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Pennisetum purpureum extracts and fractions enhanced antioxidant defense mechanism in *Drosophila melanogaster*

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ABSTRACT

Oxidative stress markers are significantly elevated and also correlate with disease complications in aging, diabetes, cardiovascular diseases, among other disorders. The study is aimed at determining the antioxidant effect of the extracts and fractions of *Pennisetum purpureum* (Napier grass) against hydrogen peroxide-induced oxidative stress in *Drosophila melanogaster.* The LC_{50} was determined to establish a safe and effective dose range for pharmacological evaluation. The extracts and fractions of *P. purpureum* were evaluated by the induction of hydrogen peroxide (H_2O_2) in *D. melanogaster* subjected to 7 days treatment regimen, after which the activity of some oxidative stress markers, namely, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were measured in treated and untreated groups. All experimental data were expressed as mean ± standard error of mean (SEM), using GraphPad Prism version 8.0.2 (263), data was considered statistically significant at $P \leq 0.05$. The result revealed that plant extracts and fractions protect against oxidative stress markers and also enhance the antioxidant capacity in *D. melanogaster*. The observed upregulation of antioxidant enzymes (CAT, GSH, and SOD) in the treatment groups suggests that *P. purpureum* extracts enhance the fly's endogenous antioxidant defence system. In conclusion, the study underscores the potential of *P. purpureum* extracts in enhancing antioxidant defence and reducing oxidative stress markers in *D. melanogaster*. The upregulation of key antioxidant enzymes and the concomitant reduction in antioxidant stress markers highlight the therapeutic prospects of *P. purpureum* as a natural antioxidant source.

Keywords: Oxidative stress*, Pennisetum purpureum*, antioxidant enzymes, hydrogen peroxide, *Drosophila melanogaster*, stress markers.

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INTRODUCTION

Oxidative stress has emerged as a fundamental mechanism underlying the onset, progression, and complications of numerous chronic diseases. Current evidence indicates that excessive generation of reactive oxygen species (ROS) disrupts cellular redox homeostasis, leading to lipid peroxidation, protein modification, mitochondrial dysfunction, DNA damage and ultimately cell death. These molecular events have been strongly associated with the pathophysiology of metabolic disorders, neurodegenerative diseases, cardiovascular dysfunction, inflammatory conditions and aging-related degenerative changes (Pizzino et al., 2017).

Although the human body possesses an elaborate antioxidant defense network, overwhelming or prolonged oxidative challenges often exceed these physiological systems, making the supplementation or discovery of additional antioxidant sources essential for combating ROS-mediated damage. In recent years, plants have

attracted growing attention as alternative sources of potent natural antioxidants due to their phytochemical diversity, lower toxicity, and multifunctional biological effects compared to synthetic antioxidants, which are increasingly limited by safety concerns (Birben et al., 2021). *Pennisetum purpureum*, widely used in ethno medicine across Africa, contains a variety of bioactive compounds including flavonoids, phenolic acids, tannins, and alkaloids—many of which are associated with strong antioxidant properties (Heuze et al., 2020). However, despite its broad traditional uses, there is limited scientific evidence validating its antioxidant activities or its potential therapeutic role in conditions associated with elevated oxidative stress. Generating such evidence is crucial because identifying novel antioxidant rich plants contributes significantly to drug discovery, preventive medicine, and functional food development. This study therefore provides a scientific basis for understanding the antioxidant ± mechanism of *P. purpureum*, bridging the gap between traditional use and modern biomedical validation.

METHOD

Collection identification and authentication of *Pennisetum purpureum*

The plant material was collected on the 22nd January, 2025, from Mista Ali, Bassa Local Government area of Plateau State, Nigeria. It was identified in the Department of Plant Science and Biotechnology, University of Jos. A voucher specimen number JUHN23000610 was issued, and the specimen was deposited in the Faculty of Pharmaceutical Sciences, University of Jos, herbarium for reference.

Preparation of *Pennisetum purpureum* extract and fractions

The leaves of the plants were sliced and air-dried for 21 days. The dried parts were then coarsely powdered. 400 ml of the extracting solvent (methanol) was added to 20 g of *P. purpureum* (1:20,$^{w}/_{v}$) in a 1,000 ml beaker. The mixture was then macerated in a water bath (LABEC, MARRICKVILLE, AUSTRALIA) at 70°C for 40 min. Subsequently, the mixture was homogenized at a temperature of 55 to 80°C by constant shaking for 4 h, using a homogenizer (IKA, Germany). The filtrate was removed from the residue by filtration using Whatman No. 1 filter paper. This process was repeated three times to exhaustively obtain the extract. The extract solution was concentrated using a rotary evaporator (Pollab, India) at 40°C and dried at room temperature. Dried extract samples were stored in airtight containers at 4°C.

Fractionation by liquid-liquid extraction

Methanol extract of *P. purpureum* was initially dissolved in distilled water to allow for effective partitioning with the non-polar solvents in the succeeding steps. Distilled water was used for the initial dilution since the aqueous solution can easily be extracted with non-polar solvents such as n-hexane. After creating the aqueous solution, the sequential extraction was continued using n-hexane first, followed by ethyl acetate, and finally methanol, to fractionate the compounds based on their polarities (Abubakar and Haque, 2020).

Qualitative phytochemical screening

Phytochemical evaluation of the methanol extract, aqueous extract, and the various methanol fractions of *P. purpureum* was carried out to identify the presence or absence of secondary metabolites. Alkaloids, anthraquinones, cardiac glycosides, carbohydrates, flavonoids, saponins, tannins and steroids using standard methods (Trease and Evans, 2020).

Test for alkaloids

To test for alkaloids, (0.5 g) of powdered plant material was stained with 5 ml of 1% aqueous hydrochloric acid on a steam bath. The mixture was then filtered. The first part of the filtrate was treated with a few drops of Mayer's reagent, while a second portion of the filtrate (1 ml) was treated with picric acid solution. Precipitation observed with either Mayer's reagent or picric acid solution is considered preliminary evidence for the presence of alkaloids in the extract. For a test solution containing Mayer's reagents, a cream or yellow precipitate may form, indicating the presence of alkaloids. In the case of picric acid solution, **a** yellow precipitate may appear, indicating the presence of alkaloids (Trease and Evans, 2020).

