection. Thereafter, flies were transferred to food containing sublethal (LD_{50}-based) concentrations of the methanolic extracts. Survival was monitored for seven days. Itraconazole (20 mg/mL) served as the positive control.

Homogenization of flies for biochemical assays

After the survival assay, live flies were collected for biochemical analysis. Twenty flies from each treatment group were anesthetized on ice and homogenized in phosphate-buffered saline (PBS, pH 7.4) at a ratio of 1:10 (w/v). Homogenization was carried out in pre-chilled tubes using a pestle while maintaining the samples on ice. The homogenates were centrifuged at 10,000 × g for 10 min at 4 °C, and the supernatants were collected for biochemical assays (Mukherjee and Mishra, 2019).

Biochemical assays

The activities of selected oxidative stress biomarkers, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), were determined in *Drosophila melanogaster* exposed to plant extracts, standard drugs, and untreated controls (Romeo and Lemaitre, 2008).

Malondialdehyde (MDA) assay

MDA levels, an index of lipid peroxidation, were quantified using a modified Ohkawa method (Ohkawa et al., 1979). Briefly, 200 µL of tissue homogenate was mixed with 1.5 mL of 20% acetic acid (pH 3.5), 1.5 mL of

0.8% thiobarbituric acid (TBA), and 200 µL of 8.1% sodium dodecyl sulfate (SDS). The mixture was adjusted to a final volume of 4 mL with distilled water and heated in a boiling water bath for 1 h. After cooling, samples were centrifuged at 4,000 rpm for 10 min at 4 °C. The absorbance of the supernatant was read at 532 nm using a UV–Vis spectrophotometer. MDA concentration was calculated from a standard curve and expressed as nmol/mg protein (Cui et al., 2021).

Superoxide Dismutase (SOD) assay

SOD activity was determined using the nitro blue tetrazolium (NBT) method based on Beauchamp and Fridovich (1971). The 3 mL reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 0.1 mM EDTA, 75 µM NBT, and 50 µL of enzyme extract. The reaction mixtures were exposed to a 400 W light source for 15 min, and absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT reduction by 50% and was expressed as units/mg protein (Shen et al., 2013).

Catalase (CAT) assay

Catalase activity was assessed using the method of Aebi (1984), which measures the rate of hydrogen peroxide (H_2O_2) decomposition. The reaction mixture contained 0.4 mL of enzyme extract and 2.6 mL of 50 mM phosphate buffer (pH 7.0) with H_2O_2. The decrease in absorbance was monitored at 240 nm. Catalase activity was calculated using a molar extinction coefficient of 43.6 mM^{-1} cm^{-1} and expressed as µmol/min/mg protein (Alam and Kataria, 2021).

Reduced Glutathione (GSH) assay

GSH levels were measured following the method of Ellman (1959), with minor modifications. Flies were homogenized in ice-cold 10% trichloroacetic acid (TCA) and 10 mM EDTA (1:1). After centrifugation at 5,000 rpm for 10 min, 200 µL of the supernatant was mixed with 0.2 M Tris buffer (pH 8.0) and 50 µL of DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)). The mixture was incubated at room temperature for 10 min, resulting in a yellow-colored complex. Absorbance was measured at 412 nm, and GSH concentration was expressed as units/mg protein (Alam and Kataria, 2021).

Protein estimation

Total protein content was determined using the Lowry method (Lowry et al., 1951). Briefly, 200 µL of tissue homogenate was mixed with Lowry's reagent and Folin-Ciocalteu phenol reagent. After incubation at room temperature, absorbance was read at 660 nm. Protein concentration was calculated from a standard curve prepared using bovine serum albumin (BSA) (Mukherjee and Mishra, 2019).

Statistical analysis

Acute toxicity data were analyzed using probit regression. Survival data of infected flies were analyzed using Kaplan-Meier survival curves. Biochemical and antioxidant data were analyzed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p < 0.05$. All analyses were performed using GraphPad Prism version 8.0.2.

