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# Investigating the bioactive compounds in aqueous extract of *Calotropis procera* and its toxicological evaluation using *Drosophila melanogaster* model

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**Abstract**
***Background/Aim:*** *Phytochemicals are present in various plant tissues. These compounds provide beneficial effects on the plants, however, limited harmful effects have been reported. This study investigated the bioactive compounds in the aqueous extract of Calotropis procera (AECP) and their toxic effects.* ***Materials and Methods:*** *The presence of bioactive compounds in the plant was determined using standard methods and high-performance liquid chromatography, while the toxicological evaluation was done using Drosophila melanogaster. Flies were grouped into 6 (n = 5), comprising control and AECP-treated groups. Flies were homogenized after 21 days of exposure to AECP, and toxicological parameters such as total thiol, reduced glutathione, catalase, nitric oxide, geotaxis, and survival tests were investigated.* ***Results:*** *The plant extract was found to be rich in phenols, flavonoids, tannins, reducing sugars, and glycosides, with the total phenols content of 14.05 ± 0.07 mg Tannic acid/g equivalent and total flavonoids content of 9.218 ± 0.05 mg Quercetin/g equivalent. Sixteen compounds were identified in the plant with the highest abundance in the order: quercetin, cymbopogon, hydroquinone, chlorogenic acid, ferulic acid, and luteolin. It was also observed that the plant was not toxic to flies exposed to doses up to 2 mg/g diet as indicated by a non-significant difference in the parameters investigated but a significant change ($p < 0.05$) was noted in catalase, reduced glutathione and nitric oxide at 4 mg/g diet when compared with the control.* ***Conclusions:*** *The presence of the secondary metabolites could ensure the plant of its pharmacological properties, and the plant could be considered safe up to a dose of 2 mg/g diet at short and long-term exposure.*
**Keywords:** *Calotropis procera, Drosophila melanogaster, Phytochemicals, Toxicity*

## INTRODUCTION

Medicinal plants have a long history globally for their use in the treatment of several ailments. The World Health Organization estimated that about 80% of developing countries still rely on conventional medications originating from medicinal plants, as several plant species (Adeola *et al*., 2023). Numerous phytochemicals have been identified and isolated in plants, and these compounds have been linked to the health benefits possessed by these medicinal plants. Examples of such phytochemicals are flavonoids, polyphenols, saponins, carotenoids, tannins, and glycosides, which are present in various plant tissues such as leaves, flowers, roots, stems, and fruits. Such compounds have beneficial effects on the plants and have been reported to be useful against many human diseases, such as cancer, cardiovascular diseases, inflammation, and diabetes. Regular consumption of grains, vegetables, and fruits could reduce the risk of these chronic diseases due to the inherent phytochemicals known for their antioxidant and anti-inflammatory potentials. This is because these phytochemicals are involved in the regulation of oxidative stress, a significant factor in the pathogenesis of some diseases (Lopez-Ortiz *et al*., 2023). However, in addition to the safety of these medicinal plants, potential toxicities have also been documented, which necessitates more pre-clinical safety studies (Adewale *et al*., 2016).

*Calotropis procera* (Aiton) Dryand belongs to the family Apocynaceae, and it is commonly found in arid and semi-arid habitats (Kaur *et al*., 2021). It is grown in most parts of Nigeria and it is harvested due to its potential medicinal properties, as parts of the plant have been reported to be active against diseases such as bronchial asthma, dysentery, microbial infections, snake poisoning, inflammation, rheumatism, ulcer, skin conditions, leprosy, cancer, and diabetes (Kazeem *et al*., 2016). The plant has been reported to contain various phytochemicals, including alkaloids, phenols, flavonoids, saponins, lignans, flavanol glycosides, cardenolides, triterpenes, steroids, proteins, and enzymes (Amini *et al*., 2021).

*Drosophila melanogaster*, commonly known as the fruit fly, is considered an appropriate model organism in assessing the safety of medicinal plants because it is less expensive and easy to maintain, with few ethical concerns. It has about 75% of known genes linked to human disease (Oyaluna *et al*., 2021). In this study, the bioactive compounds in the aqueous extract of *Calotropis*

*procera* (AECP) and their toxic effects were investigated in *Drosophila melanogaster*

## MATERIALS AND METHODS

### *Chemicals*

All chemicals used in this study were of analytical grade.

