ABUAD INTERNATIONAL JOURNAL OF NATURAL AND APPLIED SCIENCES
ISSN: 2955-1021
AIJNAS Volume 5, Issue 2, 2025 pp 84-90
https://doi.org/10.53982/aijnas.2025.0502.02-j

https://journals.abuad.edu.ng/index.php/aijnas

ANTIOXIDANT ACTIVITY, PHYTOCHEMICAL COMPOSITION OF EXTRACTS OF *PEPEROMIA PELLUCIDA* AND ITS EFFECT ON *DROSOPHILA MELANOGASTER*

Abiodun O. **ADEJORI**[1,2], Anjolaoluwa G. **IBITOYE**[2], Victoria A, **HENRY**[2] Ifeoluwa L. **ONIFADE**[1], Scholastica O. **ANADOZIE**[1], Olusola B. **ADEWALE**[1]

[1]Drosophila Laboratory, Drug Metabolism and Toxicology Unit, Biochemistry Programme, Afe Babalola University, Km 8.5, Afe Babalola way, P.M.B 5454, Ado-Ekiti, Nigeria

[2]Department of Science Laboratory Technology, University of Medical Sciences, Ondo, Nigeria.

***Correspondence: abbeeycity01@googlemail.com; aadejori@unimed.edu.ng**

Abstract

Background/Aim: *The therapeutic influence of medicinal plants is predominantly based on the several secondary metabolites inherent in them. Peperomia pellucida, a plant with great importance in traditional medicine, have been considered as an excellent remedy for numerous diseases. In this study, the antioxidant properties and phytochemical quantification of extracts of Peperomia pellucida whole plant was carried out, and a 21-day survival and longevity study to evaluate the toxic effect.* ***Materials and Methods:*** *Qualitative and quantitative phytochemical screening, as well as in vitro antioxidant potential was done using 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity, 2,2' aminio-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP). For the in vivo study, Drosophila melanogaster (Fruit flies) was expose to AEPP (0, 0.25, 0.5, 1, and 2 mg/g diet) for 21 days. Thereafter, flies were homogenized, and parameters such as reduced glutathione, glutathione-s-transferase (GST), catalase, nitric oxide, locomotive ability, acetylcholinesterase (AChE), and glucose assays were carried out.* ***Results:*** *The DPPH and ABTS radical scavenging activities, and FRAP were found to be 87.29 ± 0.03 % ellagic acid, 65.47 ± 0.04 µg/g, and 32.17 ± 0.02 mg/100g, respectively. The phytochemical screening indicated the presence of phenols (80.75 mg/100g), saponins (61.21mg/100g), alkaloids (41.63 mg/100g), and flavonoids (28.19 mg/100g), among others. The plant extract at different doses showed no significant difference on the survival rate, reduced glutathione, GST, nitric oxide, and glucose levels, as well as acetylcholinesterase activity and climbing ability. However, a significant ($p < 0.05$) decrease in the catalase activity was noted at 1 and 2 mg AEPP/g diet, although, other parameters did not show any sign of toxicity. Further toxicological studies should be conducted to ascertain this.* ***Conclusions:*** *This study could therefore, be concluded that AEPP is not toxic, most especially at lower doses, and could therefore be considered for therapeutic applications.*

1. INTRODUCTION

The use of medicinal plants is one of the ancient therapeutic means employed in the treatment of diseases or various health challenges globally. In recent times, medicinal plants play an essential role in the manufacture of several pharmaceutical products due to their beneficial phytochemical composition, accessibility, and reliability.

Peperomia pellucida (L.) Kunth, belonging to the family Piperaceae, is known for several pharmacological properties. It is a succulent herbaceous plant that is habitable to humid and loose soils. *Peperomia pellucida* is useful in folk medicine in the treatment of several diseases including indigestion, diarrhea, skin sores, gastrointestinal infections, dysentery, abscesses, and kidney diseases. The plant has also been reported to contain antioxidant, cytotoxic, antimicrobial, antidiabetic, fracture healing, and anti-hypercholesterolemia properties (Alves *et al.*, 2019). This herb contains compounds, which include alkaloids, flavonoids, polyphenols, glycosides, tannins, saponins, and terpenoids (Tuan and Men, 2024). Phytochemicals such as ellagic acid, carotol, β-caryophyllene, phytol, dillapiole, pellucidin A, and vitexin have been identified from the plant, and these could have contributed to the various pharmacological properties exhibited by the plant (Ho *et al.*, 2022). The LD_{50} of the ethanolic extract of the plant was found to be greater than 5,000 mg/kg in mice after 7 days administration and no sub-chronic toxicity at 500 mg/kg of the extract at 28 days exposure. The ethanolic extract was also found to increase the lifespan of fruit flies against oxidative stress (Tuan and Men, 2024).

