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Original Research Article

Exploring the efficacy of *Calotropis procera* methanol leaf extract with standard antimalarial drugs in the murine malaria model

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Abstract

Purpose: The emergence and proliferation of drug-resistant *Plasmodium falciparum* has critically compromised the efficacy of traditional antimalarial treatments significantly challenging global control efforts. There is a growing scientific focus on exploring plant-based therapies as complementary or alternative options for antimalarial treatment. This investigation sought to examine the antimalarial effect of *Calotropis procera* methanol leaf extract (MLCP) alone and in combination with standard antimalarial drugs in *Plasmodium berghei*-infected mice.
Methods: Standard protocols were followed for oral acute toxicity evaluation and phytochemical tests of MLCP. Curative and prophylactic effects of MLCP individually and in combination with chloroquine (CQ), artesunate (ART), and pyrimethamine (PYR), were evaluated using recognized experimental techniques in *Plasmodium berghei*-infected mice.
Results: MLCP at 200 and 400 mg/kg doses produced a marked decrease ($p < 0.05$) in parasitemia levels in both tests. In the curative study, the combination of MLCP + CQ (200/10 mg/kg) and MLCP + ART (200/5 mg/kg) considerably ($p < 0.05$) reduced parasitemia levels. The percentage of chemosuppression produced by MLCP + CQ (86.9 %) was better than CQ alone (47.2 %). However, the MLCP + ART combination and ART alone produced similar parasite suppressive effects with percentage chemosuppression of 73.6 % and 74.7 %, respectively. In the prophylactic test, the MLCP + PYR (200/1.2 mg/kg) combination produced a chemosuppression of 78.7 % compared to PYR alone which produced a chemosuppression of 78.0 %.
Conclusion: The findings show that combining *Calotropis procera* leaves with standard antimalarial chloroquine enhanced antimalarial efficacy against *Plasmodium berghei* infection in mice.

Keywords: Antimalarial, *Calotropis procera*, *Plasmodium berghei*, Chloroquine, Artesunate, Pyrimethamine

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INTRODUCTION

Despite the intensive global efforts to curb the menace of malaria, the disease continues to be a huge health burden, globally leading to elevated morbidity and mortality rates. Reports in 2023 show that the disease was responsible for more than 263 million cases and an estimated 597,000 deaths, highlighting the limitations of current therapeutic interventions.[1] The emergence and proliferation of drug-resistant strains of

Plasmodium and insecticide resistance, particularly *Plasmodium falciparum*, has significantly undermined the effectiveness of malaria control strategies.[2] Insufficient funding, socioeconomic conditions, high cost of drug production, and the overall quality of pharmaceuticals, have adversely affected the treatment processes.[3] The combination of these factors thus highlights the critical necessity for novel strategies designed to enhance the efficacy of antimalarial treatments and mitigate the development of drug resistance.

Medicinal plants have historically been instrumental in antimalarial drug discovery, exemplified by the isolation of quinine (from Cinchona) and artemisinin (from *Artemisia annua*).[4] More recently, the exploration of plant-based compounds as adjunctive or alternative antimalarial therapies has gained considerable attention.[5] Plants contain numerous bioactive phytochemical compounds capable of modulating biological pathways to produce synergistic effects when combined with standard antimalarial drugs.[6] This synergistic effect may enhance therapeutic outcomes by increasing the efficacy of standard antimalarial agents, allowing for lower treatment dosages, and potentially delaying the emergence of drug resistance.[7]

Calotropis procera, commonly known as "rubber bush" and "Sodom apple", is traditionally valued for its therapeutic effects against various illnesses, including indigestion, dermatological conditions, wounds, diarrhoea, sinus fistula constipation, joint pain, fever, and muscular pain.[8-10] Its natural habitat spans various parts of the Middle East, Asia, and Africa, and is a member of the Apocynaceae family.[11] Numerous pharmacological characteristics of *C. procera* have been documented, such as antimicrobial, anti-cancer, wound healing, antipyretic, anti-inflammatory, antidiabetic, antioxidant, and antimalarial activities.[12-18]

Evidence from several studies have suggested that co-administration of phytochemical-based preparations with standard antimalarial drugs results in enhanced activity against *Plasmodium* strains sensitive and resistant to chloroquine.[19-21] Consequently, the study explored the antimalarial efficacy of methanol leaf extract of *Calotropis procera* (MLCP) in combination with chloroquine, artesunate, and pyrimethamine in a murine model infected with *Plasmodium berghei*. The goal was to determine whether MLCP could enhance the activity of standard antimalarial medications, resulting in greater parasitemia suppression. The combination of chloroquine or artesunate with *C. procera* leaf extract could potentially restore chloroquine sensitivity or enhance the antimalarial action of artesunate, offering a dual approach to tackle drug-resistant malaria.[22] Insights into the interactions between MLCP and these treatments could thus reveal synergistic effects, advancing the design of more efficacious and sustainable malaria therapies.