### *Collection of Plant and Preparation of Plant Extract*

*Calotropis procera* leaves were sourced from Ilorin, Kwara State, Nigeria. The plant was identified and authenticated at the Department of Plant Science and Biotechnology, Ekiti State University, Nigeria. A specimen of the plant was placed in the institute's herbarium, with a voucher number UHAE 2025011. The leaves were dried at 27 °C for 14 days, and the dried leaves were ground, and the powdered sample was stored in an airtight container at 4°C until needed. The powdered sample was weighed and soaked in distilled water for 24 hr, was thereafter filtered using a cheese cloth followed by filter paper (Whatman No. 1). The supernatant was thereafter concentrated using a water bath at 45 °C for about 48 hr, and the concentrated sample was kept at 4°C for use.

### *Experimental Design and grouping of flies*

*Drosophila melanogaster* (Harwich strain) bred at Drosophila Research Laboratory, Biochemistry program, Afe Babalola University Ado-Ekiti, Nigeria, was used for this study. The basal diet used consists of corn meal, agar (1% w/v), brewer's yeast (1% w/v), and methylparaben (0.1% v/w). The flies (1-3 day old, both genders) were grouped into 6 of 40 flies per vial (n = 5). Group 1 served as the control and was fed with basal diet only, while groups 2 to 6 were fed with basal diet plus doses of AECP (0.25, 0.5, 1, 2, and 4 mg/g diet, respectively), and the treatment lasted for 21 days.

### *Negative geotaxis test*

After the 21-day study on the flies, a geotaxis test (locomotor activity) was performed following the method described by (Adedara et al., 2016). The test involved the use of 10 ice-immobilized flies (taken from each group) transferred to a 50 ml vial each, and left standing for 5 min for the flies to recover. The vial was then tapped gently to make the flies active. The locomotor activity was noted by counting the flies that crossed a 6 cm marked line on the vial within 6 s, and the climbing ability was calculated and recorded in %.

### *Homogenization*

After the locomotive test, flies were kept in the freezer for about 20 mins, homogenized in 0.1 M potassium phosphate buffer at pH 7.4, and centrifuged at 4000 x *g* (4° C) for 10 min. The resulting supernatant was stored at -20 °C for subsequent assays.

### *Biochemical parameter investigated*

Biochemical analyses, such as total protein (Lowry et al., 1951), total thiols (Ellman, 1959), reduced glutathione. and nitric oxide (NO) (Green et al., 1982) levels, as well as catalase activity (Claiborne, 2018) were carried out in the flies' homogenate.

### *Statistical analysis*

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey post hoc test on the GraphPad Prism (version 8.1). Results were then expressed as mean ± standard deviation (SD), and values of $p < 0.05$ were considered statistically significant.

## RESULTS

### Phytochemical Screening of *Calotropis procera*

Qualitative phytochemical screening of *Calotropis procera* revealed the presence of flavonoids, phenols, tannins, reducing sugars, glycosides, and saponins (Table 1). The quantitative phytochemical analysis revealed that the total phenols were 14.05 ± 0.07 mg Tannic acid equivalent/g, while the total flavonoid content was 9.218 ± 0.05 mg Quercetin/g equivalent.

### *HPLC Analysis of Bioactive Compounds in* Calotropis procer*a*

Table 2 shows the bioactive compounds present in *Calotropis procera*. Sixteen compounds were identified, with the following five compounds having the highest abundance in this order: quercetin, cymbopogon, hydroquinone, Chlorogenic Acid, Ferulic Acid, and Luteolin.