RESULTS

Phytochemical screening of methanolic stem extracts of *Terminalia* species

Phytochemical screening of the methanolic stem extracts of *T. mantaly*, *T. catappa*, and *T. avicennioides* revealed marked differences in the presence and abundance of bioactive constituents (Table 1). Alkaloids were absent in *T. mantaly* and *T. catappa* but were clearly detected in *T. avicennioides* (++). Saponins were absent in *T. mantaly*, moderately present in *T. catappa* (++), and highly abundant in *T. avicennioides* (+++). Tannins and flavonoids were strongly represented in all species, with *T. mantaly* and *T. avicennioides* showing particularly high tannin levels (+++) and moderate-to-high flavonoid contents (++ to +++). Carbohydrates were detected in all species, with higher levels in *T. mantaly* and *T. catappa* (++). Steroids were absent in *T. mantaly*, weakly present in *T. catappa* (+), and moderately present in *T. avicennioides* (+). Anthraquinones and terpenoids were absent in *T. mantaly* but moderately detected in *T. avicennioides*. Cardiac glycosides were not detected in any of the extracts. Phenolic compounds were highly abundant in *T. mantaly* (+++), moderately present in *T. catappa* (++), and *T. avicennioides* (++).

DPPH radical scavenging activity of *Terminalia* species

All methanolic stem extracts exhibited concentration-dependent increases in DPPH radical scavenging activity (Table 2). At 6.25 µg/mL, *T. avicennioides* showed the highest scavenging activity (41.95 ± 0.07%), followed by *T. catappa* (39.49 ± 0.16%) and *T. mantaly* (36.29 ± 0.04%). With increasing concentration, *T. avicennioides* consistently demonstrated superior antioxidant activity, reaching 80.50 ± 0.33% at 100 µg/mL, compared with 74.41

Table 1. Phytochemical screening of the stem methanolic extracts of *Terminalia species*.

Constituents	*T. mantaly*	*T. catappa*	*T. avicennioides*
Alkaloids	-	-	++
Saponins	-	++	+++
Tannins	+++	++	+++
Flavonoids	++	+++	+++
Carbohyrdrates	++	++	+
Steroids	-	+	+
Anthraquinones	-	-	+
Cardiac glycosides	-	-	-
Terpenoids	+	-	+
Phenol	+++	++	+++

+ = presence, ++ = more present, +++ = highly present, - = absence.

Table 2. The percentage scavenging activity (SA_{DPPH}) of *Terminalia species* using DPPH radicals.

Plants	**6.25** µg/ml	**12.5** µg/ml	**25** µg/ml	**50** µg/ml	**100** µg/ml
T. mantaly	36.29 ± 0.04^{d}	43.16 ± 0.26^{d}	56.36 ± 0.17^{d}	59.61 ± 0.03^{d}	60.41 ± 0.13^{d}
T. catappa	39.49 ± 0.16^{c}	47.66 ± 0.12^{c}	59.61 ± 0.17^{c}	71.55 ± 0.19^{c}	74.41 ± 0.15^{c}
T. avicennioides	41.95 ± 0.07^{b}	52.92 ± 0.09^{b}	68.55 ± 0.03^{b}	73.89 ± 0.07^{b}	80.50 ± 0.33^{b}
Standard (AA)	58.55 ± 0.07^{a}	78.86 ± 0.02^{a}	83.39 ± 0.04^{a}	84.14 ± 0.03^{a}	84.35 ± 0.02^{a}
L.S.D	0.28				
P-value	<0.0001 ****				

± 0.15% for *T. catappa* and 60.41 ± 0.13% for *T. mantaly*.

The standard antioxidant, ascorbic acid, exhibited significantly higher scavenging activity at all concentrations, with 84.35 ± 0.02% at 100 µg/mL. At $p \leq 0.05$, significant differences were observed among the extracts. Values are presented as mean ± SEM, and means with the same superscript are not significantly different.

Probit analysis of Lethal Concentrations (LD_{50})

Probit analysis of the mortality of *D. melanogaster* (ISO) exposed to methanolic stem extracts of *Terminalia* species revealed clear differences in toxicity (Table 3). *T. catappa* showed the highest toxicity, with the lowest LD_{50} value (62.47 mg/mL), followed by *T. mantaly* (125.91 mg/mL) and *T. avicennioides* (134.62 mg/mL). The standard drug exhibited the greatest potency, with the lowest LD_{50} (29.07 mg/mL).

The chi-square values ($p > 0.05$) indicated a good fit of the mortality data to the probit model. The confidence limits further demonstrated the reliability of the LD_{50} estimates, with *T. catappa* showing a narrower confidence range than *T. mantaly* and *T. avicennioides*.

