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"From Antioxidants to Survival: Multifaceted Bioactivities of the Bark and Leaf of *Eucalyptus Globulus* Extracts in *Drosophila Melanogaster*"

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ABSTRACT

The study evaluated the antioxidant, negative geo-taxis, survival, and reproductive effects of Eucalyptus globulus, bark and leaf extracts in Drosophila melanogaster. This method of evaluation could help determine the potential of Eucalyptus globulus as a natural intervention against age-related decline, oxidative stress and overall health. Despite numerous studies on Eucalyptus globulus, its impact on specific functional parameters like survival, negative geotaxis, antioxidant enzymes and reproductive capacity in Drosophila melanogaster remains largely unexplored. It is against this background that the study was undertaken to investigate the antioxidant, the negative geotaxis, survival and the reproductive capacity of the plant in Drosophila melanogaster. The LC_{50} values obtained were 328 mg/10 g diet for the leaf extract and 180 mg/10 g diet for the bark extract, suggesting a wider safety margin in the leaf when related to the bark. Chronic exposure to the flies revealed the relative safety of the leaf and potentialmoderate toxicity of the bark. Negative geotaxis was unaffected by the leaf extract but significantly reduced in bark-treated flies. Both extracts suppressed fecundity, although the bark caused stronger inhibition. Biochemical analysis of the safer extract (leaf) revealed increased total protein and catalase activity with the leaf extract, alongside reduced glutathione-S-transferase (GST) activity, suggesting adaptive antioxidant responses to moderate oxidative stress. In conclusion, while the bark extract showed potential moderate toxicity, impaired locomotion and reduced reproductive capacity, the leaf extract demonstrated relative safety, sustained locomotor activity, and enhanced reproductive capacity at lower concentrations. Therefore, Eucalyptus globulus leaf stands as a safer candidate for pharmacological and nutraceutical exploration, offering antioxidant resilience without compromising fundamental biological functions.

Keywords: *Eucalyptus globulus, Drosophila melanogaster, antioxidant, toxicity, safety, oxidative stress, fecundity, survival, negative geotaxis*

INTRODUCTION

Eucalyptus globulus, a tree species widely recognised for its broad spectrum of pharmacological properties, has been the focus of numerous studies exploring its potential health-promoting effects [1]. *Eucalyptusglobulus* extracts have demonstrated strong antioxidant properties *in vitro* and *in vivo* in several studies. For example, methanol and acetone extracts from *E. globulus* leaves showed high phenolic content and strong capacity to ameliorate H_2O_2-induced oxidative stress in neuronal cell lines, increasing GSH levels and antioxidant enzyme activities while reducing lipid peroxidation [2]. Also, in rodent (mouse) studies, *E. globulus* leaf extract was found to reduce serum levels of ALT, AST, creatinine, and blood urea nitrogen, and improve antioxidant markers in a model of chemically-induced hepato-renal toxicity [3]. Despite numerous studies on *E. globulus*, the impact of *its* bark and leaf extracts on specific age-related functional parameters like negative geotaxis (a measure of locomotor ability), antioxidant enzymes, and reproductive capacity in *Drosophila melanogaster* remains largely unexplored. Therefore, there is a need to investigate the antioxidant, negative geo-taxis, survival, and reproductive effects of both bark and leaf extracts in *Drosophila melanogaster* to determine their potential as natural interventions against age-related decline, oxidative stress and general health [2]. The use of *Drosophila melanogaster* as a model organism is justified by its short lifespan, ease of genetic manipulation, and established use in ageing and toxicology research [4]. The negative geotaxis assay provides a quantitative measure of locomotor function, which declines with age and is sensitive to oxidative stress [5]. Survival assays offer a direct assessment of the impact of the extracts on lifespan, while reproductive assays provide insights into the effects on organismal fitness [4]. *E. globulus* was selected due to its known antioxidant properties and availability [1]. This approach, combining a relevant model organism, ecologically sound source materials, and functional endpoints, provides a strong justification for the study's potential to contribute valued knowledge to the field of natural antioxidants and healthy ageing [6] [7].

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METHODS

Plant Collection, Identification and Authentication

Fresh leaves and barks of *Eucalyptus globulus* were collected from a botanical garden in Jos North Local Government, Plateau State, Nigeria, in March, 2025.

The plant was identified and authenticated in the Department of Plant Science and Biotechnology, University of Jos. A voucher specimen number UJ/PCG/HSP/16M20 was issued, and the specimen was deposited in the faculty's herbarium for reference.