**Table 1:** Qualitative Phytochemical screening of aqueous extract of the plants

| S/No | Phytochemicals | AECP |
|---|---|---|
| 1 | Flavonoids | ✓ |
| 2 | Phenols | ✓ |
| 3 | Tannins | ✓ |
| 4 | Alkaloids | x |
| 5 | Triterpenoids | x |
| 6 | Starch | x |
| 7 | Reducing sugar | ✓ |
| 8 | Glycosides | ✓ |
| 9 | Saponins | ✓ |

**Table 2:** HPLC Analysis of Bioactive Compounds in ***Calotropis procera***

| S/N | Component | Retention | Area | Height | External | Units |
|---|---|---|---|---|---|---|
| 1 | Chlorogenic Acid | 1.266 | 537.9440 | 23.658 | 0.0000 | % |
| 2 | Hydroquinone | 2.750 | 1096.5090 | 14.168 | 124.5636 | ppm |
| 3 | Caffeic Acid | 4.450 | 398.9955 | 6.402 | 39.8995 | ppm |
| 4 | Catechol | 5.500 | 71.7770 | 5.305 | 0.0000 | |
| 5 | Orientin | 6.483 | 141.2970 | 6.611 | 0.0000 | |
| 6 | Delphinidine | 7.333 | 75.9420 | 3.490 | 0.0000 | |
| 7 | Lycopene | 7.950 | 127.1900 | 8.138 | 0.0000 | |
| 8 | Swertiajaponin | 9.350 | 84.5120 | 7.211 | 0.0000 | |
| 9 | Quercetin | 11.050 | 6187.4495 | 129.351 | 0.0000 | |
| 10 | Cymbopogon | 12.166 | 1498.6470 | 33.272 | 0.0000 | |
| 11 | Luteolin | 13.700 | 408.6915 | 12.167 | 0.0000 | |
| 12 | Cymbopogonol | 15.066 | 308.8140 | 7.489 | 0.0000 | |
| 13 | Apiginin | 16.850 | 66.6625 | 6.176 | 0.0000 | |
| 14 | Ferulic Acid | 17.616 | 425.0560 | 6.467 | 0.0000 | |
| 15 | Linalool | 18.750 | 73.1765 | 5.669 | 0.0000 | |
| 16 | Geranoil | 19.250 | 64.9980 | 6.367 | 0.0000 | |

## Toxicological Profile of aqueous extract of *Calotropis procera*

*Effect of AECP on the survival rate of flies*

The effect of AECP on the survival rate of *D. melanogaster* is presented in figure 1. Increased mortality was noted in flies fed with doses of AECP at 2 and 4 mg/g diet when compared with the control and other treatment groups.

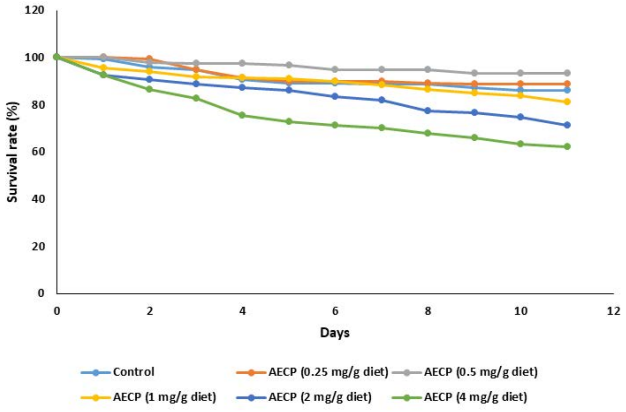


**Figure 1.** *Effect of AECP on the survival rate of D. melanogaster*
AECP: Aqueous extract of *Calotropis procera*

### *Effect of AECP on the climbing ability of flies*

The effect of AECP on the climbing ability of *D. melanogaster* is presented in figure 2. No significant difference was noted in the climbing ability of flies fed with all the doses of AECP tested when compared to the control

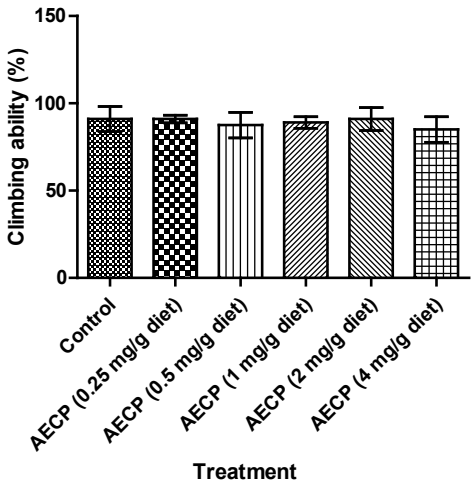


**Figure 2.** *Effect of AECP on the climbing ability of D. melanogaster*
Bars represent mean ± standard deviation (n = 5)
AECP: Aqueous extract of *Calotropis procera*