Drosophila melanogaster, also known as fruit flies, is a model used in animal research, and has been used in genetic research ranging from fundamental genetics to developmental biology (Lopez-Ortiz *et al.*, 2023). The genome of fruit flies is 60% homologous to human genome, and possess 75% of the genes responsible for human diseases (Ugur *et al.*, 2016). The major challenge to use of mammals, such as mice and rats, in conventional evaluation of toxicity of substances has been faced with challenges associated with cost, reproducibility and ethical issues regarding animal welfare. To overcome this, it is imperative to consider the alternative models compliant with the European Centre for the Validation of Alternative Methods where 3Rs standard (Replacement, Refinement and Reduction) are employed (Anadozie *et al.*, 2024).

In this study, *fruit flies* would be used in investigating the effect of aqueous extract of *Peperomia pellucida* (AEPP) whole plant, go provide further information on the safety profile using the most common and conventional mode of extraction of medicinal plants.

2. MATERIALS AND METHODS

2.1. Plant Collection and Extraction

Peperomia pellucida plants were collected from an open field at Afe Babalola University Ado-Ekiti, Nigeria in October 2024. The whole plant was rinsed to remove the sand, and other particles under running tap water. The plants were air-dried at room temperature for 10 days, and then reduced to powder using a steel grinder. About 200 g of powdered *Peperomia pellucida* was extracted in distilled water, ethanol and methanol for 72 h by maceration method and further concentrated to dryness using a water bath (45 ◦C).

2.2. Quantitative Phytochemical Studies

Phytochemical quantification of AEPP was carried out to determine the presence of metabolites such as alkaloids, anthraquinone, flavonoids, phenols, glycosides, saponins, reducing sugars, tannins, steroids and cardiac glycosides according to standard methods.

2.3. *In vitro* antioxidant potentials of *Peperomia pellucida* plant

2.3.1. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay

The radical scavenging activity of AEPP was performed using the DPPH assay, according to a method by Shirwaikar *et al.* (2019), with some modifications. Two (2) mL of AEPP, or ellagic acid (standard) was added to 2 mL 0.1 mM DPPH (in methanol). An equal volume of DPPH and methanol served as a control. The mixture was incubated in the dark at 30 C for 20 min, and the absorbance read at 517 nm. The DPPH radical scavenging activity of the samples was calculated as follows:

% DPPH radical scavenging activity = ((Abs of control – Abs of sample) / Abs of control) x 100

2.4. Fly Strain and Experimental Design

D. melanogaster Harwich strains from the Africa Centre of Excellence were bred and maintained on a simple diet prepared using corn flour, baker's yeast, agar-agar, and methylparaben. Fly laboratory temperature was kept constant at 25 °C. The animal treatment is shown in the table 1 below

Animal Treatment

Groups	Treatment
Group 1	Control (Basal diet)
Group 2	AEPP (0.25 mg/g diet)
Group 3	AEPP (0.50 mg/g diet)
Group 4	AEPP (1.00 mg/g diet)
Group 5	AEPP (2.00 mg/g diet)

Flies (both genders, 1–3 days old) were divided into five groups with each group (n = 5, 60 flies). The flies were exposed to the plant extract for 21 days, as described by Oyetayo *et al.* (2020).

2.5. Preparation of homogenate for biochemical analysis

The flies were thereafter immobilized by ice and homogenized in 0.1 M phosphate buffer, pH 7.4. The homogenates were centrifuged at 4000 × *g*, at 4 °C for 10 min. Subsequently, the supernatant was separated into a new labelled Eppendorf tube, and used for various biochemical assays.

