Vol 4, Issue 1, 2025

ISSN: 2790-9522 (Print) | 2790-9530 (Online)
Website: https://journals.jozacpublishers.com/ajbcps/index
https://doi.org/10.5281/zenodo.17488870

OPEN ACCESS

In-vivo antimalarial and anti-oxidative properties of methanol and ethylacetate stem bark extract from *Calotropis procera* (Apocynaceae) infected with *Plasmodium berghei*

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Received: May 02, 2025 | **Accepted:** July 18, 2025 | **Published:** October 30, 2025

Abstract

Calotropis procera, a commonly utilized traditional medicine in Nigeria, is reputed for its antioxidant and antimalarial properties. This study aimed to investigate the antioxidant, toxicological, and antiplasmodial effects of methanol and ethyl acetate extracts of *Calotropis procera* utilizing a rodent model to assess their suppressive, prophylactic, and curative effects against malaria, as well as to evaluate their safety profile. An antioxidant activity of 0.25 mg/ml was revealed by the DPPH assay, which revealed a robust antioxidant capacity that was higher than that of Vitamin C at a concentration of 0.25 mg/ml. Locke's method was used to evaluate acute toxicity. Additionally, toxicity assessments revealed a relatively non-toxic profile with no observed fatalities even at doses up to 5,000 mg/kg, indicating that it is relatively non-toxic based on Lorke's toxicity scale. The antimalarial efficacy of the extracts was assessed in mice infected with *Plasmodium berghei,* a model organism for malaria. The methanol extract demonstrated significant antimalarial activity without inducing adverse effects at doses of 250, 500, and 1000 mg/kg. Both the methanol stem bark extract and the ethyl acetate fraction exhibited curative and suppressive activities, although with minimal prophylactic effects. The results suggest that *Calotropis procera* extracts hold promise as potential sources for new and innovative malaria treatments. Further investigations are needed to clarify the precise mechanisms of action and to develop safe and effective therapeutic formulations.

Keywords: Acute Toxicity, Antiplasmodial, *Calotropis procera*, Lorke's method, *Plasmodium berghei*

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1. Introduction

Malaria remains a devastating global health crisis, claiming nearly one million lives annually and disproportionately affecting low-income nations. Nigeria, India, and the Democratic Republic of Congo had the highest number of malaria cases worldwide in 2016 (Ashley et al., 2018; Pradines & Robert, 2019). Transfer modes include infected blood transfusions, organ transplants, needle sharing, the disease hinders social progress and cripple's economies due to inadequate resources for prevention and treatment (Rosso et al., 2021). A multifaceted approach is essential to combat

malaria, including robust surveillance, effective vector control, quality healthcare access, ongoing research, and innovative prevention and treatment strategies (Rau, 2023; Obeagu & Obeagu, 2024).

2. Literature review

Malaria represents a considerable health challenge in Sub-Saharan Africa, primarily transmitted through the bites of infected mosquitoes, with Plasmodium falciparum being responsible for the majority of cases and fatalities, particularly among children under the age of five (Oladipo et al., 2022). The economic implications of malaria are profound, encompassing elevated healthcare expenses, diminished productivity, and increased absenteeism in both work and educational settings (WHO, 2016). Despite the implementation of control measures such as indoor residual spraying, long-lasting insecticide-treated nets, intermittent preventive treatment during pregnancy, and the use of antimalarial medications, the malaria burden persists due to several obstacles, including drug and insecticide resistance, adverse effects of antimalarial drugs, high costs, logistical challenges, and a lack of effective vaccines (Yeka et al., 2012; Olasehinde, 2016). To tackle these issues, researchers are investigating novel antimalarial agents (Onwujekwe et al., 2013; Ekor, 2014). Malaria disrupts the equilibrium between reactive oxygen species (ROS) and antioxidants, resulting in oxidative stress, inflammation, anemia, and hemolysis (Sarkar et al., 2021; Gomes et al., 2022).