### *Effect of AECP on the level of total thiol in flies*

The effect of AECP on the level of total thiol in *D. melanogaster* is presented in figure 3. No significant changes were noted in the total thiol level in flies fed with all the doses of AECP tested when compared to the control.

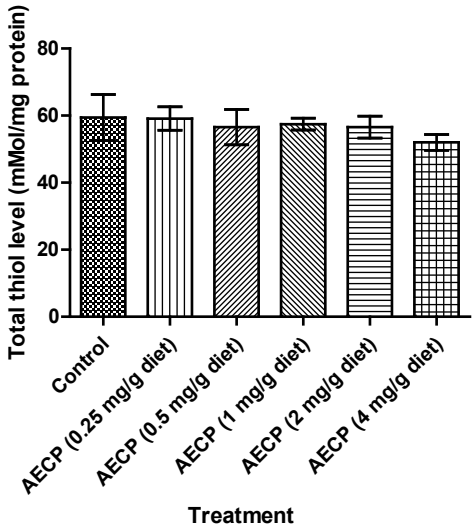


**Figure 3.** *Effect of AECP on total thiol level in flies Bars represent mean ± standard deviation (n = 5)*
AECP: Aqueous extract of *Calotropis procera*

### *Effect of AECP on the level of reduced glutathione in flies*

The effect of AECP on the level of reduced glutathione in *D. melanogaster* is presented in figure 4. No significant difference was noted in the level of reduced glutathione in flies fed with all the doses of AECP tested, except a significant ($p < 0.05$) reduction with 4 mg/g diet when compared to the control. A significant ($p < 0.05$) reduction in the level of reduced GSH was also noted in

reduced GSH in flies fed with 4 mg/g diet when compared with other doses tested.

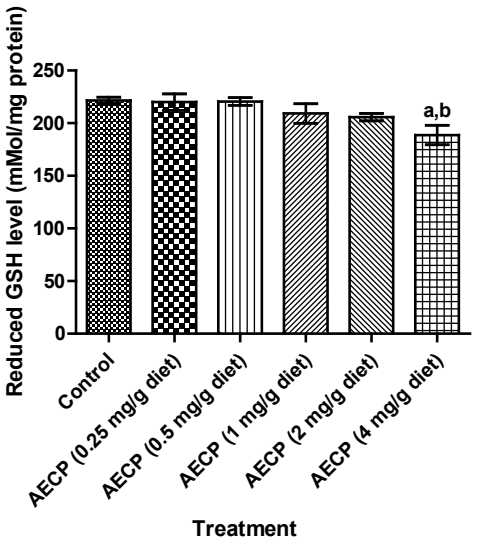


**Figure 4.** *Effect of AECP on the level of reduced glutathione in D. melanogaster*

Bars represent mean ± standard deviation (n = 5); [a]p < 0.05 when compared to control, [b]p < 0.05 when compared to AECP (0.25, 0.5 and 1 mg/g diet)

AECP: Aqueous extract of *Calotropis procera*

### *Effect of AECP on the activity of catalase in flies*

Figure 5 presents the effect of AECP on the activity of catalase in *D. melanogaster*. No significant difference was noted in the activity of catalase in flies fed with all the doses of AECP tested, except a significant ($p < 0.05$) reduction with 4 mg/g diet when compared to the control.