MATERIALS AND METHODS

Collection of *C. procera*

The plant was collected at Igabi Local Government Area, Kaduna state (Latitude: 10° 48' 21.71" N; Longitude: 7° 42' 51.95" E) in August 2023. Leaves were verified at the Botany Department, Ahmadu Bello University, Zaria, with the help of Dr. N.S. Sanusi. This was accomplished by comparing it to the voucher specimen (voucher number 900219) that had already been deposited in the Department's Herbarium Section.

Drugs and chemicals

Chloroquine Phosphate (Sigma-Aldrich, USA), Artesunate (Dialogue Pharmacy, India), Giemsa stain (Philip Harris Ltd., England), Normal Saline (Dana Pharmaceuticals Ltd, Nigeria), Trisodium citrate, Methanol, Pyrimethamine (GSK Pharmaceuticals, UK). All the reagents used were of analytical standard grade.

Equipment

Mortar and pestle, Weighing balance (Mettler Toledo, Switzerland). Water bath (HH-4, England Lab Science, England), 1mL syringe, Bunsen burner, Ethylenediaminetetraacetic acid (EDTA) bottles, Heparinized capillary tubes, Microscope (Olympus CE, Japan), Microscope Slides, Coverslip, Micropipette, Staining jar, Fixing jar, Wooden rack differential counter, Glass slide, Spreader, Round bottle conical flask, 250 mL beaker and Stoppered container.

Rodent malaria parasite – *Plasmodium berghei* (NK 65)

Plasmodium berghei strain sensitive to chloroquine (NK65) was supplied by the Microbiology Department at the National Medical Research Institute (NIMR), Lagos. Thereafter, the parasite strain was kept alive via weekly serial transition which was achieved by giving intraperitoneally naïve mice $1x10^7$ infected red blood cells (RBCs).

Preparation of standard inoculum and parasite inoculation

The study employed donor mice with a parasitemia of 30 - 35 %. A heart puncture was used to get

blood collected in a vacutainer tube containing EDTA as an anticoagulant. Based on the donor mouse's parasitemia level and a normal mouse's RBC count, collected blood was mixed with 0.9 % phosphate-buffered saline (PBS) to achieve the desired dilution. Each experimental animal then received an intraperitoneal injection of 0.2 mL infected blood containing about 1.0×10^7 parasitized red blood cells (RBCs).[23]

Preparation of methanol leaf extract of *C. procera*

Calotropis procera leaves were gathered, cleaned, allowed to air dry, and then crushed with a crusher and pestle into a fine powder. A 500 g portion of the powdered sample underwent cold maceration with methanol (1.5 L) for 10 days, with frequent shaking every 24 hours. The obtained filtrate was evaporated to dryness at 40 °C under reduced pressure using a rotary evaporator (Buchi Rota vapor, Switzerland). The methanol extract was subsequently dried to obtain a solvent-free solid crude extract, weighing 164 g. This extract, designated as methanol extract of *C. procera* (MLCP), was preserved for future experimental use in an airtight container.

Animals

Swiss albino mice weighing between 18–22 g were acquired from a certified breeder and kept in standard cages at the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. Mice were maintained on a commercially available standard diet (Vital Feeds Jos, Nigeria) and allowed unrestricted access to water. The experimental protocols complied with the ethical guidelines established by Ahmadu Bello University, Zaria Ethics Committee on Animal Use (approval number: ABUCAUC/2022/023) and Care as well as "Principles of Laboratory Animal Care".[24]

Extract/Drug administration

A total of 110 mice was employed to conduct the study with each group consisting of 5 mice. MLCP at doses of 100, 200, and 400 mg/kg was administered to the test groups. Normal saline (10 mL/kg) was administered to the untreated control group, whereas standard treatment groups received Pyrimethamine (1.2 mg/kg), Chloroquine (10 mg/kg), or Artesunate (5 mg/kg), according to the specific antimalarial test model used. All administrations were made orally through oral gavage.