Antioxidants such as vitamins C and E can mitigate this damage. In regions with limited healthcare infrastructure, traditional healthcare practitioners and medicinal plants have a crucial role (Kitua & Malebo,2004; Mahomoodally & Chintamunne, 2012). Notably, *Calotropis procera* a perennial shrub from the Apocynaceae family, has been investigated for its therapeutic attributes(Wadhwani et al, 2021), including its antimalarial, analgesic, and anti-inflammatory properties as well as its anticonvulsant and anthelmintic activities against Haemonchus contortus (Khairnar et al., 2012; Quazi et al., 2013; Cavalcante et al.,201; Dogara et al,,2023).The distinct mechanisms by which these compounds operate are especially pertinent in the context of rising antibiotic resistance (Kufer, 2005; Cock & Van Vuuren, 2020). This study seeks to explore and validate the ethnobotanical significance and potential of Calotropis procera in addressing the urgent challenges presented by malaria and its considerable health impact.

Plate1: *Calotropis procera* plant

3. Research methodology

3.1. Plant material

The stem bark of plant, *Calotropis procera* were collected at Lugbe, Abuja, Nigeria.The plant's stem bark,was presented for identification and authentication at the National Institute for

Pharmaceutical Research and Development's (NIPRD), Idu Herbarium Unit. A voucher number NIPRD/H/7070 was assigned and deposited.

3.2. Preparation of crude methanol extract

Fresh stem bark of *Calotropis procera* were washed and shade dried. The powdery plant sample (1kg) was immersed in methanol in a stoppered / tightly sealed. glass container ,The combination was allowed for three days at room temperature, Following daily stirring with fresh stem bark, No. 1 Whatman 125mm filter paper was used to filter out the extract. Using a Stuart RE 300B W13 rotary evaporator, the extract obtained at a reduced pressure and temperature (40°C) was concentrated. Ethyl acetate was used to partition the residue to produce ethyl acetate/methanol The dried extracts were later kept until required for further test.

3.3. DPPH radical scavenging assay (RSA)

To measure the methanol extract's antioxidant capacity at 517 nm a UV-visible spectrophotometer was used (Fadeyi et al., 2022). RSA was determined by measuring the percentage inhibition of DPPH discoloration (Sigma-Aldrich).

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$$RSA = \frac{Blank - Sample}{Blank} \times 100$$

3.4. Animals

Adult Swiss mice of either sex that were locally bred and weighed 20±2 g were acquired from Ahmadu Bello University Zaria's Animal House Facility They were kept in polypropylene cages at ambient environmental conditions using standard rodent feeds and water. Protocols adhered to Ahmadu Bello University, Zaria Research Policy and ethics. Drugs used included Chloroquine Phosphate, Pyrimethamine, Geimsa stain and Trisodium citrate (Krettli et al., 2009).

3.5. Acute Toxicity

Initially, three groups of mice were administered varying doses of an extract (10, 100, and 1000 mg/kg) orally in the first phase of the study. During the initial four hours, several signs of toxicity were observed in the animals, including convulsions, decreased mobility, loss of muscle tone, shallow breathing, and potentially fatal outcomes. In the second phase, the mortality rates of each group were tracked over a 24-hour period following the administration of a different dose of the plant extract. The LD50 was estimated by calculating the geometric mean of the highest dose that did not cause any deaths and the lowest dose that did result in mortality (1200, 1600, 2900, and 5000 mg/kg, respectively). These mice were further monitored for another 24 hours (Onwusonye et al., 2014).

$$LD_{50} = \sqrt{Highest}\ \ non - lethal\ dose\ \times lowest\ non\ lethal\ dose$$

3.6. Malaria parasite study

The Nigerian Institute of Medical Research in Kaduna supplied us with a strain of Plasmodium berghei NK65 (chloroquine-sensitive), which was sustained through intraperitoneal re-infection every four days. A healthy mouse was infected with P. berghei utilizing blood obtained from a donor mouse. Each mouse received approximately 0.2 mL of infected blood, which contained parasitized P. berghei erythrocytes. To prepare this solution, the parasitemia and erythrocyte count of the donor mouse were assessed, and the blood was subsequently diluted with isotonic saline (Adzu et al., 2007).

3.7. Grouping and dosing for in-vivo antiplasmodial study

Five groups of thirty mice were established for each model. Group I served as a negative control and was administered distilled water, while Groups II to IV received 250, 500 and 1000mg/kg/day of extract/fraction. Group V were administered with chloroquine phosphate and pyrimethamine as standard drugs for suppressive, curative, and prophylactic studies, delivered orally via a cannula.