2.6. Negative geotaxis/locomotor assay

The locomotive function of the flies was assessed by a negative geotaxis test following the method described by Oyaluna *et al.* (2021). Ten flies from each of the control or treated vials were immobilized and anaesthetized on ice in a new vial (length, 15 cm; diameter, 1.5cm). The flies were allowed to recover from the ice exposure (about 2 min) and the vial was gently tapped at the bottom, then the number of flies that climbed up to the 6 cm mark on the vials within 6 seconds and those that remained below the mark on the vial after the stipulated time were recorded. The scores represent the mean of the number of flies at the top (n_{top}) expressed as a percentage of the total number of flies (n_{tot}). This procedure was performed in triplicate.

2.7. Parameters investigated

Parameters such as total protein (Lowry *et al.*, 1951), total thiols (Ellman, 1959), catalase activity (Claiborne, 2018), nitric oxide (Green *et al.*, 1982), glutathione-S-transferase (Habig *et al.*, 1974), and glucose (Barham and Trinder, 1972).

2.8. Statistical Analyses

All results were reported as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) on GraphPad Prism 6.07, (GraphPad Software, Inc, San Diego, USA). Tukey's post hoc multiple comparisons test was used to determine statistical significance at $p < 0.05$ in all cases.

3. RESULTS

3.1. Phytochemical Analysis of *Peperomia pellucida* Extracts

Table 1 shows the various phytochemicals present in AEPP, with high contents of these compounds in the methanolic extracts in alkaloids, flavonoids, glycosides, phenols, and steroids in relation to both aqueous and ethanolic extracts. Ethanolic extract possesses anthraquinone, and tannin, while higher contents of saponins, reducing sugars, cardiac glycosides are found in aqueous extract compared to ethanolic and methanolic extracts.

Table 1: Phytochemical Analysis of *Peperomia pellucida* Extract

PARAMETERS (mg/100g)	Aqueous Extract	Ethanolic Extract	Methanolic Extract
Alkaloids	41.63 ± 0.01	53.30 ± 0.01	59.53 ± 0.01
Anthraquinones	5.91 ± 0.01	6.30 ± 0.01	4.58 ± 0.01
Cardiac Glycosides	3.16 ± 0.01	1.32 ± 0.01	2.08 ± 0.01
Flavonoids	28.19 ± 0.00	40.34 ± 0.00	45.23 ± 0.01
Glycosides	3.63 ± 0.00	4.53 ± 0.00	6.20 ± 0.01
Phenols	80.75 ± 0.01	86.36 ± 0.01	94.36 ± 0.01
Tannins	13.15 ± 0.01	18.67 ± 0.01	16.13 ± 0.01
Reducing Sugars	5.92 ± 0.01	5.17 ± 0.03	4.93 ± 0.01
Saponins	61.21 ± 0.01	50.58 ± 0.01	44.71 ± 0.01
Steroids	1.32 ± 0.00	1.71 ± 0.00	2.07 ± 0.01

3.2. Antioxidant properties analysis of *Peperomia pellucida* Extracts

The antioxidant properties of the aqueous, ethanolic and methanolic extracts of the plant is presented in table 2. The methanolic extract showed higher levels of DPPH, FRAP, and ABTS followed by ethanolic and aqueous extracts.

Table 2: Antioxidant properties analysis of *Peperomia pellucida* Extract

PARAMETERS	Aqueous Extract	Ethanolic Extract	Methanolic Extract
DPPH (% Ellagic acid)	87.29 ± 0.03	91.22 ± 0.02	94.35 ± 0.03
FRAP (mg/100g)	32.17 ± 0.02	36.73 ± 0.03	41.23 ± 0.02
ABTS (µg/g)	65.47 ± 0.04	67.18 ± 0.02	70.24 ± 0.02

3.3. Effect of aqueous extract of *P. pellucida* on survival rate of *D. melanogaster*

Figure 1 shows the effect of AEPP on the survival rate of *D. melanogaster*. No significant difference was noted in the survival rate of flies exposed to AEPP at all doses during the 21 days when compared to the control.