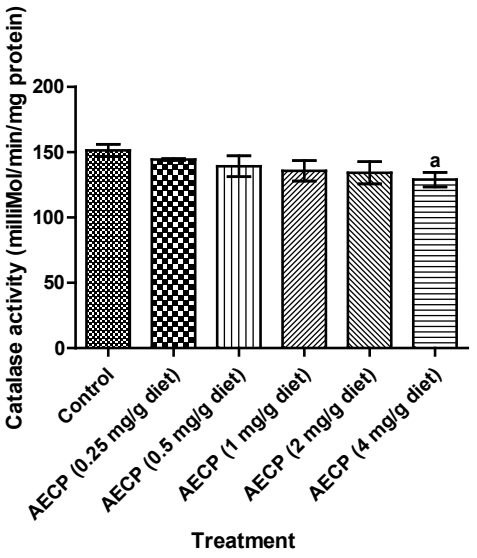


**Figure 5.** Effect of AECP on the activity of catalase in *D. melanogaster*

Bars represent mean ± standard deviation (n = 5); [a]p < 0.05 when compared to control

AECP: Aqueous extract of *Calotropis procera*

### *Effect of AECP on the level of nitric oxide in flies*

Figure 6 shows the effect of AECP on the level of nitric oxide in *D. melanogaster*. No significant difference was noted in the level of nitric oxide in flies fed with doses of AECP at 0.25 – 1 mg/g diet when compared to the control. A significant ($p < 0.05$) increase in the level of nitric oxide was noted in flies fed with 2 and 4 mg/g diet AECP when compared to the control. Also, a significant ($p < 0.05$) elevation in the level of nitric oxide was noted in flies fed with 4 mg/g diet AECP when compared with flies fed with 0.25 mg/g diet, and also with 2 and 4 mg/g diet AECP when compared with flies fed with 0.5 and 1 mg/g diet AECP.

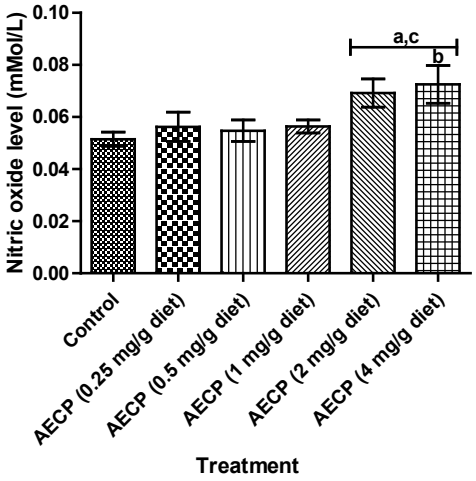


**Figure 6.** Effect of AECP on the level of nitric oxide in *D. melanogaster*

Bars represent mean ± standard deviation (n = 5), [a]p < 0.05 when compared to control, [b]p < 0.05 when compared to AECP (0.25 mg/g diet), [c]p < 0.05 when compared to AECP (0.5 and 1 mg/g diet)

AECP: Aqueous extract of *Calotropis procera*

## DISCUSSION

The presence of flavonoids, phenols, tannins, reducing sugars, saponins, and glycosides in *Calotropis procera* supports the claims by previous studies (Morsy *et al.*, 2016; Singh *et al.*, 2024). These secondary compounds have been associated with the use of medicinal plants for therapeutic interventions of several diseases. Phenolics or polyphenols, including flavonoids, are secondary metabolites of plants and have been shown to possess high levels of antioxidant properties by scavenging free radicals. These have provided plants with their medicinal properties against many diseases, including inflammation, cancer, and diabetes (Adewale *et al.*, 2014). The six highest abundant compounds identified by HPLC in the order: quercetin, cymbopogon, hydroquinone, chlorogenic acid, ferulic acid, and luteolin are known phytochemicals with a wide range of pharmacological properties. Quercetin possesses several pharmacological properties, including antioxidant, anti-