3.8. *Calotropis procera* methanol / ethylacetate fractions' antiplasmodial activity assessed via 4-day suppressive test

Each of the five mouse groups received a conventional inoculum containing 1 x 10^7 *Plasmodium berghei* -infected erythrocytes. The methanol extract and ethylacetate fraction were administered to Groups II, III, and IV as gradations of oral doses, respectively, while distilled water and chloroquine were administered to the negative control groups. The treatment lasted for four days, with blood samples taken from each mouse's tail on the fifth day. The number of parasitized erythrocytes in four randomly selected fields was used to determine the parasiteemia. Schizontocidal activity was assessed using Peters and Robinson's method (Ryley & Peters,1970).

$$\%\,Suppression = \frac{Av.parasitemia\ in\ control\ - Av.parasitemia\ in\ each\ treated\ group}{Av.parasitemia\ in\ control} \times 100$$

3.9. Curative test on schizontocidal activity of *Calotropis procera* extract /fraction and chloroquine

The schizonticidal activity of the methanol extract, ethyl acetate fraction and chloroquine against established *Plasmodium berghei* infection in mice. 0.2 mL of blood containing 1 x 10^7 *Plasmodium berghei*-infected erythrocytes was administered to each group on the first day of the experiment.The extract/ fraction were administered orally to Groups II, III, and IV seventy-two hours later after the establishment of parasitemia for 4 days. Blood films were taken from the animal's tails in order to determine the percentage chemosuppression while Group I and V received 10 ml/kg of distilled water and chloroquine 5mg/kg/day. After the animals had been fed ad- libitum for twenty-eight days, and their deaths had been recorded, the mean survival time was calculated (Mbakwe et al., 2024).

$$MST = \frac{Sum\ of\ survival\ of\ all\ mice\ in\ the\ group(days)}{Total\ number\ of\ mice\ in\ that\ group}$$

3.9.1. Prophylactic test of *Calotropis procera* extract /fraction repository activity

A plant extract's repository activity was assessed utilizing the procedure (Peters & Robinson,1970). Five groups of mice were established, with groups II-IV receiving fractional doses of 250, 500, and 1,000 mg/kg/day, respectively. Groups I and V served as controls, receiving 10 mL/kg of distilled water and 1.2 mg/kg/day of pyrimethamine. The therapy regimen spanned three consecutive days, followed by inoculation with infected red blood cells on the fourth day. Analyses of blood smears were conducted 72 hours following inoculation to assess parasitemia levels. Based on the negative control, chemosuppression percentages were calculated for each dose group (Iwalewa, 1997; Boampong, 2013).

3.9.2. Packed cell volume (PCV)

The test extract and ethyl acetate fraction were evaluated for their ability to prevent red blood cell destruction caused by increasing malaria parasites. A sampling of blood was taken from the tails of mice using heparinized capillaries blood was stuffed into capillary tubes and sealed with sealing

clay nearly. a third of their original volume.Immediately following sealing of the tubes, the tubes were centrifuged at 12,000 rpm in a micro-hematocrit centrifuge for 5 minutes. A Micro-Hematocrit Reader was used to resolve PCV after tubes were taken out of the centrifuge (Fentahun, 2017; Birru *et al.*, 2017).

$$Percent\ change\ in\ PCV\ = \frac{PCV\ day\ 4 - PVC\ day\ 0}{PCV\ day\ 4} \times 100$$

3.9.3. Statistical analysis

Dunnett's post hoc analysis was conducted in order to analyze the data after one-way analysis of variance (ANOVA). In the tables, the results are displayed as mean + standard error. Results are considered statistically significant at $p < 0.05$.