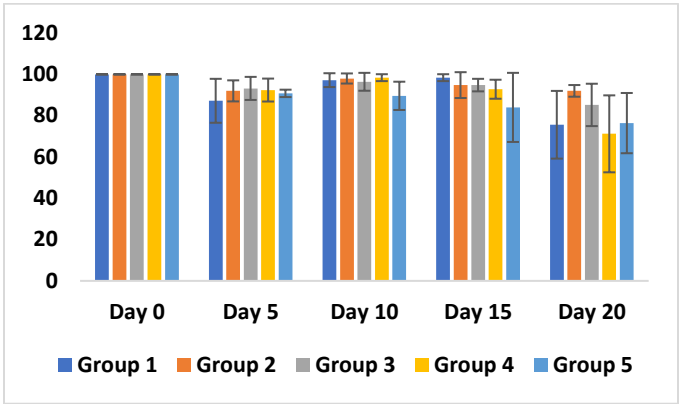

Figure 1. Effect of *P. pellucida* on survival rate of *D. melanogaster*
Bars represent mean ± SD (n = 5)
Group 1: Basal diet (control), Group 2: 0.25 mg AEPP/g diet; Groups 3: 0.5 mg AEPP/g diet, Groups 4: 1.0 mg AEPP/g diet, Groups 5: 2.0 mg AEPP/g diet

3.4. Effect of aqueous extract of *P. pellucida* on total protein concentration in *Drosophila melanogaster*

The effect of AEPP on the level of total protein in *D. melanogaster* is presented in Figure 2. No significant difference was noted in total protein level in flies exposed to AEPP at all doses when compared to the control.

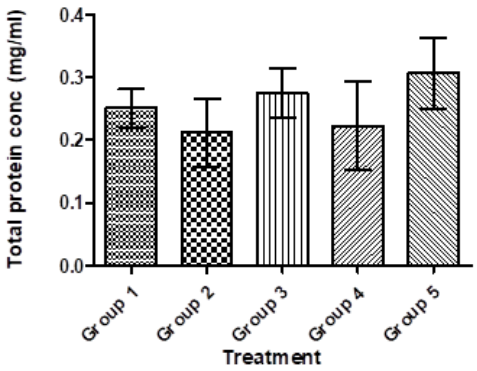

Figure 2. Effect of *P. pellucida* on total protein concentration in *Drosophila melanogaster*
Bars represent mean ± standard deviation (n = 5)
Group 1: normal diet(control), Group 2: 0.25 mg PPAQE/g diet; Groups 3: 0.5 mg AEPP/g diet, Groups 4: 1.0 mg AEPP/g diet, Groups 5: 2.0 mg AEPP/g diet

3.5. Effect of aqueous extract of *P. pellucida* on the level of reduced glutathione in *Drosophila melanogaster*

The effect of AEPP on reduced glutathione level in *D. melanogaster* is presented in Figure 3. No significant difference was noted in the level of reduced glutathione in flies exposed to AEPP at all doses when compared to the control.

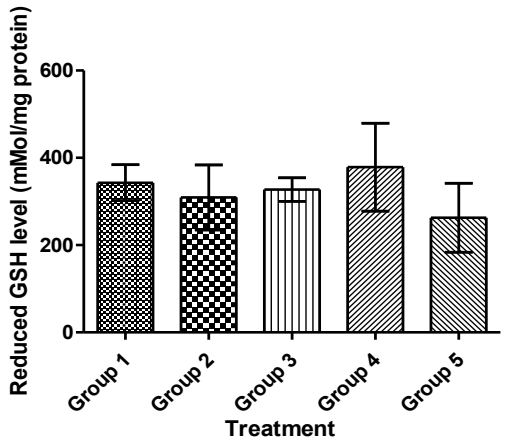

Figure 3. Effect of *P. pellucida* on the level of reduced glutathione in *Drosophila melanogaster*
Bars represent mean ± standard deviation (n = 5)
Group 1: normal diet(control), Group 2: 0.25 mg AEPP/g diet; Groups 3: 0.5 mg AEPP/g diet, Groups 4: 1.0 mg AEPP/g diet, Groups 5: 2.0 mg AEPP/g diet

3.6. Effect of aqueous extract of *P. pellucida* on the activity of glutathione-s-transferase in *Drosophila melanogaster*

The effect of AEPP on GST activity in *D. melanogaster* is presented in Figure 4. There was no significant difference in the activity of GST in flies exposed to AEPP at all doses when compared to the control.