Test for anthraquinones

Borntrager's test was employed to determine the presence of anthraquinones in both the extracts and fractions of the plant. Half a gram (0.5 g) of the methanol extract was measured into a test tube, 5 ml of chloroform was added to the test tube, shaken for 5 min, and the final filtrate was shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in ammoniacal (lower layer) indicates the presence of free hydroxyl - anthraquinones (Trease and Evans, 2020). The same procedure was repeated for the aqueous extract and all fractions of the plant extract.

Test for cardiac glycosides

The Salkowski test was employed, in which 0.5 g of the methanol extract was weighed and dissolved in 2 ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface indicates the presence of a steroidal ring of the glycone portion of cardiac glycosides. The same procedure was repeated for the aqueous extract and all fractions of the plant extract (Sofowara, 2013).

Test for flavonoids

P. purpureum methanol extract (0.5 g) was dissolved in 2 ml of dilute sodium hydroxide solution. A few drops of sulphuric acid were added, and the presence of a flavonoid was indicated if the solution became colourless (Trease and Evans, 2020). The same procedure was carried out for the aqueous extract as well as all fractions of the extract.

Test for tannins

Half a gram (0.5 g) of *P. purpureum* methanol extract was dissolved in 1 ml of distilled water, stirred and filtered. Five percent of ferric chloride was added to the filtrate, and a blue-black or green precipitation was taken as evidence for the presence of tannins (Trease and Evans, 2020). The same procedure was repeated for the aqueous extract and all fractions of the plant extract.

Test for saponins

Half a gram (0.5 g) of *P. purpureum* extract was dissolved in 5 ml of water in a test tube. The resultant mixture was shaken properly and warmed for one minute; frothing which persist on warming was taken as preliminary evidence for the presence of saponins (Trease and Evans, 2020). The same procedure was repeated for all the fractions of the crude extract.

Test for steroids and terpenes

P. purpureum (2.0 g) was dissolved in 15 ml of 95% alcohol and boiled in a steam bath; the resultant filtrate was evaporated to dryness. The residue was then dissolved in 10 ml of anhydrous chloroform and filtered. The resultant filtrate was mixed with 1 ml of acetic anhydride, followed by the addition of 1 ml of concentrated sulphuric acid run down the wall of the test tube after the test tube was inclined and a layer beneath was formed. The test tube was observed for green and reddish coloration, which is an indication of the presence of steroids and terpenes (Trease and Evans, 2020). The same procedure was repeated for all the fractions of the crude extract.

***Drosophila melanogaster* strain and culture maintenance**

The fly *Drosophila melanogaster* (Harwich) was obtained from the National Species Stock Center (Switzerland). It was maintained at constant temperature and humidity (23°C, 60% relative humidity, respectively), under 12 hours dark/light cycle. The flies were cultured by feeding them with a standard medium of the following composition: 1700 ml of corn flour, 1 g of methyl paraben dissolved in 5 ml of absolute ethanol, and 170 ml of water (Etuh et al., 2019).

Ethical and biosafety statement

Ethical approval was not required for this study, as *Drosophila melanogaster* is an invertebrate model organism and therefore exempt from animal ethics review under the research policy of the University of Jos, Nigeria**,** and the provisions of the Nigerian National Code of Health Research Ethics (NHREC, 2014)**.** Hydrogen peroxide (H_2O_2) was prepared, handled, and disposed of following institutional biosafety guidelines, using appropriate personal protective equipment (PPE), including gloves, goggles, and laboratory coats, in a well-ventilated workspace.

Determination of the 7-day survival assay

The acute toxicity testing was carried out using the method described by Abolaji et al. (2019) for seven (7) days survival assay. The fly food was prepared using the ratio of gram per die to obtain food with concentrations of 1000 mg per 10 g diet, 500 mg per 10 g diet, 250 mg per 10 g diet, 125 mg per 10 g diet and 62.5 mg per 10 g diet. The acute toxicity was carried out by placing 50 unsex flies (not more than 24 hours old) per vial in triplicate to have a total number of 150 flies per concentration. The survival of the flies was recorded for a period of 7 days, and the mortality was taken.

Determination of doses for pharmacological screening

A percentage-based dilution and direct calculation of the predetermined Lethal Concentration 50 (LC_{50}) for each extract and fraction of *P. purpureum* form the basis for the

dose determination **(**Hayes et al., 2008). The LC_{50} values were experimentally determined using a 7-day survival assay, followed by probit analysis. For pharmacological screening, a range of doses was prepared based on the LC_{50} values: 50% of LC_{50} was calculated as $LC_{50} \times 0.5$, 25% of LC_{50} as $LC_{50} \times 0.25$, and 12.5% of LC_{50} as $LC_{50} \times 0.125$. These specific dose levels (100%, 50%, 25%, and 12.5% of LC_{50}) were utilized for the pharmacological screening (Hayes et al., 2008; Islam et al., 2021; Sahu et al., 2011). Each extract and fraction was dissolved in a diet medium from the stock, and the working solutions were prepared corresponding to each calculated dose fraction by serial dilution or direct calculation. Concentrations were adjusted to match the calculated doses, ensuring uniform distribution in the test medium. The doses used as 12.5 % of the LC_{50} of the various extracts and fractions were:- 18.75 for n-Hexane, 60 for ethyl-acetate, 102.5 for residual fraction, 37.5 for methanol extract, 40 for aqueous extract and 36.13 for ascorbic acid (in mg per 10 g diet). At 25% of the LC_{50} the doses used were:- 37.5 for n-Hexane, 120 for ethyl-acetate, 205 for residual fraction, 75 for methanol extract, 80 for aqueous extract and 72.25 for ascorbic acid (in mg per 10 g diet). At 50% of the LC_{50} the doses used were:- 75 for n-Hexane, 240 for ethyl-acetate, 410 for residual fraction, 150 for methanol extract, 160 for aqueous extract and 144.5 for ascorbic acid (in mg per 10 g diet). At 100% of the LC_{50} the doses used were:- 150 for n-Hexane, 480 for ethyl-acetate, 820 for residual fraction, 300 for methanol extract, 320 for aqueous extract and 289 for ascorbic acid (in mg per 10 g diet).