Drosophila melanogaster Strain and Culture:
The Harwich strain of flies used was collected from the Drosophila Research Laboratory, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. All flies were maintained at 25°C with 12 hours on/off light cycle in vials containing standard cornmeal medium.

PREPARATION AND EXTRACTION OF PLANT MATERIAL
The fresh leaves and barks of *Eucalyptus globulus* were washed carefully with clean water and then dried at room temperature for 7 days. When the leaves and barks were dried, they were first pounded using a mortar and pestle to break down tough cell walls and to increase the surface area, which improves extraction efficiency, protects the electrical blender from damage and ensures a more uniform, high-quality final product. The coarse pieces were then processed into fine powder using the electrical grinder and sieved with a nominal mesh size of 2 mm in diameter to get the finely ground powder. The finely ground powders were separately macerated in 70% ethanol with constant stirring and thereafter filtered with a sieve and filter paper (Whatman size 1). The samples were filtered using a sieve, and the filtrates were concentrated to dryness in a drying cabinet at 40 °C. The extracts collected were weighed to be preserved in a refrigerator in a tight-sealed sample container till required.

Experimental Conditions
Adult flies of 2-3 days old were collected under mild ice anaesthesia from stock vials and distributed in groups of 5 onto experimental food conditions. Each experimental vial contained 30 flies and approximately 5ml of the appropriate food. The flies were passed to vials containing new food approximately every 48 hours to ensure consistent food quality.

PHYTOCHEMICAL SCREENING
Phytochemical analysis was conducted to detect the presence and absence of secondary metabolites. Saponins, carbohydrates, anthraquinones, tannins, flavonoids, Terpenoids, alkaloids and glycosides using standard methods [8].

Acute toxicity test/pilot study
For each of the 2 extracts, the flies were separated into seven (7) groups, with each group replicated across three (3) vials, and each vial containing thirty (30) flies. Various concentrations of the extract (10 mg, 50 mg, 100 mg, 500 mg, 1000mg, and 2000mg) were mixed into 10 g of the fly diet. This mixture was then placed into sterilised empty vials, with the first group receiving only 10 g of the diet as a negative control. The flies were introduced into the vials containing the diet and extract. Total mortality was recorded daily over a seven (7) day period, and these data were used to determine the LC_{50} of the two extracts.

Survival Assay
For each extract, five groups of 1-4 day-old flies were prepared, with each group consisting of five replicate vials, and each vial containing 50 flies.

The first group served as the negative control and was provided with their normal diet. Groups two through five were exposed to 10 mg, 50 mg, 100 mg, and 200 mg of the extracts per 10 g of fly food. Mortality was recorded daily over a 28-day period, and the data were subjected to statistical analysis.

Treatment with the Extracts
For each extract, five groups of 1-4 day-old flies were prepared, with each group consisting of five replicate vials, and each vial containing 50 flies. The first group served as the negative control and was provided with their normal diet. Groups two through five were exposed to 10 mg, 50 mg, 100 mg, and 200 mg of the extracts per 10 g of fly food. This treatment was carried out for a period of seven (7) days.

LOCOMOTOR PERFORMANCE
Locomotory performance of treated and control flies was investigated using the negative geotaxis as described by [9] [10]. Ten flies (Five males and five females) were immobilised under mild ice anaesthesiaand placed separately in labelled vertical glass columns (Length-15cm; Diameter-1.5cm). After the recovery from the exposure (about 20 minutes), the flies were gently tapped to the bottom of the column, and the number of flies that climbed up to the 6cm mark of the column in six (6) seconds, as well as those that remained below this mark after this time, were recorded. The scores represent the mean of the number of flies at the top expressed as a percentage of the total number of flies. This process was repeated three times at 1-minute intervals each.

Emergence Assay
For each of the two extracts, five pairs of flies of both male and female genders were taken from each group after treatment into a freshly prepared empty diet and left for 24 hours to allow them to mate and breed freely in the breeding bottles, during which they were allowed for courtship and mating. The bottles containing eggs were observed for pupa and the emergence of adult flies. The emergence of the flies was recorded daily for 14 days post-mating until the figures became constant for each group, as no new flies continued to emerge.

In Vivo Preparation of Samples for Assay
Following treatment with the ethanolic extracts of the leaf and bark of*Eucalyptus globulus*, the flies were anaesthetised. The flies were then rinsed several times with cold phosphate buffer solution (pH 7.4, 0.1M) to remove all traces of food that might be attached to the outside of the flies and their wings [11]and deposited in groups of five into pre-weighed Eppendorf tubes [12].The corresponding weight of the flies was recorded, and the Phosphate Buffer Solution (PBS) was added to the fly bodies in the quantity of 10 µL buffer per mg fly body [12]Flies were rapidly homogenised in PBS on ice [12].