inflammatory, anticancer, antidiabetic, antimicrobial, and antiviral properties (Aghababaei *et al*., 2022; Salehi *et al*., 2020). Cymbopogon has demonstrated several biological activities, which include antidiabetic, anti-inflammatory, antibacterial, antiviral, antineoplastic, and antiarrhythmic (Zhao *et al*., 2024). Hydroquinone is a phenolic compound used in the treatment of melasma and post-inflammatory hyperpigmentation (Truswell, 2020). Ferulic acid has been reported to possess antioxidant, anti-inflammatory, anti-fibrotic, and anti-cancer properties (Zhai *et al*., 2023). Chlorogenic acid has shown to possess antioxidant, anticancer, antibacterial, anti-inflammatory, antidiabetic, as well as hepato- and nephron-protective properties (Wang *et al*., 2022). Luteolin has been shown to possess antioxidant, anticancer, anti-inflammatory, antimicrobial, cardioprotective, neuroprotective, and antiviral properties (Taheri *et al*., 2021; Lv *et al*., 2025). Toxicological evaluation of AECP was conducted using *D. melanogaster*. Based on the survival study, increased mortality was recorded in both 2 and 4 mg/g diet AECP relative to lower doses, but did not cause mortality of the flies up to 50%. The survival study could be related to previous reports where the $LD_{50}$ of *Calotropis procera* flowers was greater than 2000 mg/kg body weight in mice (Kumar *et al*., 2022) and 5000 mg/kg body weight in rats (Mohammed et al., 2012), which suggested that the plant could be considered safe through a single oral exposure. Although the latex of the plant at 1.0 ml/kg body weight via intraperitoneal exposure was cardiotoxic and hepatoxic to rats, and the leaf extract at 60,000 mg/kg per day for 10 consecutive days was toxic to sheep (de Lima *et al*., 2011). Another study reported the $LD_{50}$ of the leaf extract in rats at 774 mg/kg body weight (200 mg/ml) via intraperitoneal administration (William *et al*., 2015). Also, the $LD_{50}$ of the leaf and stem bark extracts of this plant was greater than 3,000 mg/kg of body weight in mice, while 28-day consecutive exposure of the leaf extract at 400 mg/kg body weight could be toxic (Kinda *et al*., 2019). In this study, AECP leaf was safe up to 2 mg/g diet (2000 mg/kg diet) in fruit flies. However, oral administration of AECP could be considered toxic during long-term exposure at high doses.

Total thiols are required to maintain the redox state in an organism, which are required for cellular defense against oxidative stress. Total thiols comprise GSH (an antioxidant in cells that is critical in protecting cells against oxidative stress) and several other thiol-containing molecules in tissues. Catalase is a haeme-containing enzyme responsible for the catalysis of $H_2O_2$ to molecular oxygen and water, and is therefore, responsible for reducing or inhibiting oxidative stress (Oyetayo et al., 2020). Nitric oxide is an important signaling molecule, which is involved in a variety of physiological processes, and its increased concentrations may promote oxidative stress (Andrabi et al., 2023). In this study, the non-significant ($p < 0.05$) changes noted in various toxicological parameters, such as total thiol, reduced GSH, catalase, and NO in AECP exposed flies up to 2 mg/g diet when compared with the control could suggest the antioxidant property of the plant, thereby indicating its safety. It can be suggested that the extract could be toxic at high doses (above 4 mg/g diet) at long-term exposure based on the changes noted in the antioxidant and oxidative stress markers.

## CONCLUSION

Based on the results from this study, aqueous extract of *Calotropis procera* can be concluded to contain phytochemicals, which are known to possess antioxidant properties, and can, therefore, be considered for therapeutic interventions against many diseases. Doses of aqueous extract of *Calotropis procera* up to 2 mg/g diet can be suggested safe.

## LIST OF ABBREVIATIONS

AECP: Aqueous extract of *Calotropis procera*
ANOVA: Analysis of variance

HPLC: High-performance liquid chromatography

## DECLARATIONS

**Ethics approval and consent to participate:** Not Applicable

**Consent for publication:** Not Applicable

**Competing interests:** Not Applicable

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**Authors' contributions: Samuel S. Abegunde:** Writing – original draft, Methodology, Investigation, Data curation. **Oluwaseyi A. Akpor:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Scholastica O. Anadozie:** Writing – review & editing, Formal analysis, Methodology, Data curation. **Salmat I. Yusuf:** Writing – review & editing, Resources. **Olusola B. Adewale:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization.

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**Availability of Data and Materials:** All data generated or analysed during this study are included in this published article

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