Treatment with the extracts and fractions

For each extract and fraction, five groups of 1-4 day-old unsex flies were prepared, with each group consisting of three replicates of independent vials and each vial containing 50 flies. Outcomes were averaged across ≥3 independent experiments. The various controls were: *Toxicant control (H_2O_2 only), Vehicle/diet control* and positive antioxidant control (*ascorbic acid*). 100%, 50%, 25% and 12.5% of the LC_{50}, were administered for each of the extracts and fractions as indicated under 'KEYS' of Tables 2, 3, 4 and 5.

Oxidative stress (OS) test

The oxidative stress was induced following a modified method (Fan et al., 2017; Murthy et al., 2018; Prasad and Muralidhara, 2014; Wongchum and Dechakhamphu, 2021). Hydrogen peroxide (30% stock solution, Sigma-Aldrich USA) was introduced into each vial to yield a final concentration of 1% (v/v) H_2O_2 in vials containing various concentrations of the extracts and fractions of *P. purpureum.* The *P. purpureum* extracts and fractions treated flies were considered as the treated group, the control vials received diet without extract or H_2O_2, while the toxicant group received only hydrogen peroxide. Fifty flies were maintained in each group in three replicates per treatment. Prior to the induction of oxidative stress with hydrogen peroxide, the flies were maintained in *P. purpureum* extracts and fractions treated culture media and were aged for 7 days. The experiment was carried out using four(4) different concentrations of the extracts and fractions, which were predetermined based on the acute toxicity study.

Biochemical estimations

The activity of some oxidative stress markers, namely, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were measured in treated and untreated groups. The enzyme activity was studied in 7 days of the treated flies, having four (4) different concentrations. Both flies that have been exposed to different concentrations of the prepared samples and unexposed flies, as explained above, were immobilized under ice followed by homogenization in 0.1M sodium phosphate buffer (pH 7.4). A centrifuge (Eppendorf-Germany Model AG, 5227R) was set to revolve 4000 times per minute for 600 seconds to spin the fly homogenate. The supernatant was micro-pipetted into the labelled Eppendorf tube for the determination of malondialdehyde (MDA) activity, and the activities of superoxide dismutase (SOD), reduced glutathione (GHS), and catalase (CAT).

Malondialdehyde (MDA) assay

Lipid peroxidation was evaluated by estimating malondialdehyde (MDA) formation in *Drosophila melanogaster* homogenates using the thiobarbituric acid reactive substances (TBARS) method as described by Buege and Aust (1978), with slight modification. The assay quantifies the pink chromogen formed by the reaction of MDA with thiobarbituric acid nm.

A standard calibration curve (TBA) under acidic and high-temperature conditions, measured spectrophotometrically at 532, was prepared using serial dilutions of 1, 1, 3, 3-tetraethoxypropane (TEP)**,** which yields MDA upon hydrolysis. The resulting MDA standards (0–10 nmol/ml) were treated identically to the samples to ensure accurate calibration. The standard curve equation:

$$y = mx + b$$

Where y = absorbance at 532 nm, m = the slope of the line, x = MDA concentration (nmol/mL) and b = the y-intercept.

Sample MDA concentrations were extrapolated from the linear regression of this curve.

To normalize to protein content, MDA concentration (nmol/ml) was converted to nmol MDA per mg protein (nmol/mg pro.) using the relationship:

$$\text{MDA (nmol/mg protein)} = \frac{[[MDA] \times V_{assay}]}{Protein\ content}$$

Where;-
• *[MDA]* = concentration from the standard curve (nmol/ml),
• V_{assay} = 0.2 ml (assay volume),
• *Protein* = 0.5 mg (per sample, determined by the Bradford method)

Thus
MDA (nmol/mg protein) = [MDA] × 0.4
Final results were expressed as nmol MDA/mg protein, reflecting lipid peroxidation intensity relative to total cellular protein content.

Superoxide dismutase (SOD)

SOD activity was measured by using the NBT (Nitro blue tetazolium) method (Yilgor and Demir, 2024). Three mls (3 ml) reaction mixture was prepared by adding 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 0.1 mM EDTA (Ethylenediaminetetraacetic acid), 75 µM NBT and 50 µl of enzyme extract. All the tubes were exposed to 400 W bulbs for 15 min, and their absorbance was read at 560 nM. The 50% inhibition of the reaction between riboflavin and NBT in the presence of methionine was considered as one unit of SOD activity, and it was expressed in units/mg of protein.

Catalase (CAT)

CAT activity was measured by following the standard protocol (Yilgor and Demir, 2024). The activity was measured based on the quantity of the hydrogen peroxide substrate remaining after the action of the CAT present in the enzyme extract. To measure this, 0.4 ml of enzyme extract was mixed with 2.6 ml of phosphate buffer along with 30 % hydrogen peroxide. The activity was measured by determining the decomposition of hydrogen peroxide at 240 nm. CAT activity was calculated by using the millimolar extinction coefficient of 43.6 and expressed in terms of µm/min/mg of protein.

Reduced glutathione (GSH)

The activity of GSH was measured by using the DTNB (5, 5-dithio-bis-(2-nitrobenzoic acid) method (Yilgor and Demir, 2024). To measure the enzyme activity, flies were homogenized in ice-cold 10 % TCA (Trichloroacetic acid) and 10 mM EDTA solution (1:1). Then, the homogenate was centrifuged at 5000 rpm. Further enzyme reaction mixture was prepared by adding 200 µl of supernatant, 0.2 M tris-buffer (pH 8.0) and 50 µl of DTNB. The reaction mixture was incubated for 10 min at room temperature to get yellow colored complex. The absorbance was read at 412 nm, and the activity was expressed as µg/mg protein.

Data analysis

All data were expressed as mean ± standard error of the mean (SEM) from three independent replicates (n = 3 per group). Statistical analyses were performed using a two-way analysis of variance (ANOVA) to evaluate the main and interactive effects of treatment (extracts and fractions) and dose level (12.5%, 25%, 50% and 100% LC_{50}) on biochemical indices. Prior to ANOVA, data were assessed for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test), and both assumptions were satisfied ($p > 0.05$). When significant main or interaction effects were observed, comparisons between experimental groups and the oxidative stress control (H_2O_2-treated group) were made using Dunnett's post hoc test, with adjustment for multiple comparisons. Significance was accepted at $p \leq 0.05$, and adjusted p-values (p^a) were reported where applicable. The effect size for each ANOVA factor was computed as partial eta squared (η^2) to estimate the magnitude of treatment and dose effects, interpreted as small (0.01 to 0.05), medium (0.06 to 0.13), or large (≥0.14).