The homogenates were then centrifuged for 10 minutes at 4000rpm (rotations per minute), at a temperature of 4°C in an Eppendorf centrifuge that has been pre-chilled to 4°C (Abolaji et al., 2017). The resulting supernatants were pipetted using micropipettes into corresponding pre-labelled Eppendorf tubes and stored at 20°C [12]. Subsequently, the supernatants were used for glucose quantification, total protein, total thiol assay, catalase and Glutathione-S Transferase (enzymatic) activity.

Total Protein Determination
Total protein concentrations across different samples were quantified via the Bradford method utilising the Randox protein

assay kit provided by Randox Laboratory in Crumlin, County Antrim, United Kingdom, following the established protocol issued by the manufacturer.

This methodology is based on Coomassie dye binding to protein molecules within each sample, leading to colour development which can be quantitatively analysed using a microplate reader. After combining and equilibrating reagents for 30 minutes at room temperature (25 ^{0}C), absorbance readings were collected at 546 nm over a duration of 120 seconds with successive intervals every ten seconds [4].

Determination of Total Thiol Content

The total thiol concentration was determined using the Ellman method, as modified by [9].The assay utilised a reaction mixture with a final volume of 210 µL, containing 10 µL of the sample, 180 µL of 100 mM potassium phosphate buffer (pH 7.4), and 10 µL each of 10 mM DTNB and GSH. This mixture was incubated for 30 minutes at a temperature of 25 ^{0}C. Absorbance measurements were taken at intervals over a period ranging from zero to two minutes, with readings recorded every ten seconds at an absorbance wavelength of 412 nm.

Determination of Catalase (CAT) Activity

Catalase activity was quantified following Aebi's method as described [9].The working solution consisted primarily of potassium phosphate buffer (100 mL, pH 7.0) incorporated with hydrogen peroxide at a concentration level equivalent to that found in Solution A (19 mM H_2O_2; specifically, using a volume standardisation where 190 µL equals this molarity). For the actual measurement, the fly tissue homogenate, amounting to approximately 10 µL, is added to 590 µL of Object of Solution A. The decrease in the solution's absorbance due to the decomposition of hydrogen peroxide was continuously monitored at 340 nm, and the return to its baseline rate was carefully documented at 25 ^{0}C.

Determination of Glutathione-S Transferase (GST) Activity

The determination of the GST content of the sample was carried out according to the method described by[9]with little modifications. Briefly, the assay reaction mixture was made up of 200 µL of a solution containing (7 µL mL of 0.25 M potassium phosphate buffer (pH 7.0) with 7 µL of 2.5 mM Ethylenediaminetetraacetic acid (EDTA), 3 µL of distilled water and 160 µL of 0.1 M GSH at 25 ^{0}C, 20 µL of a sample (1:4 dilution) and 3 µL of 25mM CDNB (1-chloro-2, 4- dinitrobenzene). The reaction was monitored for 2 min (10s intervals) at 340 nm in a spectrophotometer.

ANALYSIS

Each of the Biochemical assays was run in a SPECTROstar Nano BMG LABTECH Germany spectrometer using a 96 micro plate reader labelled according to the individual assays and analysed using Mars software coupled with the SPECTROstar Nano device. The statistical analysis was carried out using the GraphPad Prism Version 8.0.2. ANOVA and the Turkey post-hoc was used to determine statistical significance.

RESULTS

Phytochemical Analysis

Table 1: Results of Phytochemical Analysis

Phytochemicals	*Eucalyptus globulus* leaves	*Eucalyptus globulus* bark
Alkaloids	-	-
Saponin	+	+
Tannins	+	+
Flavanoids	+	+
Carbohydrates	+	+
Terpenoids	+	+
Anthraquinones	-	+
Cardiac glycosides	+	+