Table 3. Probit analysis of the sub lethal doses (LD_{50}) of the mortality rate of *D. melanogaster* (iso) on the extracts of *Terminalia species*.

Plants	Regression equation	Chi square (P > 0.05)	LD_{50}	Lower	Upper
T. catappa	Y = 0.2157*x+7.04	0.34	62.47	0.712	1.573
T. mantly	Y = 0.1542*x+5.63	1.07	125.91	0.561	1.468
T. avicennioides	Y = 0.1766*x+1.96	0.67	134.62	0.836	1.894
Standard drug	Y = 0.3364*x+7.80	20.71	29.07	1.037	1.855

Acute toxicity of *D. melanogaster* exposed to *Terminalia* extracts

Acute toxicity analysis revealed significant concentration and time-dependent mortality in *D. melanogaster* over seven days ($p \leq 0.05$). Kaplan-Meier survival curves showed that increasing extract concentration and exposure duration reduced fly survival in all treatment groups.

T. catappa exhibited the highest toxicity, with mortality increasing from 13.33% at 6.25 mg/mL to 60% at 100 mg/mL (Figure 1). *T. mantaly* caused moderate toxicity,

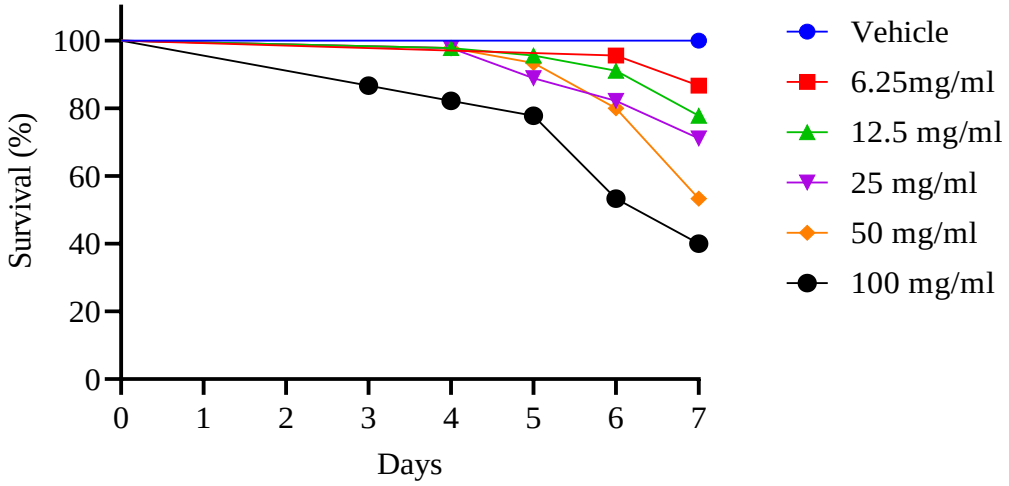

Figure 1. Kaplan–Meier survival curve for flies exposed to methanol extract of *T. catappa*.

with mortality ranging from 6.64% to 42.22% (Figure 2), while *T. avicennioides* was the least toxic, with mortality between 2.44% and 41% (Figure 3). In contrast, ascorbic acid caused 0% mortality at 6.25 mg/mL and 100% mortality at 100 mg/mL by day five (Figure 4).

In vivo antifungal efficacy against *A. niger*

Methanolic stem extracts of *Terminalia* species significantly improved the survival of *D. melanogaster* infected with *A. niger* ($p \leq 0.05$) at their respective sublethal (LD_{50}-based) doses. All treated groups showed survival rates above 50%, whereas the untreated infected control exhibited 100% mortality by day five.

T. avicennioides showed the highest antifungal efficacy (77.78% survival), followed by *T. mantaly* (64.44%) and *T. catappa* (53.33%). Ascorbic acid, used as the positive control, produced the highest survival rate (86.67%), confirming its strong antioxidant-mediated protective effect (Figure 5).