RESULTS

Phytochemical screening of the extracts and fractions of *Pennisetum purpureum*

The qualitative phytochemical screening of *Pennisetum purpureum* extracts and solvent fractions revealed the presence of various secondary metabolites, as presented in Table 1. The methanol extract contained all the tested phytochemical classes, including alkaloids, saponins, tannins, flavonoids, phenols, steroids, anthraquinones, cardiac glycosides, and terpenoids. The aqueous extract showed a similar pattern, though anthraquinones and terpenoids were absent. The n-hexane fraction tested positive for flavonoids, steroids, cardiac glycosides, and terpenoids, but lacked alkaloids, saponins, tannins, and phenols. The ethyl acetate fraction contained flavonoids, steroids, anthraquinones, cardiac glycosides, and terpenoids, while the residual methanol fraction showed the presence of alkaloids, saponins, tannins, flavonoids, and phenols but lacked steroids, anthraquinones, and terpenoids.

Effect of the extracts and fractions of *Pennisetum purpureum* on antioxidant enzyme parameter in *Drosophila melanogaster* under oxidative stress

Malondialdehyde (MDA) assay (nmol/mg/pro.) of Drosophila melanogaster exposed to different treatments for 7 days with induction of oxidative stress

After converting all data to nmol MDA/mg protein, clear dose–dependent and fraction-specific patterns emerged (Table 2). Across treatments, MDA levels, a marker of lipid peroxidation, declined progressively with increasing extract concentration, reflecting enhanced antioxidant protection against H_2O_2 induced oxidative stress. At lower doses (12.5 to 50% LC_{50}), most fractions produced modest reductions in MDA (≈ 2.0 to 2.8 nmol/mg protein) compared to the oxidative-stress control (4.0 nmol/mg), indicating partial scavenging of reactive oxygen species (ROS). However, at the highest dose (100% LC_{50}), a pronounced divergence in activity was observed among the fractions. The ethyl-acetate fraction exhibited the strongest lipid peroxidation inhibition, reaching 0.4 ± 0.2 nmol MDA/mg protein, followed by the n-hexane fraction (0.8 ± 0.4 nmol/mg). These represent reductions of approximately 90 and 80%, respectively, relative to the H_2O_2 control, suggesting potent dose-responsive antioxidant activity. The methanol and aqueous extracts showed intermediate effects (1.6 to 2.0 nmol/mg), while the residual fraction displayed comparatively mild protection (≈ 2.0 nmol/mg across all doses), implying limited dose sensitivity. Interestingly, the dose–response trend plateaued or slightly inverted at 100 % LC_{50} for some extracts, particularly the methanol and aqueous fractions, where MDA levels rose marginally relative to their 50% values. This inversion may indicate pro-oxidant effects or saturation of antioxidant defences at higher concentrations, a phenomenon often reported with polyphenolic compounds when redox balance is exceeded.

Overall, the ethyl-acetate fraction demonstrated the most consistent and dose-dependent suppression of lipid peroxidation, followed by the n-hexane fraction, confirming that semi-polar and lipophilic constituents of *Pennisetum purpureum* contribute most significantly to membrane stabilization and ROS quenching under oxidative stress.

Reduced glutathione (GSH) assay (units /mg/pro.) of Drosophila melanogaster exposed to extracts and fractions of Pennisetum purpureum for 7 days with induction of oxidative stress

Exposure of *Drosophila melanogaster* to extracts and fractions of *Pennisetum purpureum* for seven days under oxidative stress induction produced a dose-dependent increase in reduced glutathione (GSH) concentration (Table 3). Generally, all extract-treated groups showed significantly higher GSH levels compared with the positive (H_2O_2) control (0.53 ± 0.13 µmol/mg protein), indicating effective restoration of endogenous antioxidant defense. Among the fractions, the ethyl-acetate fraction displayed the most remarkable elevation in GSH levels, reaching 52.07 ± 0.18 µmol/mg protein at 50% LC_{50}, a value roughly 100-fold higher than the oxidatively stressed control and even higher than the ascorbic-acid standard (21.20 ± 0.12 µmol/mg protein at the same concentration). This fraction also maintained appreciable GSH elevation at lower doses (8.26 ± 0.18 and 16.27 ± 0.16 µmol/mg protein at 12.5 and 25% LC_{50}, respectively), suggesting a potent dose-responsive antioxidant activity probably linked to medium-polarity phytochemicals such as flavonoids and phenolic esters. The aqueous extract also showed consistently high GSH values across the lower concentrations, peaking at 66.60 ± 0.31 µmol/mg protein (50% LC_{50}), followed by a slight reduction at 100% LC_{50} (16.47 ± 0.29 µmol/mg protein), possibly reflecting saturation or mild pro-oxidant effects at excessive concentration. Similarly, the methanol extract maintained substantial antioxidant potential, with GSH increasing progressively from 12.40 ± 0.23 to 35.87 ± 0.29 µmol/mg protein across the tested doses. Non-polar fractions such as the n-hexane and residual methanol fractions exhibited moderate yet significant increases in GSH content (17.86 ± 0.29 and 79.53 ± 0.23 µmol/mg protein at 100% LC_{50}, respectively), indicating that both lipophilic and hydrophilic antioxidant constituents contributed to redox protection.

Overall, the rank order of GSH elevation at the effective dose (50% LC_{50}) was:- ethyl-acetate > aqueous > residual > methanol > n-hexane > ascorbic acid ≫ H_2O_2 control.

These results collectively demonstrate that the extracts and fractions of *Pennisetum purpureum* enhance the cellular pool of reduced glutathione, thereby reinforcing endogenous defense against reactive oxygen species (ROS). The exceptionally high GSH values in the ethyl-acetate and aqueous fractions suggest that the bioactive antioxidant principles are likely concentrated in these medium- to polar-phase constituents.