4. Data analysis

Table 1: Antioxidant activity of methanol extract of *Calotropis procera*

Conc in mg/ml	% I CP	Vit C
0.5	66.46	77.88
0.25	86.76	77.69
0.125	71.84	72.50
0.0625	72.92	60.38
0.03125	43.23	77.88

Table 2: Determination of the intra-peritoneal/oral LD_{50} of methanol extract

First phase

Doses (mg/kg)	Number of mice used	Mortality	Toxicity sign
10	3	0/3	None
100	3	0/3	None
1000	3	0/3	None

Table 3: Determination of the Intra-peritoneal/ Oral LD $_{50}$ of the methanol extract

Second phase **Doses (mg/kg)**	**Number of mice used**	**Mortality**	**Toxicity sign**
1200	1	0/1	None
1600	1	0/1	None
2500	1	0/1	None
5000	1	0/1	None

Table 4: Impact of *Calotropis procera* fraction and methanol extract on the average survival duration of mice infected with *Plasmodium berghei(* Suppressive test)

Test Samples	**Doses (mg/kg)**	**Mean Survival Time (MST, days)**
DW	10	10.53 ± 1.73
A	250	19.70 ± 1.54*
	500	23.41 ± 1.63*
	1000	23.69 ± 0.13*
B	250	18.90 ± 1.22*
	500	22.32 ± 1.45*
	1000	23.39 ± 0.64*
CQ	5	28.60 ± 0.53*

Key: DW (Distilled water)
A: Methanol
B: Ethylacetate
CQ:Chloroquine

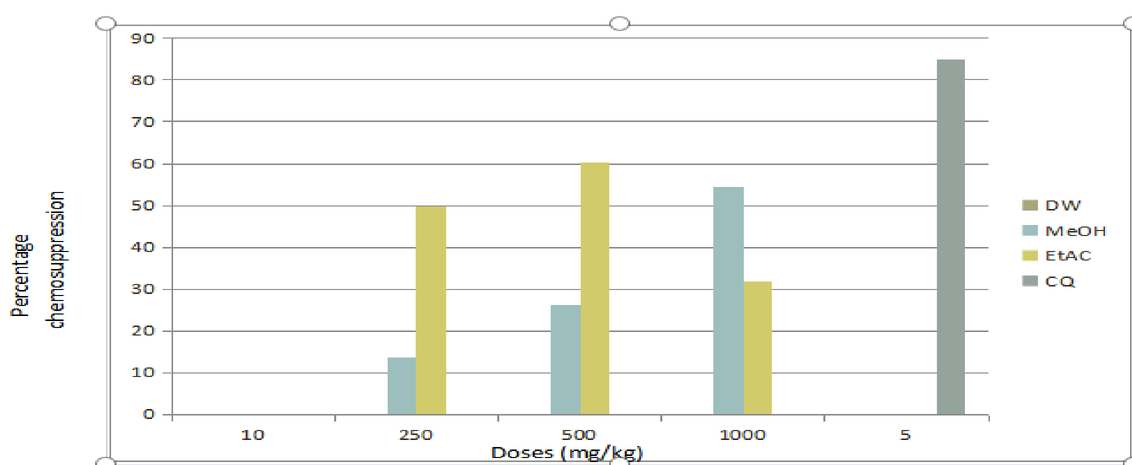

Figure 1: Effect of methanol extract / ethylacetate fraction of *Calotropis procera* on mean survival time

Table 5: Effects of methanol extract / fraction of *Calotropis procera* on early *Plasmodium berghei* infection in mice (Suppressive test)

Test Samples	Treatment (mg/kg)	Average Parasitemia ± SEM	% Chemosuppression
DW	10	20.30 ± 0.31	-
A	250	17.77 ± 0.28*	12.46
	500	10.23 ± 0.44*	49.61
	1000	15.77 ± 0.41*	22.32
B	250	13.40 ± 0.72*	33.99
	500	16.57 ± 0.81*	18.72
	1000	10.53 ± 0.66*	48.13
CQ	5	6.40 ± 0.69*	68.47