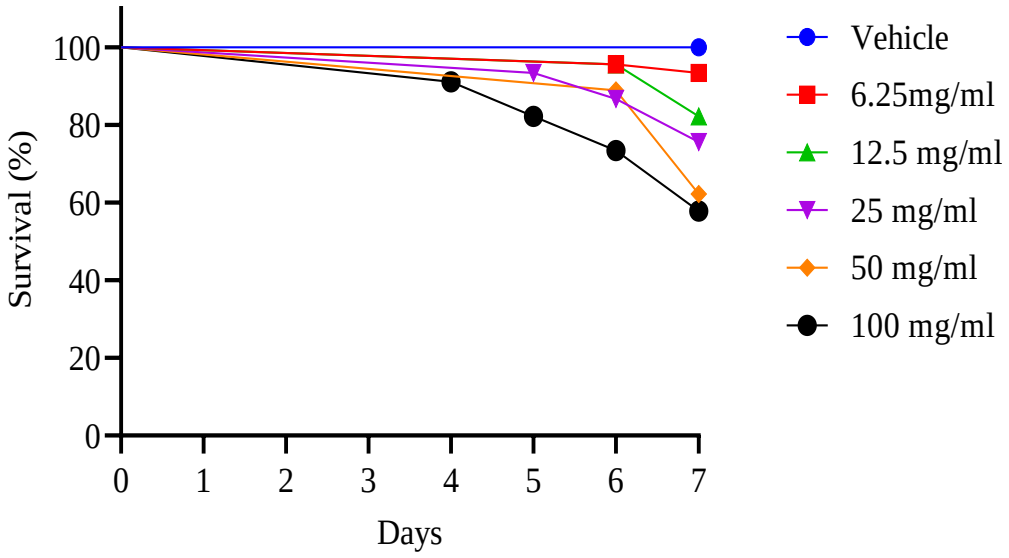

Figure 2. Kaplan–Meier survival curve for flies exposed to methanol extract of *T. mantaly.*

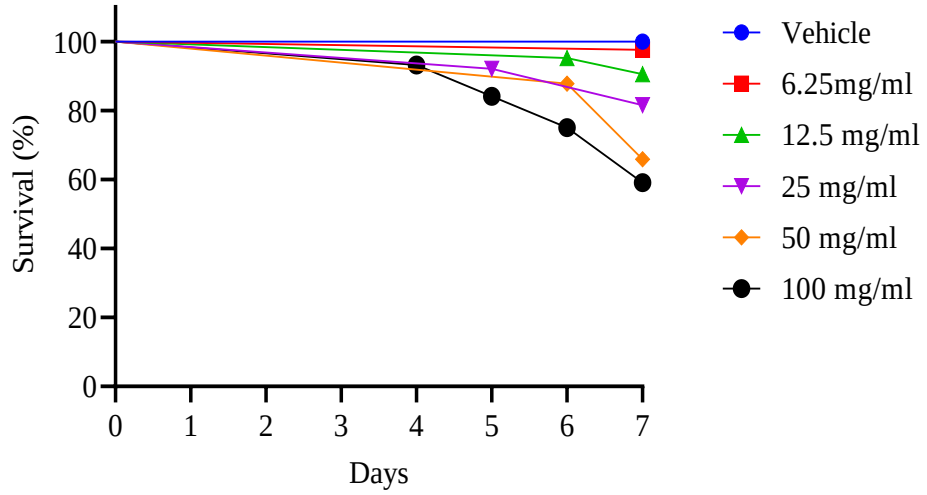

Figure 3. Kaplan–Meier survival curve for flies exposed to methanol extract of *T. avicennioides.*

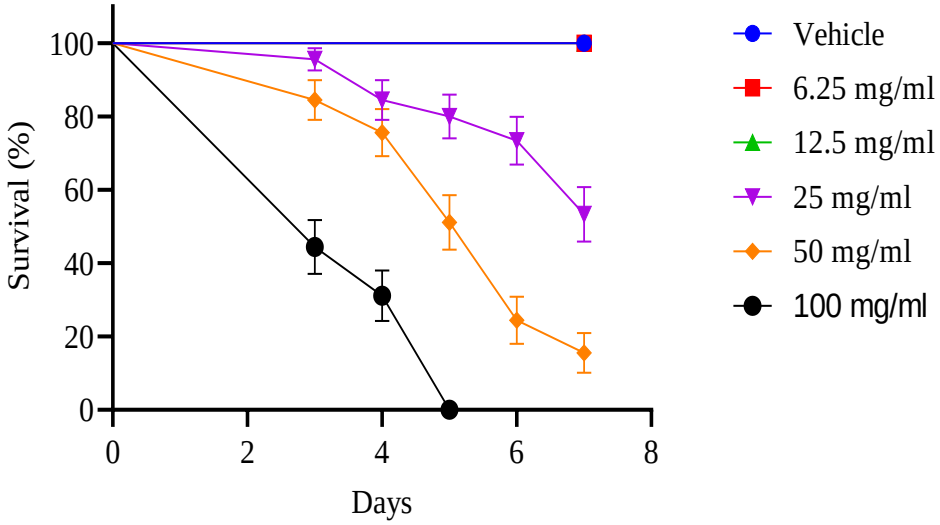

Figure 4. Kaplan–Meier survival curve for flies exposed to standard antioxidant ascorbic acid.