Superoxide dismutase (SOD) Assay (U/mg protein) of Drosophila melanogaster exposed to extracts and fractions of Pennisetum purpureum for 7 days with induction of oxidative stress

The Superoxide Dismutase (SOD) activity of *D. melanogaster* exposed to *Pennisetum purpureum* extracts and fractions for 7 days under oxidative stress is presented in Table 4. Exposure to 1% H_2O_2 (toxicant control) resulted in a marked reduction in SOD activity (0.80 ± 0.06 U/mg protein) compared to the normal diet control (9.76 ± 0.40

U/mg protein), confirming the induction of oxidative stress. Treatment with the extracts and fractions caused significant ($P \leq 0.05$) variations in SOD activity across concentrations. The methanol extract and n-hexane fraction exhibited the highest activities at 12.5% LC_{50} (13.74 ± 0.06 and 11.20 ± 0.02 U/mg protein, respectively), which were not only significantly higher than the toxicant control but also comparable to or greater than the normal diet group. However, increasing the concentration of these fractions caused a progressive decline in SOD activity, suggesting a concentration-dependent or biphasic (hormetic) response. The ethyl acetate and residual fractions produced moderate increases in SOD activity (3.0 to 4.8 U/mg protein) at lower concentrations, while the aqueous fraction showed minimal improvement, indicating weaker antioxidant potential. The antioxidant control showed the highest activity at low concentration (15.96 ± 0.04 U/mg protein), validating the assay sensitivity. Overall, ANOVA confirmed that the methanol extract and n-hexane fraction significantly restored SOD activity toward or above baseline levels, demonstrating strong antioxidant capacity against H_2O_2 induced oxidative stress in *D. melanogaster*.

Catalase (CAT) assay (µmol min⁻¹ mg⁻¹ protein) of Drosophila melanogaster exposed to extracts and Fractions of Pennisetum purpureum for 7 days with induction of oxidative stress

The CAT assay results showed significant differences ($P \leq 0.05$) in CAT activity in *D. melanogaster* exposed to different concentrations of the extracts and fractions of *P. purpureum* (Table 5). At 12.5% LC_{50}: All treatment groups (n-hexane: 1.716 ± 0.04, ethyl-acetate: 1.077 ± 0.01, methanol fraction: 1.046 ± 0.01, aqueous extract: 1.066 ± 0.10, methanol extract: 0.973 ± 0.01 and ascorbic acid: 0.994 ± 0.10) had much higher CAT activity than the positive control. Treatment with all extracts and fraction were found to be comparable and even higher than the negative control. At 25% LC_{50}: All treatments (n-hexane: 1.382 ± 0.06, ethyl-acetate: 1.070 ± 0.01, methanol fraction: 1.062 ± 0.01, aqueous extract: 1.071 ± 0.01, methanol extract: 1.130 ± 0.01, ascorbic acid: 1.008 ± 0.10) remained significantly higher than the positive control, as well as comparable and even higher than the negative control. At 50% LC_{50}: All groups (n-hexane: 1.053 ± 0.01, ethyl-acetate: 1.158 ± 0.01, methanol: 1.133 ± 0.03, aqueous: 1.089 ± 0.02, methanol extract: 1.314 ± 0.01, ascorbic acid: 1.038 ± 0.04) continued to show elevated CAT activity compared to the positive control. All treatments were also found to be comparable and even significantly higher than the negative control. At 100% LC_{50}: All treatments (n-hexane: 0.975 ± 0.01, ethyl-acetate: 1.266 ± 0.02, methanol: 1.146 ± 0.01, aqueous: 1.136 ± 0.10, methanol extract: 1.314 ± 0.01, ascorbic acid: 1.086 ± 0.03) were still much higher than the positive control. All treatments were also found to be comparable and even significantly higher than the negative control.

Table 1. Phytochemical screening of the extracts and fractions of *Pennisetum purpureum*.

Constituents	Methanol extract	Aqueous extract	n-Hexane fraction	Ethyl-acetate fraction	Residual methanol fraction
Alkaloids	+	+	-	+	+
Saponins	+	+	-	-	+
Tannins	+	+	-	-	+
Flavonoids	+	+	+	+	+
Phenol	+	+	-	-	+
Steroids	+	+	+	+	-
Anthraquinones	+	-	-	+	-
Cardiac glycosides	+	+	+	+	+
Terpenoids	+	-	+	+	+

Keys: + = Detected; - = Not detected

Table 2. Malondialdehyde (MDA) assay (nmol MDA/mg protein) of *Drosophila melanogaster* exposed to extracts and fractions of *Pennisetum purpureum* for 7 days with induction of oxidative stress.

Treatment	12.5% LC_{50}	25% LC_{50}	50% LC_{50}	100% LC_{50}
n-Hexane fraction	2.4 ± 0.04*	2.4 ± 0.04*	2.0 ± 0.04*	0.8 ± 0.4*#
Ethyl-acetate fraction	2.8 ± 0.04*	2.8 ± 0.04*	2.4 ± 0.04*	0.4 ± 0.2*#
Residual fraction	2.0 ± 0.12*	2.0 ± 0.12*	2.0 ± 0.08*	2.0 ± 0.04*
Aqueous extract	2.8 ± 0.04*	2.8 ± 0.08*	2.4 ± 0.04*	2.0 ± 0.08*
Methanol extract	2.0 ± 0.04*	2.0 ± 0.08*	1.6 ± 0.8*	1.6 ± 0.4
Ascorbic acid	3.2 ± 0.08	3.6 ± 0.08	2.8 ± 0.8	3.2 ± 0.8
Negative control	1.2 ± 0.08	1.2 ± 0.8	1.2 ± 0.8	1.2 ± 0.8
Positive control (H_2O_2)	4.0 ± 0.08	4.0 ± 0.08	4.0 ± 0.08	4.0 ± 0.08

Means in the same column marked with an asterisk (*) are significantly different from the H_2O_2 group at P ≤ 0.05.
Means in the same column marked with hash (#) are comparable (same) and have even significantly higher intended effect than the normal diet group at P ≤ 0.05 value, are mean ± SEM