Key: DW (Distilled water)
A: Methanol
B: Ethyl acetate
CQ:Chloroquine

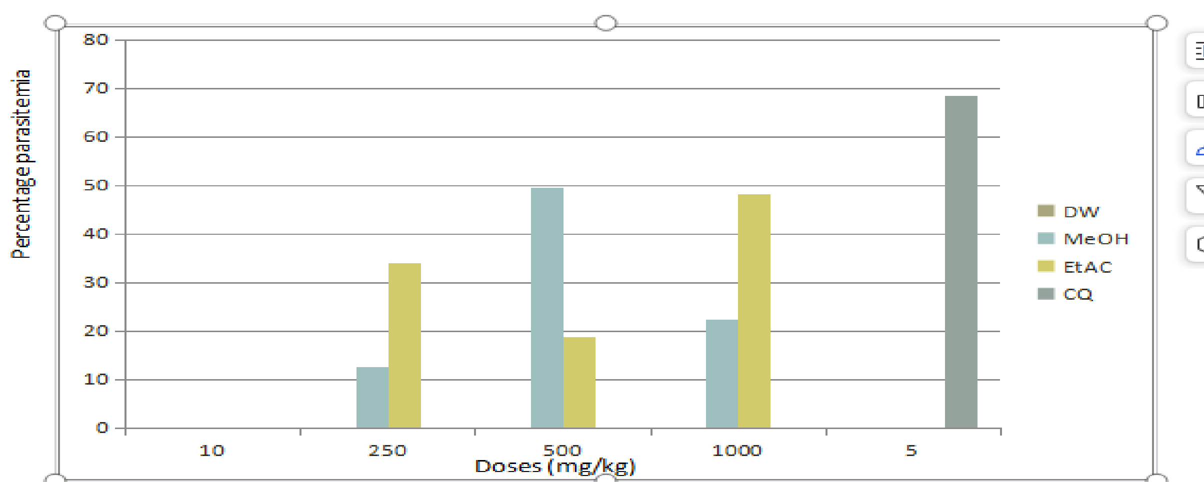

Figure 2: Average parasitemia of methanol/ethylacetate fraction of *Calotropis procera* in early *plasmodium berghei* infection in mice(Suppressive test).

Table 6: Effects of methanol extract /fraction of *Calotropis procera* in *Plasmodium berghei* infected in mice (Prophylactic test)

Test Samples	Treatment(mg/kg)	Average parasitemia±SEM	Percentage Chemosuppression(%)
DW	10ml/kg	20.47±0.45	-
A	250	17.80±0.24*	13.40
	500	10.83±0.68*	47.09
	1000	7.87±0.55*	61.55
B	250	10.53±0.62*	48.56
	500	7.57±0.98*	63.02
	1000	6.70±0.46*	67.27
PYR	1.2	2.97±0.13*	85.49

Key: DW (Distilled water)
A: Methanol
B: Ethyl acetate
PRY:Pyrimethamine

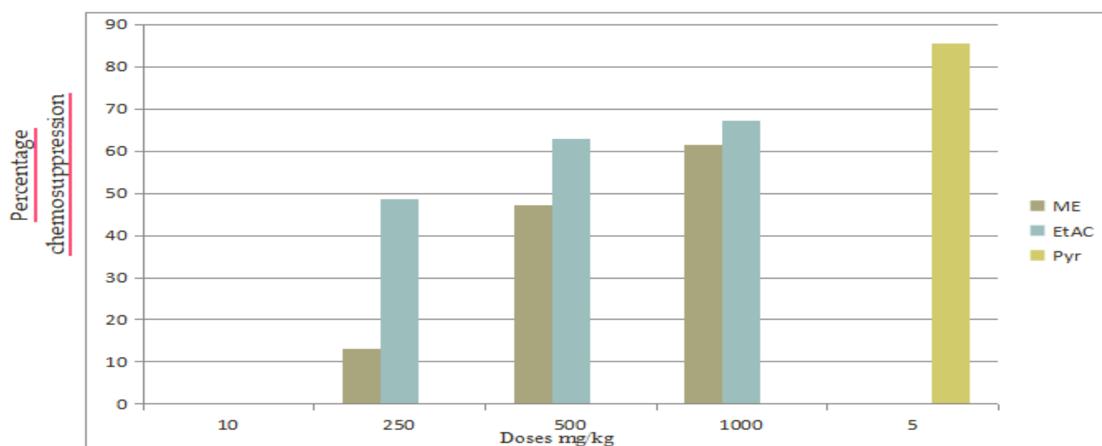

Figure 3: Prophylactic activity of methanol extract/ ethyl acetate fraction of *Calotropis procera* in *Plasmodium berghei* Infected in mice (Repository test)

Table 7: Effects of methanol extract /fraction of *Calotropis procera* in established *Plasmodium berghei* infected mice (curative test)