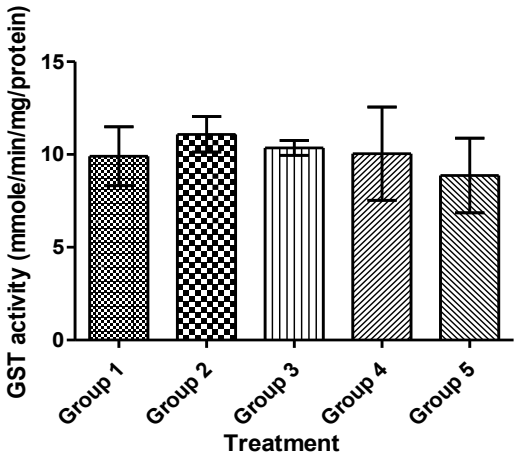

Figure 4. Effect of *P. pellucida* on the activity of glutathione-s-transferase in *Drosophila melanogaster*
Bars represent mean ± standard deviation (n = 5)
Group 1: normal diet(control), Group 2: 0.25 mg AEPP/g diet; Groups 3: 0.5 mg AEPP/g diet, Groups 4: 1.0 mg AEPP/g diet, Groups 5: 2.0 mg AEPP/g diet

3.7. Effect of aqueous extract of *P. pellucida* on catalase activity in *Drosophila melanogaster*

Figure 5 shows the effect of AEPP on the activity of catalase in *D. melanogaster*. No significant difference was noted in catalase activity in flies exposed to AEPP at all doses when compared to the control, except a significant ($p < 0.05$) reduction in the activity of this enzyme at doses 1 and 2 mg/kg diet.

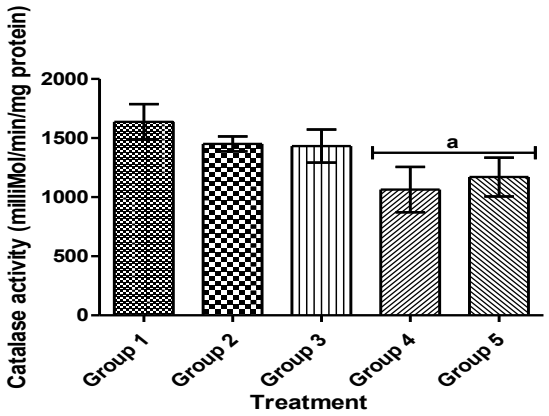

Figure 5. Effect of *P. pellucida* on catalase activity in *Drosophila melanogaster*
Bars represent mean ± standard deviation (n = 5), $^{a}p < 0.05$ when compared to control. Group 1: normal diet(control), Group 2: 0.25 mg AEPP/g diet; Groups 3: 0.5 mg AEPP/g diet, Groups 4: 1.0 mg AEPP/g diet, Groups 5: 2.0 mg AEPP/g diet

3.8. Effect of aqueous extract of *P. pellucida* on nitric oxide level in *Drosophila melanogaster*

Figure 6 shows the effect of AEPP on the level of nitric oxide in *D. melanogaster*. No significant difference was noted in nitric oxide level in flies exposed to AEPP at all doses when compared to the control.

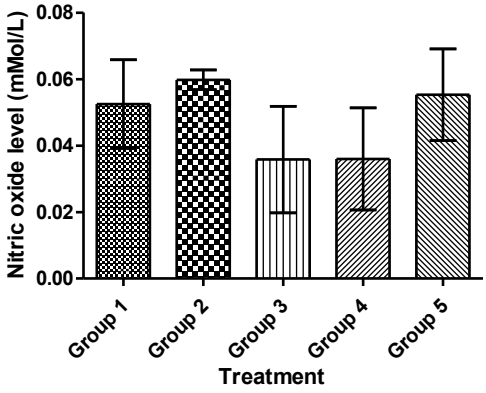

Figure 6. Effect of *P. pellucida* on nitric oxide level in *Drosophila melanogaster*
Bars represent mean ± standard deviation (n = 5)
Group 1: normal diet(control), Group 2: 0.25 mg AEPP/g diet; Groups 3: 0.5 mg AEPP/g diet, Groups 4: 1.0 mg AEPP/g diet, Groups 5: 2.0 mg AEPP/g diet

3.9. Effect of aqueous extract of *P. pellucida* on the activity of acetylcholinesterase in *Drosophila melanogaster*

Figure 7 shows the effect of AEPP on the activity of AChE in *D. melanogaster*. No significant difference was noted in AChE activity in flies exposed to AEPP at all doses when compared to the control.