KEY:
12.5% LC_{50} = 18.75 for n-Hexane, 60 for ethyl-acetate, 102.5 for residual fraction, 37.5 for methanol extract, 40 for aqueous extract and 36.13 for ascorbic acid (in mg per 10 g diet)
25% LC_{50} = 37.5 for n-Hexane, 120 for ethyl-acetate, 205 for residual fraction, 75 for methanol extract, 80 for aqueous extract and 72.25 for ascorbic acid (in mg per 10 g diet)
50% LC_{50} = 75 for n-Hexane, 240 for ethyl-acetate, 410 for residual fraction, 150 for methanol extract, 160 for aqueous extract and 144.5 for ascorbic acid (in mg per 10 g diet)
100% LC_{50} = 150 for n-Hexane, 480 for ethyl-acetate, 820 for residual fraction, 300 for methanol extract, 320 for aqueous extract and 289 for ascorbic acid (in mg per 10 g diet)

Table 3. Reduced Glutathione (GSH) assay (µmol GSH/mg) of *Drosophila melanogaster* exposed to extracts and fractions for 7 days with induction of oxidative stress **(**Protein = 0.5 mg, Assay volume = 200 µl).

Treatment	12.5% LC_{50}	25% LC_{50}	50% LC_{50}	100% LC_{50}
n-Hexane fraction	8.53 ± 0.29*#	9.07 ± 0.18*#	14.06 ± 0.29*#	17.86 ± 0.29*#
Ethyl-acetate fraction	8.26 ± 0.18*#	16.27 ± 0.16*#	52.07 ± 0.18*#	34.93 ± 0.24*#
Residual fraction	6.26 ± 0.12*#	9.00 ± 0.16*#	19.40 ± 0.16*#	79.53 ± 0.23*#
Aqueous extract	38.73 ± 0.16*#	48.27 ± 0.18*#	66.60 ± 0.31*#	16.47 ± 0.29*#
Methanol extract	12.40 ± 0.23*#	19.60 ± 0.12*#	31.47 ± 0.12*#	35.87 ± 0.29*#
Ascorbic acid	5.27 ± 0.12	20.67 ± 0.16	21.20 ± 0.12	31.33 ± 0.18
Diet control	5.47 ± 0.37	5.47 ± 0.37	5.47 ± 0.37	5.47 ± 0.37
Toxicant control	0.53 ± 0.13	0.53 ± 0.13	0.53 ± 0.13	0.53 ± 0.13

Means in the same column marked with an asterisk (*) are significantly different from the H_2O_2 group at P ≤ 0.05.
Means in the same column marked with hash (#) are comparable (same) and have even significantly higher intended effect than the normal diet group at P ≤ 0.05 value, are mean ± SEM.

KEY:
12.5% LC_{50} = 18.75 for n-Hexane, 60 for ethyl-acetate, 102.5 for residual fraction, 37.5 for methanol extract, 40 for aqueous extract and 36.13 for ascorbic acid (in mg per 10 g diet)
25% LC_{50} = 37.5 for n-Hexane, 120 for ethyl-acetate, 205 for residual fraction, 75 for methanol extract, 80 for aqueous extract and 72.25 for ascorbic acid (in mg per 10 g diet)
50% LC_{50} = 75 for n-Hexane, 240 for ethyl-acetate, 410 for residual fraction, 150 for methanol extract, 160 for aqueous extract and 144.5 for ascorbic acid (in mg per 10 g diet)
100% LC_{50} = 150 for n-Hexane, 480 for ethyl-acetate, 820 for residual fraction, 300 for methanol extract, 320 for aqueous extract and 289 for ascorbic acid (in mg per 10 g diet)

Conversion note for Table 3

Conversion based on Randox DTNB GSH kit calibration (ε_{412} = 13.6 mM^{-1} cm^{-1}) 200 µl reaction volume **and** 0.5 mg protein/sample
To express GSH activity in standard molar units (µmol GSH mg^{-1} protein), the original kit readings (µg mg^{-1} protein) obtained from the Randox GSH kit were recalculated using the molar mass of reduced glutathione (307.32 g mol^{-1}) and the assay parameters. Each reaction contained 200 µL total assay volume and 0.5 mg total protein per sample.

$$\text{GSH (µmol/mg protein)} = \frac{\text{GSH (µg/mg protein)}}{307.32}$$

Table 4. Superoxide dismutase (SOD) activity (U/mg protein) in flies exposed to extracts and fractions of *Pennisetum purpureum* for 7 days under oxidative stress.

Treatment	12.5% LC_{50}	25% LC_{50}	50% LC_{50}	100% LC_{50}
n-Hexane Fraction	11.20 ± 0.02*#	4.72 ± 0.04*	2.64 ± 0.02*	2.30 ± 0.04*
Ethyl-acetate Fraction	4.76 ± 0.04*	3.38 ± 0.20*	3.32 ± 0.04*	3.10 ± 0.04*
Residual Fraction	0.04 ± 0.02	2.94 ± 0.02*	3.02 ± 0.02*	0.44 ± 0.02
Aqueous Fraction	0.90 ± 0.02*	0.56 ± 0.02	0.40 ± 0.04	0.32 ± 0.02
Methanol Extract	13.74 ± 0.06*#	9.94 ± 0.04*	3.32 ± 0.02*	0.12 ± 0.02
Ascorbic acid	15.96 ± 0.04	3.96 ± 0.04	2.54 ± 0.04	2.34 ± 0.04
Normal Diet (Control)	9.76 ± 0.40	9.76 ± 0.40	9.76 ± 0.40	9.76 ± 0.40
Toxicant Control	0.80 ± 0.06	0.80 ± 0.06	0.80 ± 0.06	0.80 ± 0.06

Means in the same column marked with an asterisk (*) are significantly different from the H_2O_2 group at P ≤ 0.05.
Means in the same column marked with hash (#) are comparable (same) and have even significantly higher intended effect than the normal diet group, at P ≤ 0.05 value are mean ± SEM.