Assessment of phytochemical constituents of *C. procera* methanol leaf extract

Standard procedures for the qualitative detection of phytochemical constituents utilizing the method of Trease and Evans were used to screen the plant extract.[25]

Acute oral toxicity test

In a two-phase experimental design with a total of 13 animals, Lorke's method[26] was applied to estimate the oral median lethal dosage (LD_{50}) of MLCP in mice.

Phase 1: Graded oral doses of 10, 100, and 1,000 mg/kg of the extract were given to three animal groups, containing three animals per group. Within the first four hours and then intermittently throughout the next twenty-four, observations were taken for indications of toxicity and mortality.

Phase 2: The extract was administered orally to four more mice in graded doses of 1200, 1600, 2900, and 5000 mg/kg. For the first four hours and the next twenty-four hours, the mice were similarly monitored for toxicity and mortality.

Using Equation 1, the LD_{50} value was derived by computing the geometric mean between the highest administered dose that did not induce mortality and the lowest dose that resulted in lethality among the test animals.

$LD_{50} = \sqrt{}$ (highest dose with no mortality × lowest dose that caused death) -- Equation 1

Grouping and dosing of animals

For the prophylactic and curative evaluation, twenty-five (25) animals were randomly distributed into five groups of five mice each. Group I was administered normal saline (10 mL/kg), and Group V received chloroquine at a dose of 10 mg/kg as the reference treatment in the curative test.

Groups II to IV were treated orally with graded doses (100, 200, and 400 mg/kg) of *Calotropis procera* leaf extract. In the prophylactic study, animals in Group V received pyrimethamine (PYR) 1.2 mg/kg in the prophylactic study. The interaction investigations were then conducted using the highest effective dose.

Curative study

Twenty-five (25) mice were weighed on day 0 and intraperitoneally injected with 0.2 mL of $1x10^7$ *P. berghei*-infected erythrocytes. A 72-hour observation period was allowed post-inoculation for the development of detectable parasitemia. Animals were then randomly assigned into five experimental groups (n=5) and administered extract and drug orally for four days. Treatment

was assigned as follows: Group I - normal saline (10 mL/kg), Group V - chloroquine (10 mg/kg), and Groups II, III, and IV - *C. procera* methanol leaf extract at 100, 200, and 400 mg/kg orally. On Day 7, animals were weighed and blood drawn from the tail vein was smeared on slides, fixed, and treated with 3% Giemsa stain (pH 7.2) for microscopic analysis.

In five randomly chosen fields, parasitemia levels were quantified as the ratio of infected erythrocytes to 100 total erythrocytes observed in a microscopic field.[27] Parasitemia was assessed microscopically under oil immersion (×100 objective) and calculated using Equation 2 below. Mice were observed for 28 days with *ad libitum* feeding and deaths that occurred were recorded. Mean survival time (MST) was computed using equation 3 to assess the duration of survival post-infection.

$$\text{Percentage parsitemia} = \frac{\text{Number of parasiticized eryhtrocytes}}{\text{Total number of eryhtrocytes}} \times 100 \text{ -- Equation 2}$$

$$\text{Mean Survival Time} = \frac{\text{Cumulative number of days survived by all mice in a given group (days)}}{\text{Total number of mice in the group}} \times 100\text{- Equation 3}$$

Prophylactic study

The residual infection procedure was used to test MLCP for preventative efficacy.[28] A total of 25 mice after weighing were distributed into five treatment groups comprising three (3) extract-treated groups and two (2) control groups each containing five (5) mice. Group I served as the negative control group (administered normal saline 10 mL/kg) and Group V served as the standard treatment group (received Pyrimethamine 1.2 mg/kg.

Groups II - IV received 100, 200, and 400 mg/kg MLCP respectively. On the third day, mice were inoculated with 1×10^7 *P. berghei*-infected erythrocytes and monitored. Seventy-two hours (72 hours) post-infection, blood smears obtained from each mouse were prepared and parasitemia levels were determined in 5 fields on day 7.