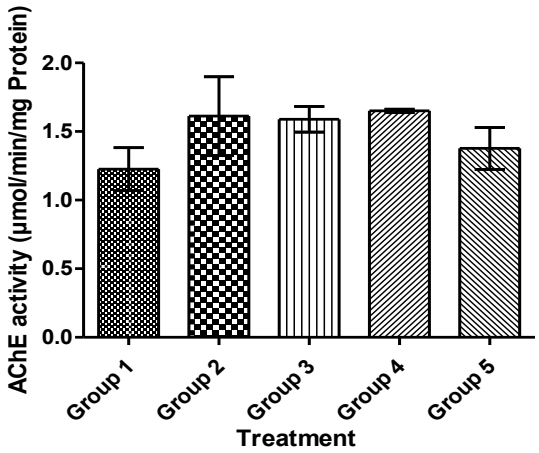

Figure 7. Effect of *P. pellucida* on the activity of acetylcholinesterase in *Drosophila melanogaster*
Bars represent mean ± standard deviation (n = 5)
Group 1: normal diet(control), Group 2: 0.25 mg AEPP/g diet; Groups 3: 0.5 mg AEPP/g diet, Groups 4: 1.0 mg AEPP/g diet, Groups 5: 2.0 mg AEPP/g diet

3.10. Effect of aqueous extract of *P. pellucida* on the climbing rate of *Drosophila melanogaster*

The effect of AEPP on the climbing or locomotive ability in *D. melanogaster* is presented in Figure 8. No significant difference was noted in locomotive ability of flies exposed to AEPP at all doses when compared to the control.

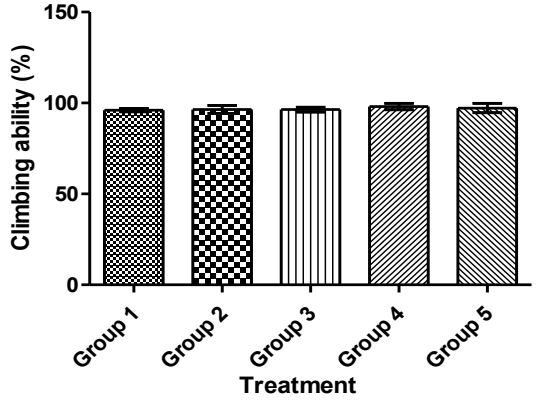

Figure 8. Effect of *P. pellucida* on the climbing rate of *Drosophila melanogaster*
Bars represent mean ± standard deviation (n = 5)
Group 1: normal diet(control), Group 2: 0.25 mg AEPP/g diet; Groups 3: 0.5 mg AEPP/g diet, Groups 4: 1.0 mg AEPP/g diet, Groups 5: 2.0 mg AEPP/g diet

3.11. Effect of aqueous extract of *P. pellucida* on glucose level in *Drosophila melanogaster*

Figure 9 shows the effect of AEPP on glucose level in *D. melanogaster*. No significant difference was noted in the level of glucose in flies exposed to AEPP at all doses when compared to the control.

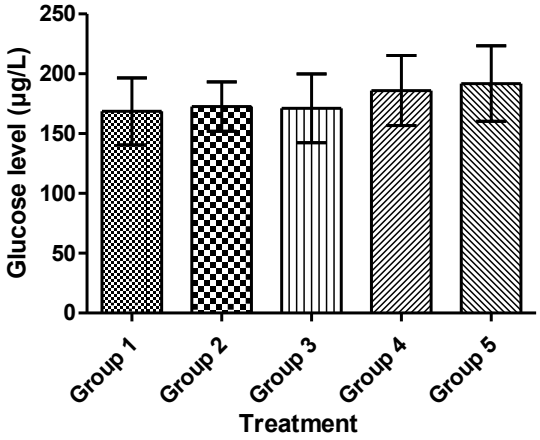

Figure 9. Effect of *P. pellucida* on glucose level in *Drosophila melanogaster*
Bars represent mean ± standard deviation (n = 5)
Group 1: normal diet(control), Group 2: 0.25 mg AEPP/g diet; Groups 3: 0.5 mg AEPP/g diet, Groups 4: 1.0 mg AEPP/g diet, Groups 5: 2.0 mg AEPP/g diet

4. DISCUSSION

Medicinal plants play crucial roles in the treatment of various ailments globally (Nwanna, 2021), which may be due to several compounds inherent in them. Although, there are several benefits in the therapeutic applications of these plants, which include availability, affordability and general assumption of safety. However, despite the medicinal relevance, no adequate information on the safety of many of these plants and study on the therapeutic doses required for the treatment of these diseases are still a concern. In this study, toxicological evaluation of the AEPP, including immediate, continuous or delayed effect, was carried out in *Drosophila melanogaster*.