Extract/Fraction	Treatment (mg/kg)	Average parasitemia D3	Average parasitemia D7	% Change PCV
DW	10	20.22±0.98	21.20 ± 0.58*	--
A	250	20.06 ± 0.19	18.33 ± 0.30*	13.54
	500	20.13 ± 0.36	15.63 ± 0.77*	26.27
	1000	20.46 ± 0.37	9.67 ± 0.35*	54.39
B	250	20.83 ± 0.26	10.67 ± 0.25*	49.67
	500	20.57 ± 0.38	8.40 ± 0.66*	60.38
	1000	14.43 ± 0.26	14.43 ± 1.04*	31.93
CQ	5	20.83 ± 0.53	3.17 ± 0.47*	85.05

Key: DW (Distilled water)
A: Methanol
B: Ethylacetate
CQ: Chloroquine

Table 8: Impacts of *Calotropis procera* extracts on *Plasmodium berghei:* a prophylactic study using methanol and ethyl acetate fractions

Extract/Fraction	Treatment (mg/kg)	Avg PCV Day 4 (±SD)	Avg PCV Day 7 (±SD)	% Change PCV
DW	10	37.60 ± 1.14	33.17 ± 1.01	-14.10
A	250	37.83 ± 1.65	36.33 ± 1.63	-5.32
	500	37.50 ± 1.52	34.83 ± 1.77	-6.61
	1000	36.67 ± 0.67	33.83 ± 1.30	-6.55
B	250	38.17 ± 1.22	36.83 ± 1.51	-2.74
	500	35.83 ± 1.08	34.67 ± 1.36	-2.41
	1000	38.50 ± 1.57	36.50 ± 1.34	-5.84
PYR	1.2	37.67 ± 1.17	41.33 ± 0.99*	+8.37

Key: DW (Distilled water)
A: Methanol
B: Ethylacetate
PYR: Pyrimethamine

Table 9: Suppressive effects of *Calotropis procera* extracts on packed cell volume in *Plasmodium berghei*-infected mice: assessment of average pcv and percent change

Test Sample	Treatment ml/kg	Average PCV (D0)	Average PCV (D4)	% Change in PCV
DW	10	31.33 ± 1.12	27.67 ± 1.12	-12.71
A	250	35.50 ± 1.41	31.83 ± 1.17	-5.32
	500	36.83 ± 1.49	33.83 ± 1.47	-8.62
	1000	34.00 ± 1.81	32.67 ± 1.54	-4.68
B	250	29.83 ± 1.08	26.67 ± 1.28	-10.59
	500	35.83 ± 1.35	32.00 ± 1.71	-10.29
	1000	32.00 ± 1.32	30.67 ± 1.17	-4.65
CQ	5.0/1.2	37.67 ± 1.84	38.67 ± 1.54	+1.74

Key: DW (Distilled water)
A: Methanol
B: Ethylacetate
CQ:Chloroquine

Table 10: Curative effects of *Calotropis procera* extracts on Packed Cell Volume in *Plasmodium berghei*-infected mice: Evaluation of methanol and ethyl acetate fractions

Test Sample	Dose (mg/kg)	PCV Day 4 (Mean ± SEM)	PCV Day 7 (Mean ± SEM)	% Change in PCV
DW	10	71.17 ± 1.35	32.50 ± 1.05	-14.68
A	250	37.83 ± 1.40	33.83 ± 1.17	-12.11
	500	36.83 ± 1.49	33.83 ± 1.47	-8.62
	1000	38.33 ± 1.05	36.67 ± 1.54	-7.88
B	250	39.17 ± 1.49	34.50 ± 1.85	-11.86
	500	36.33 ± 0.99	33.33 ± 0.99	-9.03
	1000	38.33 ± 0.88	34.33 ± 1.12	-10.58
CQ	5.0/1.2	37.67 ± 1.84	38.67 ± 1.54	+1.74