Interactive studies with standard antimalarial drugs

Using the curative model, thirty mice were inoculated with 1 $x10^7$ *P. berghei*-infected erythrocytes on the first day followed by observation for a duration of seventy-two hours for induction of parasitemia. Mice were then divided randomly into 5 groups each containing 6 mice. Animals in the first group (Group I) received normal saline (10 mL/kg); Groups II, III, and IV received the most effective dose of *C. procera* leaf extract (200mg/kg), ART (5 mg/kg), and CQ (10 mg/kg) respectively. Animals in group V received 200 mg/kg of the *C. procera* leaf extract/ART (5 mg/kg) while group VI animals were given 200 mg/kg of the *C. procera* leaf extract/CQ (10 mg/kg). Blood was collected from the tail of each mouse, fixed, stained with Giemsa on day seven and parasitemia levels were assessed in 5 fields.

For the prophylactic interactive study, twenty (20) mice were grouped into four experimental sets, each comprising five mice. Normal saline (10 mL/kg) was administered to Group I, whereas Group II was given the extract at 400 mg/kg (the most effective prophylactic dose); Group III was given 400 mg/kg MLCP and PYR (1.2 mg/kg); Pyrimethamine at 1.2 mg/kg was administered to Group IV, with all groups receiving treatment daily over a period of three days. A 0.2 mL inoculum comprising 1×10^7 erythrocytes infected with *P. berghei* was administered to the mice on the third day through the intraperitoneal route. On the sixth day of the experiment, blood collected from the tail vein was used to prepare smears, which were then fixed and stained for parasite detection in five fields.

Calculation of percentage parasitemia

For each study, the average percentage of parasite inhibition compared to the control was determined using the equation (Equation 4) by Iwalewa et al.,[29] :

$$\%\ \text{Suppression} = \frac{\text{Mean baseline parasitemia in negative control group} - \text{Mean parasitemia in each treated group}}{\text{Mean baseline parasitemia in negative control group}} \times 100 \text{ -- Equation 4}$$

Data analysis

Experimental data were presented as mean ± SEM. Statistical significance ($p<0.05$) was determined using one-way ANOVA, followed by Dunnett and Tukey post hoc analysis. The statistical evaluation of data was conducted using SPSS (Statistical Package for the Social Sciences) Statistics version 23.0 (IBM Corp., 2015).

RESULT AND DISCUSSION

Resistance to current antimalarial agents has prompted increased scientific interest in combining these drugs with medicinal plants. This strategy aims to optimize treatment effectiveness while exploring the pharmacological potential of plant-based compounds. From the literature, these combinations are reported to offer advantages such as minimizing cytotoxic effects, mitigating or delaying the emergence of drug resistance, and

improving the overall potency and effectiveness of antimalarial therapies. [7, 30]

Percentage yield and phytochemical evaluation of *C. procera* methanol leaf extract

The macerated 500 g of powdered *C. procera* leaves yielded 32.8 %w/w of the methanol leaf extract after extraction and concentration. Secondary metabolites found in MLCP are shown in Table 1. Notably, anthraquinones and steroids were not detected in the extract. Similar phytochemicals in the leaves of *Calotropis procera* have been reported by several studies.[31-34]

Table 1: Phytochemical profile of *C. procera* methanol leaf bark extract

Constituents	Test	Inference
Carbohydrates	Molisch	+
Anthraquinones	Bontrager	-
Steroids	Salkowski	-
Triterpenes	Liebermann-Burchard	+
Cardiac glycosides	Keller-Killiani	+
Saponins	Froth	+
Tannins	Ferric-Chloride	+
Flavonoids	Shinoda	+
Alkaloids	Dragendorff	+

+ = Present; - + Absent

Due to their diverse biological and therapeutic properties, plant secondary metabolites have been utilized in traditional medicine for ages.[35] Antimalarial alkaloids, such as quinine, inhibit the ability of the parasite to convert toxic heme into non-toxic hemozoin in its food vacuole, while also disrupting protein synthesis, thereby causing cell death.[36] Artemisinin and related terpenoids exert their antimalarial effects by reacting with iron in the parasite vacuole via an endoperoxide bridge, forming harmful heme-products that disrupt parasite function.[37] Flavonoids inhibit plasmepsin II, a key enzyme involved in hemoglobin digestion within the malaria parasite. This inhibition results in an accumulation of undigested hemoglobin, which interferes with the parasite's metabolism and survival.[38] Saponins isolated from various plants have demonstrated antimalarial activity through two primary mechanisms: inducing hemolysis, which directly leads to parasite death, and modulating oxidative stress by enhancing antioxidant defense mechanisms.[39] Therefore, the antiplasmodial activity observed in MLCP could have been derived from a single or synergistic effect of these metabolites.