Peperomia pellucida (L.) Kunth, has been reported to contain compounds, which include alkaloids, flavonoids, polyphenols, glycosides, tannins, saponins, and terpenoids (Tuan and Men, 2024). High contents of these compounds were also found in the results of the phytochemical screening of our study in addition to other compounds, except terpenoids that was not seen in our study. These compounds could contribute to the pharmacological relevance exhibited by the plant. In the same vein, the *in vitro* antioxidant capabilities of the plant extracts were in the following order methanolic > ethanolic > aqueous. This indicates that the methanolic extract possessed greater ability to scavenge free radicals. This is line with the results of the phytochemical screening. In all, methanolic extract can be suggested as the most potent, although, all the extracts have appreciable phytochemicals and antioxidant capabilities Medicinal plants are mostly prepared locally for therapeutic purposes in form of herbal preparations, and water is the most widely used solvent. Hence, the reason for considering aqueous extract in the *in vivo* studies.

On exposure of flies to any substance, the immediate effect can be noticed through survival study, where the mortality of flies is recorded. In this study, there was no significant changes in the mortality rate of flies at all doses tested when compared to the control, which indicates that the AEPP, at all doses, did not cause death of the flies at 21 days of exposure. This is in line with an acute toxicity study and 28 days exposure of ethanolic extract where the LD_{50} was found to be greater than 5,000 mg/kg in mice after 7 days administration and no toxic effect up to 500 mg/kg at 28 days exposure was noted. The extract was also reported to increase the lifespan of fruit flies against oxidative stress (Tuan and Men, 2024).

Antioxidant (reduced GSH, GST, and catalase) and oxidative stress (MDA) markers are important toxicological indices used to access the general well-being of an organism. Thiols, containing sulfhydryl (-SH) groups, can conjugate free radicals and electrophiles, thereby converting them to less harmful forms (Balogun *et al.*, 2021; Oyaluna *et al.*, 2021). The conversion hydrogen peroxide (a radical) to water and oxygen is catalysed by catalase, and any impairment in the activity of this enzyme could result in elevated level/accumulation of this radical, and induce oxidative damage (Adesanoye *et al.*, 2021). A non-significant difference in the level of nitric oxide also indicated no redox imbalance. A balanced redox status is responsible for normal cellular metabolism and physiological function in biological system (Balogun *et al.*, 2021), while an imbalance results in oxidative stress. In this study, no alteration was noted in these parameters in flies exposed to AEPP at all tested. Although, a significant reduction was noted with high doses of the extract (1 and 2 mg/g diet) in comparison with the control. However, there was no other parameter that was altered.

The normal physiological activity of an organism is reflected by its behaviour. The locomotive ability of flies and the activity of AChE coordinate their neurological functions. Acetylcholinesterase catalyses the conversion of acetylcholine to acetate and choline, and this neurotransmitter (choline) participates in the regulation of locomotion, memory, and motor function (Oyaluna *et al.*, 2021). In this study, the non-significant difference in the locomotive ability and AChE activity in flies on exposure to AEPP could suggest that the plant extract did not alter the behaviour of the flies and could therefore, protect the brain against damage.

Glucose is the major metabolic fuel of most organs in the body. Persistent increase in its blood level indicates hyperglycemia, which could result in diabetes. The non-significant difference in the level of glucose in this study on exposure of flies to AEPP at all tested doses could suggested no derangement in glucose metabolism in the flies.

5. CONCLUSION

Based on the results of this study, it can be concluded that aqueous extract of *Peperomia pellucida* could be considered safe for consumption up to 2 mg/g diet, and this could further support its traditional use in the treatment of diseases.