key + = Present - = Absent

Acute Toxicity

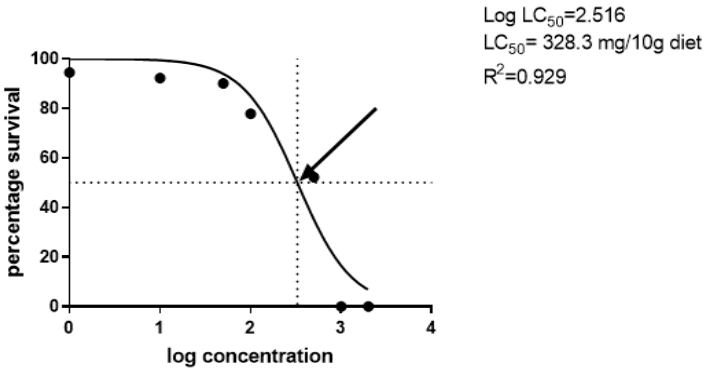

Figure 1: LC_{50} of Eucalyptus globulus leaf

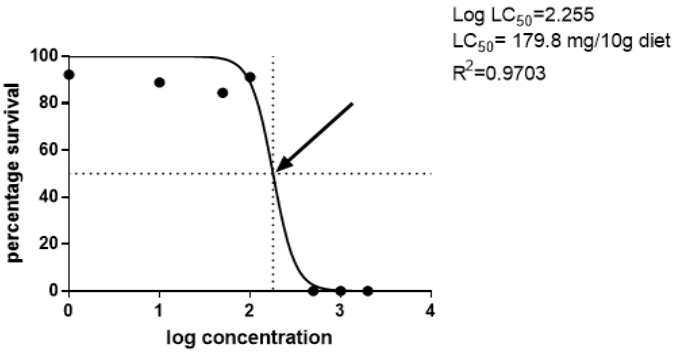

Figure 2: LC_{50} of Eucalyptus globulus bark

28-Days Survival Assay

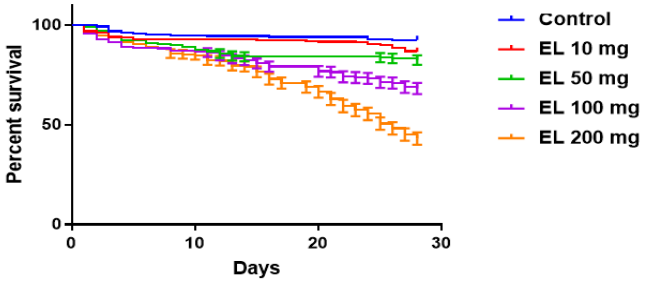

Figure 3: Kaplan-mier Survival Curve after 28-Day Treatment with EL Extract

Control = Diet EL= Eucalyptus globulus leaf

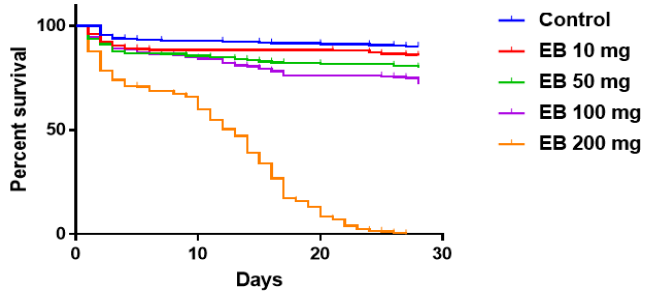

Figure 4: Kaplan-mier survival Curve after 28-Day Treatment with EB Extract

Control = Diet EB= Eucalyptus globulus Bark

Climbing Assay

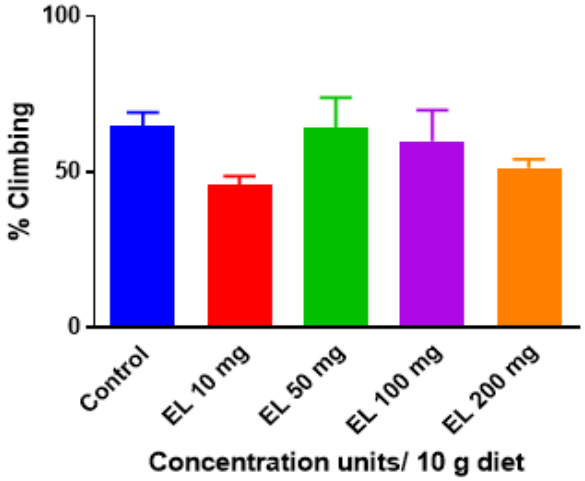

Figure 5: Effect of Eucalyptus globulus leaf extract on Climbing Performance ofDrosophila melanogaster

** implies a significant (P<0.05) difference compared to the control group.*
Data are presented as mean ± SEM (standard error of mean). EL = Eucalyptus globulus leaf; control= diet.
The leaf extract of Eucalyptus globulus showed a similar trend in activity as the control group.