Effect of acute oral exposure to *C. procera* methanol leaf extract in mice

Over 24 hours following oral administration of MLCP in the first phase, no overt symptoms of toxicity or animal death were noted. Death did, however, occur during the study's second phase. The oral median lethal dose was determined as 1385.6 mg/kg. Determining the acute toxic effects of extracts is essential to ascertain their safe use in animals. To ensure that extracts are safe for use in animals, it is crucial to identify their acute toxic effects. As a common indicator of short-term toxicological effects, the oral median lethal dose (LD_{50}) value shows the amount of a substance needed to kill 50 % of test subjects under certain conditions.[26] A chemical is considered harmful if its LD_{50} values fall between 500 mg/kg and 5000 mg/kg (Lorke, 1983). MLCP may therefore be categorized as being slightly toxic. According to several other studies, the oral median lethal dose (LD_{50}) for *Calotropis procera* leaves in mice has been documented as 2600 mg/kg for the ethanol extract,[40] 3000 mg/kg for the aqueous extract,[41] and 2750 mg/kg for the methanol extract.[42] Variability in plant collecting time, plant extract preparation (extraction solvent employed, drying techniques), and testing methodologies can account for the observed differences in these results. However, the general conclusion from this is that *Calotropis procera* leaves are mildly hazardous because all of the previously published LD_{50} values from earlier studies and the value obtained in the current study fell within the dose range of 500–5000 mg/kg.

Curative efficacy of *C. procera* methanol leaf extract against *Plasmodium berghei* infection in mice

Across all tested dose levels of MLCP administered, a reduction in parasitemia was observed. However, only the 200 mg/kg dose led to a marked decrease ($p < 0.05$) in parasitemia levels (percentage chemosuppression 42.8%) compared to the control. Treatment with chloroquine at 10 mg/kg led to a chemosuppression of 58.3%. (Table 2). Many antimalarial drugs, including mefloquine, halofantrine, chloroquine, and derivatives of artemisinin, were discovered and tested using mouse models.[23] These models have facilitated preclinical studies by offering insights into drug efficacy and parasite-host interactions.

Table 2: Curative potential of *C. procera* methanol leaf extract in mice infected with *P. berghei*

Treatment groups	Dose (mg/kg)	Average parasitemia level		Percentage chemosuppression (%)
NS	10 mL/kg	21.20 0.23	±	0
MLCP	100	17.47 0.57	±	29.1
MLCP	200	10.87 0.41*	±	42.8
MLCP	400	13.40 0.72	±	31.3
CQ	10	8.83 1.74*	±	58.3

Data are presented as mean ± SEM; n = 5 per group. Comparisons were made using one-way ANOVA with Dunnett's post hoc test. *p < 0.05 indicates a significant difference versus the NS (normal saline) control. MLCP = Methanol Leaf Extract of *Calotropis procera*; CQ = Chloroquine; all treatments administered orally.

In this study, the antiplasmodial effect of MLCP was first ascertained using the curative and prophylactic models after which the most effective doses were combined with conventional antimalarial drugs to assess the parasitemia suppressive effects of the combinations. The capacity of the extract to treat an established infection was evaluated using Rane's test. The maximum parasite suppression effects of MLCP were recorded at 200 mg/kg (42.8 %). Compounds that produce 30 % or more parasitemia suppression are considered to have good antiplasmodial activity.[44] Thus, MLCP can be considered to possess antiplasmodial activity.

Mean survival time

Death occurred earlier (day 15) in the negative control (normal saline-treated) group compared to the extract-treated groups. The group treated with 200 mg/kg demonstrated superior efficacy as they lived longer compared to the 100 mg/kg and 400 mg/kg treatment groups. Animals in the chloroquine-treated group survived for 28 days (Table 3). One crucial parameter for assessing the anti-malarial efficacy of substances or extracts is the mean survival time. According to Peter and Anantoli, [45] substances are thought to have good parasite suppression properties if they can extend the lifespan of mice infected with *Plasmodium berghei* longer than the negative control. Mice at all dosage levels survived for longer periods after receiving MLCP, which was accompanied by a noticeable reduction in parasitemia, indicating the possible antimalarial effectiveness of the extract.