LIST OF ABBREVIATIONS

AEPP: Aqueous extract of *Peperomia pellucida*

DECLARATIONS

Ethics approval and consent to participate: Not Applicable

Consent for publication: Not Applicable

Competing interests: Not Applicable

Funding: No funding was obtained for this study.

REFERENCES

Adesanoye, O. A., Farodoye, O. M., Adedara, A. O., Falobi, A. A., Abolaji, A. O., & Ojo, O. O. (2021). Beneficial actions of esculentin-2CHa(GA30) on high sucrose-induced oxidative stress in Drosophila melanogaster. *Food and Chemical Toxicology, 157*, 112620. doi:https://doi.org/10.1016/j.fct.2021.112620

Alves, N. S. F., Setzer, W. N., & da Silva, J. K. R. (2019). The chemistry and biological activities of Peperomia pellucida (Piperaceae): A critical review. *Journal of Ethnopharmacology, 232*, 90-102. doi:https://doi.org/10.1016/j.jep.2018.12.021

Anadozie, S. O., Aduma, A. U., & Adewale, O. B. (2024). Alkaloid-rich extract of Buchholzia coriacea seed mitigate the effect of copper-induced toxicity in Drosophila melanogaster. *Vegetos, 37*(2), 460-468. doi:10.1007/s42535-023-00760-9

Balogun, O., Abolaji, A., Adedara, A. O., Akinsanmi, A. O., & Alemika, T. (2021). Ameliorative Role of Plectranthus esculentus on 4-Vinylcyclohexene Monoepoxide-Induced Oxidative Stress in Drosophila melanogaster. *Biointerface Research in Applied Chemistry, 11*, 9432-9442. doi:10.33263/BRIAC112.94329442

Barham, D., & Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst, 97*(151), 142-145. doi:10.1039/an9729700142

Claiborne, A. (2018). Catalase Activity. In *Handbook Methods For Oxygen Radical Research* (pp. 283-284): CRC Press.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Arch Biochem Biophys, 82*. doi:10.1016/0003-9861(59)90090-6

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Analytical Biochemistry, 126*(1), 131-138. doi:https://doi.org/10.1016/0003-2697(82)90118-X

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases. The first enzymatic step in

mercapturic acid formation. *The Journal of Biological Chemistry, 249*(22), 7130-7139.

Ho, K. L., Yong, P. H., Wang, C. W., Kuppusamy, U. R., Ngo, C. T., Massawe, F., & Ng, Z. X. (2022). Peperomia pellucida (L.) Kunth and eye diseases: A review on phytochemistry, pharmacology and toxicology. *Journal of Integrative Medicine, 20*(4), 292-304. doi:https://doi.org/10.1016/j.joim.2022.02.002

Lopez-Ortiz, C., Gracia-Rodriguez, C., Belcher, S., Flores-Iga, G., Das, A., Nimmakayala, P., . . . Reddy, U. K. (2023). Drosophila melanogaster as a Translational Model System to Explore the Impact of Phytochemicals on Human Health. *International Journal of Molecular Sciences, 24*(17), 13365.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry, 193*(1), 265-275.

Oyaluna, Z., Abolaji, A., & Babalola, C. (2021). Effects of Ruzu Herbal Bitters, a Traditional Nigerian Polyherbal Drug, on Longevity and Selected Toxicological Indices in Drosophila melanogaster. *Biointerface Research in Applied Chemistry, 11*. doi:10.33263/BRIAC112.96389645

Oyetayo, B. O., Abolaji, A. O., Fasae, K. D., & Aderibigbe, A. (2020). Ameliorative role of diets fortified with Curcumin in a Drosophila melanogaster model of aluminum chloride-induced neurotoxicity. *Journal of Functional Foods, 71*, 104035. doi:https://doi.org/10.1016/j.jff.2020.104035

Tuan, C. T., & Men, T. T. (2024). Peperomia pellucida 's Ingredients, Antioxidant Properties, and Safe Usage as Food and Herbal Medicine. *Journal of Microbiology and Biotechnology, 34*(11), 2321-2330. doi:10.4014/jmb.2406.06025

Ugur, B., Chen, K., & Bellen, H. J. (2016). Drosophila tools and assays for the study of human diseases. *Dis Model Mech, 9*(3), 235-244. doi:10.1242/dmm.023762