Biochemical responses in *D. melanogaster*

Significant treatment-dependent changes were observed in oxidative stress biomarkers ($p \leq 0.05$). Lipid peroxidation (MDA) was highest in flies treated with *T. mantaly* (0.062 ± 0.01 nmol/mg protein) but was markedly lower in *T. catappa* (0.0041 ± 0.002) and *T. avicennioides* (0.0052 ± 0.001), values comparable to ascorbic acid (0.0036 ± 0.002) and the normal control (0.003 ± 0.001) (Table 4).

SOD activity was highest in *T. catappa* (2.301 ± 0.002 µg/mg protein), followed by *T. avicennioides* (1.691 ± 0.005), and lowest in *T. mantaly* (0.971 ± 0.04). Ascorbic acid and the normal group showed intermediate values (1.454 ± 0.002 and 0.582 ± 0.004, respectively).

GSH levels were highest in *T. avicennioides* (135.79 ± 1.51 units/mg protein) compared with *T. mantaly* (52.44 ± 0.12) and *T. catappa* (52.38 ± 0.06), while ascorbic acid (601.32 ± 0.10) and the normal control (452.66 ± 2.89) showed markedly higher non-enzymatic antioxidant capacity.

Catalase activity was highest in the normal control (1.54 ± 0.06 µmol/min/mg protein), reduced in the ascorbic acid group (0.605 ± 0.01), and lowest among extract-treated groups, with *T. mantaly* (0.460 ± 0.02), *T. catappa* (0.351 ± 0.001), and *T. avicennioides* (0.221 ± 0.02).

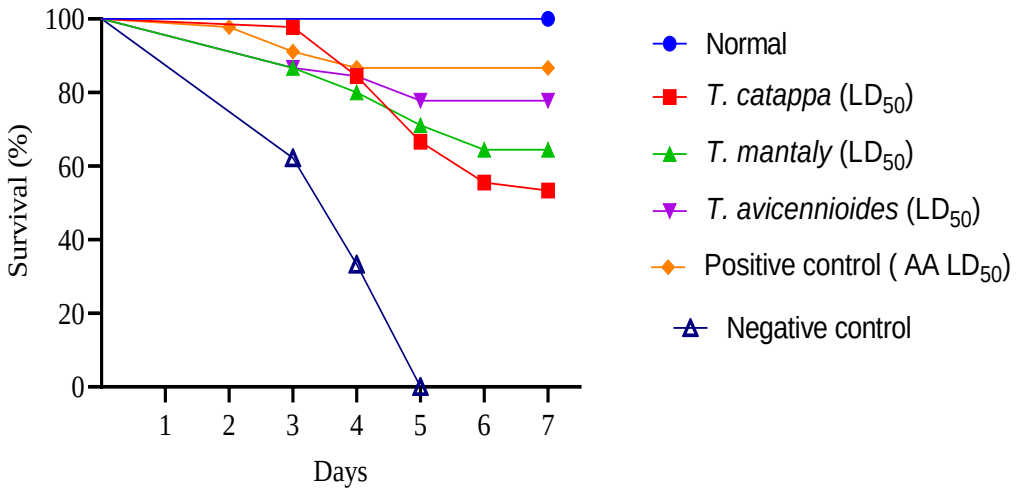

Figure 5: Kaplan–Meier survival curve for flies infected with *A. niger* and treated on methanol extracts of *Terminalia species*.

Table 4. Combined biochemical assay of *D. melanogaster* exposed to methanol stem extracts of *Terminalia species*.