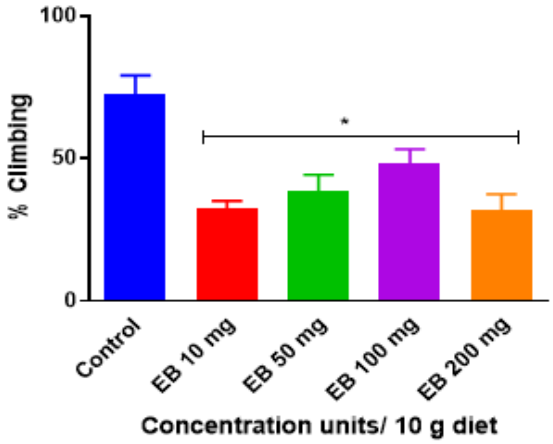

Figure 6: Effect of Eucalyptus globulus Bark Extract on Negative Geotaxis in Drosophila melanogaster

** implies a significant (P<0.05) difference compared to the control group. Data are presented as mean ± SEM (standard error of mean). EB = Eucalyptus globulus bark; control = diet*
The percentage climbing (negative geotaxis) of flies treated with the bark extracts of Eucalyptus globulus showed significantly reduced climbing ability for all concentrations of the bark at (p<0.05).

Emergence

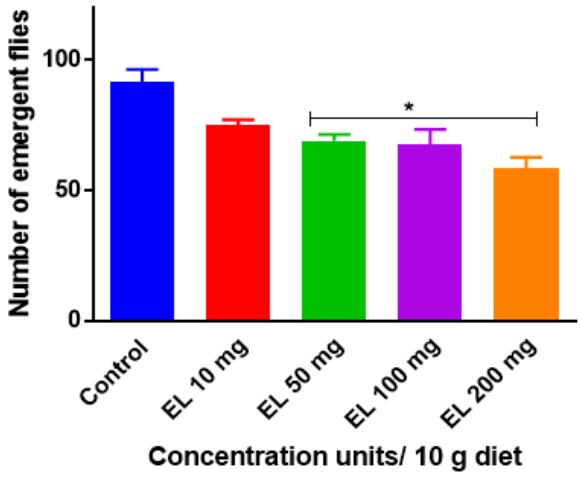

Figure 7: Effect of Eucalyptus globulus Leaf extract on Fecundity in Drosophila melanogaster

**implies a significant (p<0.05) difference compared to the control group.*
Data are presented as mean ± SEM (standard error of mean). EL = Eucalyptus globulus leaf, control = diet.
The result showed a significant suppression of reproduction in the flies at (p < 0.05).

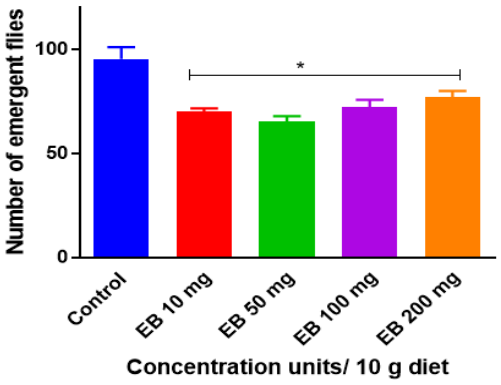

Figure 8: Effect of Eucalyptus globulus Bark Extract on Fecundity in Drosophila melanogaster

**implies a significant (p<0.05) difference compared to the control group. Data are presented as mean ± SEM (standard error of mean). EL = Eucalyptus globulus bark; control = diet.*
The result of the bark extract also showed significant suppression of reproduction in the flies at (p < 0.05).

Total protein

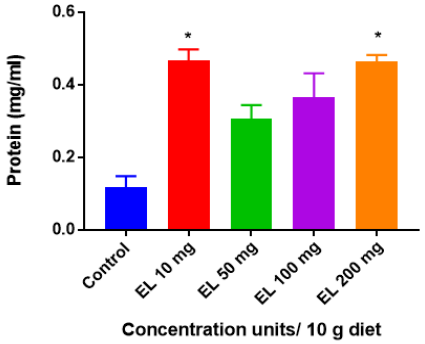

Figure 9: Effect of the Leaf Extracts of Eucalyptus globulus on Protein Levels in Drosophila melanogaster after Seven Days of Treatment

**implies a significant difference when compared to the control group.*
Data are presented as mean ± SEM (standard error of mean). EL = Eucalyptus globulusleaf; control = diet.
Following exposure of Drosophila melanogaster to the Eucalyptus globulus leaf extract, total protein levels increased significantly at 10 mg and 200 mg (0.466 ± 0.04 mg/mL and 0.47 ± 0.02 mg/mL, respectively), compared with the corresponding control group (0.14 ± 0.04 mg/mL).