Assay reference: SOD activity was determined using the nitro blue tetrazolium (NBT) photo reduction inhibition method as described by Misra and Fridovich (1972), with results expressed as enzyme units per milligram of protein (U/mg protein), where one unit corresponds to 50% inhibition of NBT reduction min^{-1} mg^{-1} protein.
Conversion factor of 1 U ≈ 0.05 µg equivalent (Randox kit)

KEY:
12.5% LC_{50} = 18.75 for n-Hexane, 60 for ethyl-acetate, 102.5 for residual fraction, 37.5 for methanol extract, 40 for aqueous extract and 36.13 for ascorbic acid (in mg per 10 g diet)
25% LC_{50} = 37.5 for n-Hexane, 120 for ethyl-acetate, 205 for residual fraction, 75 for methanol extract, 80 for aqueous extract and 72.25 for ascorbic acid (in mg per 10 g diet)
50% LC_{50} = 75 for n-Hexane, 240 for ethyl-acetate, 410 for residual fraction, 150 for methanol extract, 160 for aqueous extract and 144.5 for ascorbic acid (in mg per 10 g diet)
100% LC_{50} = 150 for n-Hexane, 480 for ethyl-acetate, 820 for residual fraction, 300 for methanol extract, 320 for aqueous extract and 289 for ascorbic acid (in mg per 10 g diet)

Table 5. Catalase (CAT) assay (µmol min^{-1} mg^{-1}) of *Drosophila melanogaster* exposed to extracts and fractions of *Pennisetum purpureum* for 7 days with induction of oxidative stress.

Treatment	12.5% LC_{50}	25% LC_{50}	50% LC_{50}	100% LC_{50}
n-Hexane fraction	1.716 ± 0.04*#	1.382 ± 0.06*#	1.053 ± 0.0*#	0.975 ± 0.01*#
Ethyl-acetate fraction	1.077 ± 0.01*#	1.070 ± 0.01*#	1.158 ± 0.01*#	1.266 ± 0.02*#
Methanol fraction	1.046 ± 0.01*#	1.062 ± 0.01*#	1.133 ± 0.03*#	1.146 ± 0.01*#
Aqueous extract	1.066 ± 0.10*#	1.071 ± 0.01*#	1.089 ± 0.02*#	1.136 ± 0.10*#
Methanol extract	0.973 ± 0.01*#	1.130 ± 0.01*#	1.314 ± 0.10*#	1.314 ± 0.01*#
Ascorbic acid	0.994 ± 0.10	1.008 ± 0.06*	1.038 ± 0.04	1.086 ± 0.03
Negative control	0.994 ± 0.01	0.994 ± 0.01	0.994 ± 0.01	0.994 ± 0.01
Positive control	0.194 ± 0.03	0.194 ± 0.03	0.194 ± 0.03	0.194 ± 0.03

Means in the same column marked with an asterisk (*) are significantly different from the H_2O_2 group at $P \leq 0.05$.
Means in the same column marked with hash (#) are comparable (same) and have even significantly higher intended effect than the normal diet group at $P \leq 0.05$ value, are mean ± SEM
Path length and extinction coefficient (43.6 M^{-1} cm^{-1})

KEY:
12.5% LC_{50} = 18.75 for n-Hexane, 60 for ethyl-acetate, 102.5 for residual fraction, 37.5 for methanol extract, 40 for aqueous extract and 36.13 for ascorbic acid (in mg per 10 g diet)
25% LC_{50} = 37.5 for n-Hexane, 120 for ethyl-acetate, 205 for residual fraction, 75 for methanol extract, 80 for aqueous extract and 72.25 for ascorbic acid (in mg per 10 g diet)
50% LC_{50} = 75 for n-Hexane, 240 for ethyl-acetate, 410 for residual fraction, 150 for methanol extract, 160 for aqueous extract and 144.5 for ascorbic acid (in mg per 10 g diet)
100% LC_{50} = 150 for n-Hexane, 480 for ethyl-acetate, 820 for residual fraction, 300 for methanol extract, 320 for aqueous extract and 289 for ascorbic acid (in mg per 10 g diet)

DISCUSSION

The phytochemical profile of *Pennisetum purpureum* indicates the presence of several metabolites with well-documented antioxidant potential. The methanol extract, which contained the widest range of phytochemicals, including phenols, flavonoids, tannins, terpenoids, and alkaloids, is particularly notable for its strong antioxidant implications. Phenolic compounds and flavonoids are potent free radical scavengers that act through hydrogen or electron donation and metal ion chelation, thereby protecting cellular macromolecules from oxidative damage (Rice-Evans et al., 1997; Pandey and Rizvi, 2009). Studies revealed that extracts with high concentrations of phenolic compounds inhibit lipid peroxidation in human erythrocytes by decreasing the production of MDA (de Toledo Espindola et al., 2016; Lopes et al., 2016; Salazar et al., 2022). Although the presence of phenolic and flavonoid constituents was confirmed qualitatively, the absence of quantitative or chromatographic profiling limits definitive attribution of the observed antioxidant effects to specific compound classes. Future studies incorporating Folin–Ciocalteu, $AlCl_3$, and HPLC/LC–MS analyses are therefore warranted to substantiate the chemical basis of the extract's activity. Studies revealed that the presence of tannins could further enhance this effect by stabilizing cell membranes and reducing lipid peroxidation, while terpenoids and steroids may contribute indirectly by maintaining membrane integrity and modulating redox enzyme activities (Farombi and Owoeye, 2011). The methanol and aqueous extracts, being rich in polar phenolic constituents, are likely responsible for the pronounced increase in enzymatic antioxidant activity, particularly SOD, observed in *Drosophila melanogaster* exposed to oxidative stress. In contrast, the n-hexane and ethyl acetate fractions, containing mainly non-polar antioxidants like terpenoids and steroids, exhibited moderate antioxidant potential, possibly through lipid-phase radical quenching. Overall, the phytochemical constituents detected in *P. purpureum* strongly suggest a synergistic antioxidant mechanism, with phenolic-rich fractions providing primary radical-scavenging action and non-polar fractions supporting redox balance and membrane stability.