Treatments	**MDA (**nmol/mg/pro.)	**SOD (**µg/mg/pro.**)**	**GSH (**units**/**mg/pro.**)**	**CAT (**µmol/min/mg/pro.**)**
T. mantaly	0.062± 0.01^{a}	0.971± 0.04^{d}	52.44± 0.12^{d}	0.460± 0.02^{c}
T. catappa	0.0041± 0.002^{b}	2.301± 0.002^{a}	52.38± 0.06^{d}	0.351± 0.001^{d}
T. avicennioides	0.0052± 0.001^{b}	1.691± 0.005^{b}	135.79± 1.51^{c}	0.221± 0.02^{e}
Stardard (AA)	0.0036± 0.002^{c}	1.454± 0.002^{c}	601.32± 0.10^{a}	0.605± 0.01^{b}
Normal	0.003± 0.001^{c}	0.582± 0.004^{e}	452.66± 2.89^{b}	1.54± 0.06^{a}
L.S.D	0.006	0.040	2.40	0.020
P-value	<0.0001 ****			

At P≤0.05 there was a significant difference in the biochemical assay of the *D.melanogaster* (iso strain) exposed to different methanol stem extracts of *Terminalia species*. Values are presented as mean±standard error of means. Ranking was done across the different plants and values with the same superscript are not significant.

DISCUSSION

The phytochemical variations observed in the methanolic stem extracts of *T. mantaly*, *T. catappa*, and *T. avicennioides* are consistent with previous reports indicating that *Terminalia* species are rich sources of phenolic compounds and flavonoids, which contribute substantially to their pharmacological properties (Mwangi et al., 2024). Wanyo et al. (2024) reported that tannins and flavonoids are dominant secondary metabolites in several *Terminalia* species and are closely associated with antioxidant, anti-inflammatory, and antimicrobial activities, supporting the strong presence of these compounds across the three species examined. The differential distribution of alkaloids and saponins observed in this study also agrees with earlier findings. Moomin et al. (2023) reported low alkaloid abundance in certain *Terminalia* species, which corresponds with their absence in *T. mantaly* and *T. catappa* in this study, whereas the notable presence of alkaloids in *T. avicennioides* suggests enhanced therapeutic potential, given the documented analgesic and anti-inflammatory roles of alkaloids. Similarly, Xiao et al. (2025) associated saponins with antimicrobial and cholesterol-lowering effects, supporting the potential biomedical relevance of their high abundance in *T. avicennioides* and moderate levels in *T. catappa*. The presence of phenols, terpenoids, and steroids in varying concentrations further indicates distinct biochemical and pharmacological capacities among the species. Hazra et al. (2021) demonstrated the strong free-radical scavenging capacity of phenolic compounds, which corresponds with the high phenol content observed in *T. mantaly*, while Aliyu-Amoo and Isa (2023) reported anti-inflammatory and immunomodulatory effects associated with terpenoids and steroids, consistent with their moderate detection in *T. avicennioides*.

The concentration-dependent antioxidant activity observed in the methanolic stem extracts of *T. avicennioides*, *T. catappa*, and *T. mantaly* is consistent

with previous studies demonstrating strong free-radical scavenging capacity in *Terminalia* species. Olugbami et al. (2015) reported potent DPPH radical-scavenging activity in methanolic extracts of *Terminalia*, attributing this effect to their high phenolic and flavonoid content, which aligns with the superior antioxidant activity of *T. avicennioides* observed in this study. Mwangi et al. (2024) similarly reported that tannins and flavonoids contribute significantly to the radical-quenching capacity of *Terminalia* species, supporting the concentration-dependent increase in antioxidant activity recorded here. The moderate-to-high antioxidant activities of *T. catappa* and *T. mantaly* also corroborate previous reports linking the antioxidant capacity of *T. catappa* to its phenolic content (Das et al., 2020) and describing *T. mantaly* as having moderate antioxidant potential due to lower concentrations of certain bioactive compounds (Yunusa et al., 2024).

The observed differences in toxicity among the three *Terminalia* species are consistent with earlier studies highlighting species-specific variations in phytochemical composition and biological potency. *T. catappa* has frequently been reported to exhibit higher toxicity, particularly toward invertebrates, a property attributed to its elevated concentrations of phenolics and tannins that can disrupt cellular and metabolic processes (Zanna et al., 2018; Meles et al., 2019). In contrast, *T. mantaly* and *T. avicennioides* generally exhibit lower toxicity, likely due to reduced concentrations of certain secondary metabolites, including alkaloids (Nwanze et al., 2020; Agbafor et al., 2016). Although less potent than synthetic standards, *Terminalia* extracts consistently demonstrate measurable bioactivity, supporting their potential as natural insecticidal or therapeutic agents (Sulaimon et al., 2020). The reproducible toxicity profile of *T. catappa* in particular suggests that it could serve as a reliable source of biologically active compounds.