Catalase Activity

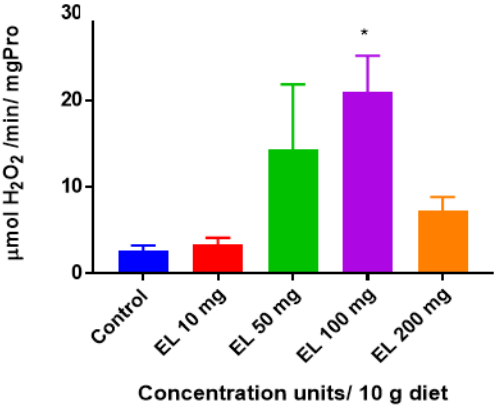

Figure 10: Effect of the leaf Extract of Eucalyptus globulus on Catalase Activity in Drosophila melanogaster after Seven Days of Treatment

** implies a significant (p<0.05) difference compared to the control group.*
Data are presented as mean ± SEM (standard error of mean). EL = Eucalyptus globulus leaf; control = diet. After exposure of fruit flies to the extracts, the Catalase activity increased significantly at 100 mg/10 g diet of the Eucalyptus globulus extract, rising to 20.9 ± 4.21 µmol H_2O_2/min/mg Protein.

Glutathione-S Transferase (GST) Activity

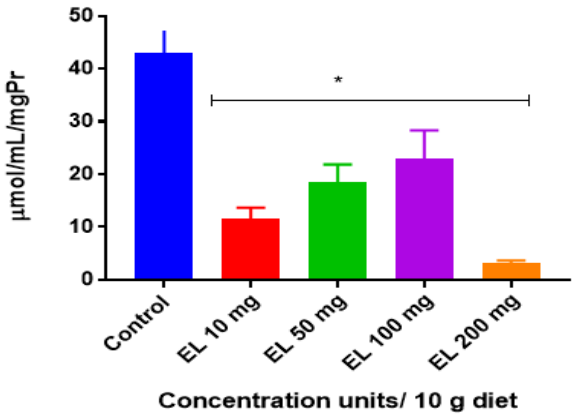

Figure 11: Effect of the leaf Extract of Eucalyptus globulus on Glutathione-S-Transferase Activity in Drosophila melanogaster after Seven Days of Treatment

**implies a significant (p<0.05) difference compared to the control group.*
Data are presented as mean ± SEM (standard error of mean). EL = Eucalyptus globulus leaf; GST, Glutathione-S-Transferase; control = diet
After exposure of fruit flies to the extract, the GST activity decreased significantly for all concentrations of the Eucalyptus globulus extract.

Total Thiol

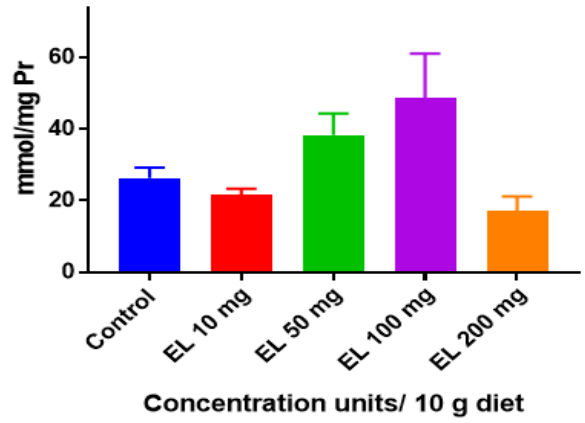

Figure 12: Effect of the Leaf Extract of Eucalyptus globulus on Total thiol levels in Drosophila melanogaster after Seven Days of Treatment

**implies a significant (p<0.05) difference compared to Control group. Data are presented as mean ± SEM (standard error of mean). EL = Eucalyptus globulus leaf.*
After exposure of fruit flies to the extracts, the total thiol levels increased significantly, there was a decline in Total thiol content at the highest treatment concentration (200 mg/10 g diet).

DISCUSSION

The seven-day survival and Kaplan–Meier curves revealed that the bark extract exhibited a lower LC_{50} (180 mg/10 g diet) than the leaf extract (328 mg/10 g diet) (see Figures 1 and 2), indicating greater lethality. These findings suggest that the bark contains higher concentrations of potentially toxic secondary metabolites, whereas the leaf extract demonstrates a wider safety margin [13] [14].This relative difference likely reflects inter-part distinction in phytochemical composition and the concentrations of bioactive constituents [15]. Therefore, within the context of this *Drosophila* model, the leaf extract is relatively safer than the bark extract. However, calling either part "safe" in absolute terms would be premature: safety was determined by expected exposure (dose), route (dietary, dermal, inhalation), duration (acute versus chronic), and the target species. National Research Council, 2004 [16].For instance, mammalian acute-toxicity data show substantially higher oral LD_{50} values for 1, 8-cineole in eucalyptus oil than the insect LC_{50}s reported here, indicating lower acute susceptibility in standard rodent models compared with insects on a mg per mass basis. Nevertheless, mammalian sub-acute and sub-chronic studies have revealed liver and neurological effects at repeated high doses, and human accidental ingestions (particularly in children) have resulted in moderate to severe poisoning in some cases, so human safety still requires conservative exposure control [16]Numerous studies revealed that 1,8-cineole,(the dominant toxicologically important constituent of many *Eucalyptus* oils) is present at differing concentrations in leaf versus bark extracts. Our *Drosophila* assay revealed a greater toxicity in the bark; these findings corroborate the study according to[14]which indicates intra-plant variation in phytochemical compositions and their concentrations.