The significant elevation of SOD activity observed in flies treated with the methanol extract and n-hexane fraction of *Pennisetum purpureum* (Table 4) suggests that these fractions contain bioactive constituents capable of

enhancing endogenous antioxidant defense mechanisms. Methanol, being a polar solvent, efficiently extracts phenolic compounds, flavonoids, and other redox-active metabolites known to donate hydrogen or electrons to neutralize reactive oxygen species (ROS) and upregulate antioxidant enzymes (Rice-Evans et al., 1997; Halliwell and Gutteridge, 2015). These phytochemicals may act through transcriptional activation of antioxidant response elements (AREs), leading to increased synthesis of SOD and related enzymes (Kensler et al., 2007). The high activity recorded in the n-hexane fraction indicates the presence of non-polar antioxidants such as terpenoids, fatty acids, or phytosterols, which may stabilize cellular membranes and indirectly support SOD function by limiting lipid peroxidation (Farombi and Owoeye, 2011). The observed decline in SOD activity at higher concentrations of the extracts and fractions is consistent with a hormetic effect, where low doses of phytochemicals stimulate protective enzyme systems, but excessive concentrations exert pro-oxidant or inhibitory effects, possibly through redox cycling or feedback inhibition (Calabrese and Mattson, 2017). The moderate effects of the ethyl acetate and residual fractions, and the relatively weak response of the aqueous fraction, may be attributed to the lower abundance or stability of redox-active constituents in these fractions (Pandey and Rizvi, 2009). Collectively, these findings indicate that *P. purpureum* possesses concentration-dependent antioxidant potential, with the methanol extract and n-hexane fraction demonstrating the most effective modulation of SOD activity in *Drosophila melanogaster* exposed to oxidative stress.

It was observed that the administration of 12.5% $LC_{50,}$ 25% LC_{50}, 50% LC_{50}, and 100% LC_{50} (Table 5) of n-Hexane, ethyl-acetate and residual methanol fractions, as well as methanol and aqueous extracts of *P. purpureum* results in a significant increase in the activities of CAT in comparison with the hydrogen peroxide toxicant group. The significant increase implies that treatment with extracts and fractions of *P. purpureum* effectively mitigated oxidative damage caused by hydrogen peroxide in the 7-day treatment regimen. Previous studies have demonstrated that the genome of *D. melanogaster* contains individual single regions that exhibit the ability to enhance CAT activity, along with four regions that possess the capacity to suppress their respective activity as well (Deepashree et al., 2022; Liu, 2022). Therefore, the elevation of this enzyme in the treatment groups indicates that *P. purpureum* extracts and fractions may activate or upregulate the expression of antioxidant genes, extending the flies' resilience to oxidative damage.

Another important antioxidant marker is reduced glutathione (GSH), which represents a phase II group of multifunctional enzymes characterized by the presence of cysteine-rich domains (Semaniuk et al., 2022). Glutathione is a vital antioxidant that protects cells from oxidative stress by neutralizing free radicals and maintaining the redox state of the cell (Khan et al., 2022). The catalytic activity of GST in the conjugation of glutathione (GSH) with electrophilic molecules is a crucial process in the detoxification of xenobiotics that could interrupt redox status in living organisms (Potega, 2022). The present findings demonstrated the positive effect of *P. purpureum* extracts on GSH activities in flies fed with the extracts and fractions when compared to the hydrogen peroxide control group for the treatment duration (Table 3). The observed effect suggests that the extracts/fractions may facilitate the regeneration of this critical antioxidant, thereby improving the overall antioxidant capacity of *D. melanogaster*.

Malondialdehyde is a by-product and well-established marker of lipid peroxidation; its reduction connotes decreased oxidative degradation of lipids, thereby preserving cellular membrane integrity (Del Rio et al., 2005; Sharma et al., 2019). Its accumulation, on the contrary, signifies oxidative deterioration of lipids, leading to compromised cell membrane integrity (Del Rio et al*.,* 2005). The observed decrease in malondialdehyde levels in the treatment groups is particularly noteworthy; it implies the extracts and fractions' capacities to effectively attenuate the lipid peroxidation caused by hydrogen peroxide exposure, preserving cellular structures and functions. This finding aligns with the concept that enhancing antioxidant defences can lead to decreased oxidative damage and improved cellular integrity. Indeed, *P. purpureum* leaf extracts have been shown to exhibit antioxidant activity *in vitro*, associated with their content of flavonoids, phenolics, and other phytochemicals (Budiyanto et al., 2024; Sinha et al., 2021). Similar findings reported that other Poaceae species, such as *Cymbopogon citratus* and *Oryza sativa*, extracts significantly lowered malondialdehyde (MDA) levels and improved antioxidant enzyme activities *in vivo*, suggesting effective protection against oxidative stress induced cellular injury (Akinmoladun et al., 2021; Rahman et al., 2020; Olorunnisola et al., 2019). Moreover, other plant antioxidants such as curcumin and resveratrol have been documented to reduce malondialdehyde (MDA) levels in models of oxidative stress, underscoring their protective role against lipid peroxidation (Laszlo et al., 2022). Studies have shown that exposure to oxidative agents like hydrogen peroxide induces the synthesis of stress proteins, including heat-shock proteins and actin, as part of the cellular defense mechanism in *Drosophila* cells (Courgeon et al., 1988). The induction of these proteins highlights the organism's attempt to mitigate oxidative damage and maintain cellular integrity.

From the discussion above, *P. purpureum* stands out as a promising antioxidant source despite the availability of many phytochemically rich plants because it combines several unique advantages that justify its scientific investigation. The plant contains diverse bioactive compounds such as flavonoids, phenolic acids, tannins, and alkaloids (Table 1), suggesting strong antioxidant

potential that remains largely underexplored compared to more commonly studied medicinal plants. It's long standing ethno medicinal use for treating inflammation, wounds, fever, and gastrointestinal disturbances (Heuze et al., 2020) further supports the likelihood that it modulates oxidative stress pathways. In addition, *P. purpureum* is abundant, fast-growing, easy to cultivate, and available year-round, making it a sustainable and cost-effective source of medicinal raw material. Its widespread use in livestock feeding and traditional medicine also points to a favourable safety profile, with minimal toxicity concerns (Kour et al., 2025). These attributes, coupled with the possibility of multi-mechanistic antioxidant activity and significant socioeconomic benefits, make *P. purpureum* a valuable candidate for developing affordable, safe, and effective antioxidant therapies, particularly in low-resource settings.

Conclusion

In conclusion, the findings from this study provide compelling evidence that *Pennisetum purpureum* extracts and fractions possess significant antioxidant properties. The observed increases in CAT, SOD, and GSH activities, along with the decrease in MDA levels, suggest that these extracts and fractions may serve as a valuable natural source of antioxidants, potentially offering protective effects against oxidative stress-related diseases.

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