Key: DW (Distilled water)
A: Methanol
B: Ethyl acetate
CQ:Chloroquine

5. Discussion

As a natural remedy for oxidative stress and malaria, *Calotropis procera* has been demonstrated to have significant potential. It works primarily by increasing glutathione levels, decreasing inflammation, and inhibiting the growth of parasites such as *Plasmodium falciparum* (Sharma *et al.*,2012; Singh *et al.*,2024).This action may enhance the effectiveness of conventional antimalarial treatments(*Padhy* et al. ,2007) .Based on a comprehensive evaluation of its antioxidant properties,polar methanol extract exhibited a higher activity compared to the less polar ethyl acetate extract, particularly showing superior capacity for DPPH radical scavenging. The methanol extract proved to be especially effective in scavenging free radicals, particularly at lower

concentrations in comparison to ascorbic acid, thus establishing it as a valuable source of antioxidants. Toxicity evaluations carried out during the study revealed a positive safety profile for *Calotropis procera,* with no deaths recorded even at elevated doses of up to 5,000mg/kg. As previously reported, the results of this study are consistent with those obtained from previous studies involving *Bauhinia strychnifolia* and *Entandrophragma angolense* leaf and stem bark extracts (Chutoam et al., 2015; Kamku et al., 2020). According to Lorke's toxicity scale (Tepongning et al., 2011), the extract is considered relatively nontoxic (Tepongning et al., 2011). A comparison of the survival times of mice treated with distilled water, methanol extract, ethylacetate fraction and chloroquine (Table 4). Distilled water at a dosage of 10 mg/kg resulted in the shortest mean survival time (MST) of 10.53 days. In contrast, methanol extract and ethylacetate fraction administered at higher doses of 250, 500, and 1000 mg/kg significantly prolonged the MST to approximately 19–23 days, although the benefits began to rise at higher dosages. Notably, chloroquine, at a mere 5 mg/kg dose, achieved the longest MST of 28.60 days, demonstrating remarkable potency. To investigate the antimalarial activity of *Calotropis procera,* this study employed a rodent malaria parasite model, which is renowned for its reliability and reproducibility (Pierrot et al., 2003; Otto et al., 2014). Four-weekold mice were selected to minimize the confounding effects of anaemia, commonly seen in older animals. Oral administration of the extract and fraction was chosen to mimic traditional administration methods and likely routes of clinical evaluation, ensuring a relevant assessment of the plant's antimalarial properties (Waako et al., 2005).

According to Peters' 4-day suppression test, both the methanol extract and ethyl acetate fraction of *Calotropis procera* exhibited substantial chemo-suppressive effects, significantly reducing parasitemia level (Table 5), exhibiting a notable reduction in parasite burden and alleviation of malaria-related symptoms, such as fever and chills, ranging from 70.4% to 90.3% at various doses. Specifically, the methanol extract and ethyl acetate fraction displayed significant ($p < 0.05$) .The methanol extract suppressed by 12.46%, 49.61%, and 22.32%, while the ethyl acetate fraction suppressed by 33.99%, 18.73%, and 48.13% In comparison to the control group.

Calotropis procera methanol extract and fraction showed dose-dependent reductions in parasitemia in the prophylactic testing (Table 6). Specifically, the methanol extract resulted in a parasitemia suppression of 13.04%, 48.99%, and 61.55%, while the fraction exhibited a suppression of 48.56%, 63.02%, and 67.27%. In comparison, the standard antimalarial drug pyrimethamine achieved a chemo-suppression rate of 85.49%.

In this study, the methanol extract and the ethyl acetate fraction of Calotropis procera were tested for their ability to inhibit malarial growth, (Table 7). Both showed notable antimalarial activity, significantly lowering parasitemia levels ($p < 0.05$). The extract produced clearance rates of 13.54%, 26.27%, and 54.39%, while the fraction achieved clearance rates of 49.67%, 60.38%, and 31.93%. In comparison, chloroquine, a well-known antimalarial medication, exhibited an 85.05% parasite clearance At a dosage of 5 mg/kg. The extract at 1,000 mg/kg and the fraction at 500 mg/kg achieved clearances of 54.39% and 60.38%, respectively, consistent with earlier findings on Brassica nigra (L.) Koch. seeds against Plasmodium berghei infection in mice (Muluye *et al.*, 2015).