The Kaplan–Meier survival curve for *Eucalyptus globulus* leaf (EL) treatment over 28 days indicates a gradual decline in survival rate relative to the control diet, suggesting that exposure to the leaf extract produced mild to moderate toxic effects over time (Figure 3). The observed survival pattern implies that mortality occurred progressively rather than abruptly, which is characteristic of moderate toxicity rather than acute lethality. Such patterns often reflect the presence of bioactive but less aggressive phytochemicals such as 1,8-cineole and flavonoids, known to exhibit both therapeutic and dose-dependent toxic actions [13] [14]. The relatively prolonged survival period in the leaf-treated group suggests that the extract may be relatively safe within the tested concentration range, though sub-lethal physiological stress may have occurred. The bark extract demonstrates a steeper decline in survival probability over the 28-day period compared to the control (see Figure 4). This indicates a higher degree of toxicity, likely due to the elevated levels of phenolic compounds, condensed tannins, and other secondary metabolites typically concentrated in bark tissues [14]. The sharper decline in survival suggests that exposure to the bark extract induced cumulative or progressive toxic effects that reduced longevity.

The climbing (negative geotaxis) performance of *Drosophila melanogaster* treated with the *Eucalyptus globulus* leaf extract showed no significant deviation from the control group (Figure 5), indicating that locomotor activity and neuromuscular coordination were largely preserved after exposure. The similarity in climbing behaviour suggests that the leaf extract did not impair motor functions or induce neurotoxic effects within the tested concentrations. This finding implies a relativelysafe neurophysiological profile, reflecting the milder biochemical composition of the leaf extract compared with other plant parts [14]. The result aligns with prior studies showing that moderate doses of *E. globulus* essential oil or phenolic-rich extracts may exert mild antioxidant and protective effects on neural tissues rather than neurotoxicity [15]. Therefore, the observed outcome suggests that *E. globulus* leaf extract supports or maintains normal climbing ability, reinforcing its relative safety within the concentration range tested. In contrast, flies treated with the *Eucalyptus globulus* bark extract exhibited a significant reduction ($p < 0.05$) in climbing ability at all tested concentrations (see Figure 6), suggesting an adverse impact on neuromuscular functionor motor coordination. This decline in negative geotaxis is indicative of potential neurotoxicity or locomotor impairment, which may result from the higher levels of secondary metabolites, such as tannins and Terpenoids, often concentrated in bark tissues [13] [14]. The marked reduction in climbing activity implies that prolonged exposure or higher doses of bark extract could disrupt motor performance, possibly through oxidative stress or interference with neurotransmission in dopaminergic pathways — mechanisms previously associated with plant-derived Phenolics and Terpenes in *Drosophila* models [15].

Hence the bark extract appears to possess a narrower safety margin, indicating potential neurotoxic risk at elevated concentrations.

The *Eucalyptus globulus* leaf extract caused a significant suppression of reproduction ($p < 0.05$) in *Drosophila melanogaster*, indicating an inhibitory effect on fecundity (Figure 7). This suggests that certain phytochemicals in the leaf extract may interfere with reproductive physiology, possibly by altering hormonal signalling, oogenesis, or oxidative balance. Such a reduction in reproductive output may stem from oxidative stress or metabolic disruption caused by bioactive Terpenoids and phenolics present in eucalyptus leaves [13]. Previous reports in insect models have similarly shown that moderate exposure to plant secondary metabolites can compromise reproductive fitness or delay emergence rates [15]. Despite this suppression, the lack of a corresponding decline in locomotor performance implies that the reproductive impairment might occur at sub-lethal physiological levels rather than through general toxicity. The bark extract also produced a significant ($p < 0.05$) suppression of reproduction in *Drosophila melanogaster*, suggesting strong inhibitory or cytotoxic effects on reproductive tissues or gametogenesis (Figure 8). Bark tissues generally contain higher concentrations of polyphenols, tannins, and alkaloids, compounds known to exert antifertility effects through oxidative and endocrine-mediated mechanisms [14]. The pronounced reduction in fecundity observed here indicates that the bark extract exerts a reproductive toxic effect, potentially linked to elevated oxidative stress or disruption of the antioxidant–pro-oxidant balance in reproductive cells. This reproductive suppression may serve as a biomarker of systemic toxicity when evaluating plant-derived formulations for safety [16].