The acute toxicity observed in *D. melanogaster* exposed to methanolic stem extracts of *Terminalia* species further supports the dose and time dependent nature of plant-derived bioactive compounds. Previous studies have shown that mortality in *Drosophila* is strongly influenced by both concentration and duration of exposure to phytochemicals (Singh et al., 2022; Lopez-Ortiz et al., 2023). In agreement with these findings, *T. catappa* exhibited the highest toxicity, reflecting its potent phytochemical profile, a pattern also reported in other studies on *Terminalia species* (de Oliveira et al., 2024), while *T. avicennioides* showed the lowest toxicity, consistent with reports that differences in flavonoid and tannin content modulate toxicological responses among *Terminalia* species (Amou et al., 2025). *T. mantaly* demonstrated intermediate toxicity, reinforcing the notion that *Terminalia* species vary widely in their biological effects depending on their phytochemical composition (Selvamurugan et al., 2025). The dose-dependent effects of ascorbic acid further validate the suitability of *Drosophila* as a sensitive model for evaluating both plant-based and synthetic
compounds.

The improved survival of *D. melanogaster* infected with *A. niger* following treatment with *Terminalia* extracts supports earlier evidence that plant-derived compounds possess antifungal properties against opportunistic filamentous fungi. Extracts of *Terminalia* species have been shown to inhibit the growth of *Aspergillus* spp., reinforcing their potential to reduce fungal pathogenicity in vivo (Samantaray et al., 2025). Although *T. catappa* is known to possess antifungal compounds, the greater protective effect observed for *T. avicennioides* in this study is consistent with reports that species specific phytochemical profiles influence antifungal potency and spectrum of activity (Ahmad et al., 2024). The moderate protection afforded by *T. mantaly* and *T. catappa* aligns with reports that antifungal activity within the genus varies depending on the abundance of phenolics, flavonoids, and related antimicrobial compounds (Jaafaru et al., 2024). The strong protective effect of ascorbic acid further confirms its role in enhancing host resistance to oxidative stress during fungal infection.

The biochemical responses observed in *D. melanogaster* exposed to *Terminalia* extracts indicate treatment-dependent modulation of oxidative stress and antioxidant defenses, consistent with earlier reports on plant-derived redox modulation (Oyeniran et al., 2022). The elevated MDA level in flies treated with *T. mantaly* suggests increased lipid peroxidation compared with *T. catappa* and *T. avicennioides*, a pattern consistent with reports that *T. mantaly* can induce oxidative stress in vivo despite having in vitro antioxidant activity (Oyetayo and Ogundare, 2013; Gomes et al., 2023). In contrast, SOD activity was highest in *T. catappa*, followed by *T. avicennioides*, reflecting the capacity of their bioactive compounds to stimulate enzymatic antioxidant defenses (Singh et al., 2022). GSH levels were highest in *T. avicennioides*, indicating superior non-enzymatic antioxidant protection, whereas the standard and normal groups showed markedly higher baseline antioxidant capacity. Catalase activity was highest in the normal group and lowest in extract-treated flies, particularly those treated with *T. avicennioides*, indicating differential regulation of hydrogen peroxide detoxification among treatments.

CONCLUSION

This study demonstrates that methanolic stem extracts of *Terminalia* species possess diverse phytochemical profiles and biologically relevant activities. All extracts exhibited concentration-dependent antioxidant activity, with *T. avicennioides* showing the strongest free-radical scavenging capacity. In vivo, *T. avicennioides* conferred the greatest antifungal protection against *Aspergillus*

niger infection in *D. melanogaster*, highlighting its potential as a natural antifungal and antioxidant agent. Biochemical analyses further revealed distinct antioxidant responses, with *T. avicennioides* showing elevated glutathione levels and *T. catappa* exhibiting the highest superoxide dismutase activity. Collectively, these findings support the therapeutic potential of *Terminalia* species, particularly *T. avicennioides*, as sources of bioactive compounds for managing oxidative stress and fungal infections.

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