Extracts of *Calotropis procera* in methanol and ethyl acetate demonstrate encouraging anti-anemic and antimalarial properties, with packed cell volume (PCV) reductions ranging from 5.32% to 6.61% across different doses, while smaller reductions showed better performance at 2.74% to 5.84%. Treatment with PYR notably raised PCV by 8.37%. (Table 8)

The impact of methanol and ethyl acetate extracts, along with chloroquine (CQ), on PCV was examined in comparison to distilled water (DW) over four days, DW resulted in a significant PCV decrease of 12.71%. Methanol extract led to reductions in PCV of 4.68% to 8.62%, with the least decline of 4.68% noted at the highest dosage of 1000 mg/kg. The ethyl acetate extract showed more fluctuation, reducing PCV from 4.65% to 10.59%, also performing best at the 1000 mg/kg dosage (Table 9).In contrast, chloroquine (CQ) improved PCV by 1.74%, distinguishing it from the other treatments as it not only mitigated the PCV decline but also encouraged an increase.

The (Table 10) the impact of distilled water(control), methanol extract and ethylacetate fraction (administered at 250, 500, and 1000 mg/kg), and Chloroquine (at dosages of 5.0/1.2 mg/kg) on packed cell volume (PCV) in an animal study. DW exhibited the most significant decline in PCV, dropping by 14.68% from Day 4 (71.17) to Day 7 (32.50), indicating a lack of beneficial effects. In contrast, methanol extract and ethylacetate fraction resulted in smaller decreases in PCV, ranging from 7.88% to 12.11%, with higher doses generally providing better outcomes .Chloroquine proved to be the most effective treatment, leading to a slight increase in PCV by 1.74%, from 37.67 to 38.67, suggesting its ability to mitigate the factors reducing PCV. The higher doses of methanol extract and ethylacetate fraction, along with the lower dose of chloroquine, demonstrated protective effects, with it being the most powerful option.

Overall, these extracts effectively reduced parasitemia, prevented anemia, and prolonged survival time in infected mice. Furthermore, they led to a significant increase in survival time ($p<0.05$) and exhibited an impressive chemotherapy suppression of 30% or greater, meeting the established efficacy criteria (Adugna et *al.*, 2014).

The study concluded that *Calotropis procera* extracts exhibited robust curative, prophylactic, and suppressive activities against *Plasmodium berghei* infections in mice. The considerable parasite clearance achieved correlates positively with increased survival times, underscoring the extract's antimalarial efficacy. This activity can be attributed to the presence of bio-active compounds such as flavonoids, alkaloids, saponins, and tannins, which may interact with and inhibit various pathways in *Plasmodium infections* (Turkson, 2021; Nawaz *et al.*, 2024). Notably, despite containing hemolytic saponins, the extracts did not induce hematological abnormalities in sub-acute toxicity studies, further supporting their therapeutic potential.

6. Implication of the study

The methanol extract is safe up to 5000 mg/kg, with no observed toxicity or hematological abnormalities. It is classified as relatively safe on Lorke’s toxicity scale. The extract shows both curative and prophylactic properties. It effectively alleviates malaria-induced anemia.It significantly reduces parasitemia levels. Treated groups exhibit prolonged survival compared to controls. The extract’s chemo-suppressive effects surpass chloroquine in parasite clearance across multiple doses.

7. Recommendation

The study demonstrated the promising antimalarial activity of *Calotropis procera*'s methanol extract and fraction in preventing PCV reduction, suppressing parasitemia, by preventing the development of anemia in mice infected with *Plasmodium berghei.* This suggests that the active compounds in the extract, such as flavonoids, alkaloids, saponins, and tannins, target various pathways to stimulate antimalarial activity.

8. Conclusion

To cultivate a deeper comprehension of the bioactive principles of *Calotropis procera*, further investigations are imperative, particularly to elucidate their specific antimalarial mechanisms of action. The study underscored the promising potential of the methanol extract and ethylacetate fraction in the treatment of malaria, attributable to their marked antimalarial efficacy observed in rodent malaria models. The extract's capacity to mitigate anemia, suppress parasitemia, and prolong survival duration in infected murine subjects indicates its promise as a novel antimalarial agent.

9. Acknowledgments

The authors express their profound gratitude to the Department of Chemistry at the University of Abuja, as well as the management of the Sheda Science and Technology Complex (SHESTCO), for facilitating access to the laboratories and for providing various reagents utilized in this study.

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