A significant increase in total protein levels was observed at 10 mg and 200 mg/10 g diet concentrations of *E. globulus* leaf extract relative to the control (Figure 9). Elevated protein levels can reflect enhanced metabolic or stress-responsive protein synthesis as part of compensatory mechanisms against phytochemical exposure. Increased protein content is often observed during activation of antioxidant defence pathways or detoxification enzymes, as the organism attempts to restore homeostasis [14]. This response could also be related to the mild stimulatory effect sometimes seen at low doses of plant extracts (hormesis) [15]. Hence, the increase in total protein following leaf extract exposure suggests a sub-lethal adaptive response rather than overt toxicity.

Catalase activity increased significantly at 100 mg/10 g diet (Figure 10), indicating induction of antioxidant defence mechanisms. Elevated catalase activity reflects enhanced capacity to decompose hydrogen peroxide, suggesting that the leaf extract induced mild oxidative stress, prompting adaptive upregulation of enzymatic antioxidants [13]. Activation of catalase is consistent with the known pro-oxidant/antioxidant duality of *Eucalyptus* phytochemicals, including 1,8-cineole and polyphenols, which can trigger redox balancing mechanisms at moderate exposure levels [14]. Therefore, the rise in catalase activity implies a **protective physiological adjustment**that helps mitigate oxidative damage in *Drosophila* tissues exposed to the extract.

Glutathione-S-transferase activity decreased significantly across all tested concentrations (see Figure 11), signifying possible enzyme inhibition or depletion of intracellular glutathione reserves. GST plays a crucial role in conjugating xenobiotics and protecting against oxidative damage; thus, its reduction suggests compromised detoxification capacity [14]. The decrease may result from enzyme saturation or oxidative depletion of GSH by reactive phytochemical intermediates. This pattern aligns with observations that certain plant extracts can suppress phase II detoxification enzymes when oxidative load exceeds adaptive capacity[15]. Hence the reduction in GST activity reflects a potential toxic stress response, marking a point where antioxidant compensation begins to fail.

Although the document excerpt did not provide numerical values, changes in total thiol content generally indicate shifts in redox homeostasis. Thiols (–SH groups) are critical for maintaining cellular redox equilibrium, and their depletion would imply oxidative stress or protein oxidation [13]. Given the observed GST inhibition and catalase induction, it is plausible that total thiol levels were affected correspondingly (see Figure 12), representing either consumption during detoxification or compensatory synthesis of thiol-containing molecules. Such fluctuations reflect the balance between oxidative challenge and antioxidant response, integral to evaluating safety and toxicity [16].

CONCLUSIONS

These findings unveil a multifaceted bioactivity spectrum where beneficial oxidative modulation coexists with concentration-dependent toxicity. The *E. globulus* leaf thus stands as a safer candidate for pharmacological and nutraceutical exploration, offering antioxidant resilience without compromising fundamental biological functions. In contrast, the bark embodies the plant's defensive chemistry—potent, bioactive, but demanding respect in dosage and application. Collectively, these results suggest that although both plant parts contain bioactive compounds, the leaf possesses a broader safety margin than the bark under comparable conditions.

RECOMMENDATIONS

Chemical Profiling:

Comprehensive GC–MS and LC–MS analyses of both leaf and bark extracts should be conducted to identify and quantify the specific compounds responsible for the observed antioxidant and toxic effects.

Dose Refinement and Standardisation:

Establishing safe concentration thresholds and standardised extract formulations will enable controlled use in pharmacological or nutraceutical contexts.

Chronic and Sub-lethal Studies:

Future studies should investigate long-term exposure effects, focusing on reproduction, neurobehavioral function, and lifespan modulation to better delineate the margin between adaptation and toxicity.

Cross-Species Validation:

Comparative studies using mammalian models are essential to evaluate translational relevance, considering the interspecies differences in metabolism and detoxification highlighted by the National Research Council (US) Committee (2004).

Therapeutic Exploration:

Given the leaf extract's strong antioxidant and moderate metabolic activation effects, it may serve as a promising template for developing safe natural antioxidant formulations or insect model screening platforms.

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