Table 3: Effect of *C. procera* methanol leaf extract on survival duration in mice infected with *P. berghei*

Treatment groups	Dose (mg/kg)	MST (days)
NS	10 mL/kg	15.8 ± 2.2
MLCP	100	22.4 ± 0.3
MLCP	200	27.6 ± 0.2*
MLCP	400	26.4 ± 2.3*
CQ	10	28.0 ± 0.0*

Data are presented as mean ± SEM; n = 5 per group. Comparisons were made using one-way ANOVA with Dunnett's post hoc test. *p < 0.05 indicates a significant difference versus the NS (normal saline) control. MST = Mean Survival Time; MLCP = Methanol Leaf Extract of *Calotropis procera*; CQ = Chloroquine; all treatments administered orally.

Effect of coadministration of methanol leaf extract of *C. procera* with chloroquine and artesunate on parasitemia level in the curative model

To determine specific group differences, Tukey's multiple comparison test was conducted following ANOVA. All treatments were effective compared to the negative control (Figure 1). Parasite suppressive effects produced by MLCP 200 and CQ 10 were not significantly different from each other, an important observation notable in the search for alternative or adjunct antimalarial therapies. Coadministration of MLCP/CQ (200/10 mg/kg) produced a better reduction in parasitemia levels compared to CQ (10 mg/kg) alone and MLCP/ART (200/5 mg/kg) combination (Figure 1). Coadministration of MLCP with CQ and ART further improved the parasite suppression. Chemosuppression of 86.9 % and 73.7 % were observed for MLCP + CQ and MLCP + ART combinations respectively. Chloroquine exerts its antimalarial effects by blocking the polymerization of hemozoin, thereby disrupting its formation.[46] On the other hand, artemisinin derivatives work by alkylating heme and parasite proteins and also by blocking the parasite's sarcoplasmic reticulum Ca^{2+}-transporting ATPase (SERCA).[4, 47] In this study, the co-administration of the plant extract with standard antimalarial drugs displayed potent blood schizonticide (curative) activity, significantly reducing parasite density at the administered doses.

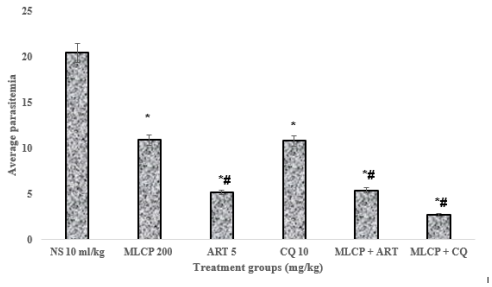

Figure 1: Curative efficacy of coadministration of *C. procera* methanol leaf extract with chloroquine and artesunate in mice infected with *P. berghei*
Data are presented as mean ± SEM; n = 5 per group. Comparisons were made using one-way ANOVA followed by Tukey's post-hoc test; * = $p < 0.05$ versus NS control; # = compared to CQ; NS = Normal Saline; MLCP = Methanol Leaf Extract of *Calotropis procera*; CQ = Chloroquine; ART = Artesunate; all treatments administered orally.

The observed percentage of chemosuppression was notably greater than that achieved by chloroquine, artesunate, or plant extract when used independently in the *P. berghei* murine infection model. A synergistic effect was observed in the combination of MLCP+CQ, leading to enhanced parasitemia clearance, indicating potent synergistic antimalarial activity between MLCP and chloroquine. The findings of this study align closely with existing reports on plant-drug interactions in antimalarial therapy, which have demonstrated that certain plants enhance the antimalarial efficacy of standard antimalarial drugs.[30, 48] For instance, co-administration of *Aloe camperi* leaf latex with the hydroalcoholic fruit extract of *Balanites aegyptiaca* enhanced the parasitemia suppression efficacy of chloroquine.[49] Also, kaempferol when combined with CQ, reduced parasitemia levels in *P. berghei* -infected mice.[50]
There was no additive or synergistic curative effect observed from the combination of extract and ART in the present study. In animals administered with the MLCP + ART combination, chemosuppression observed (73.6 %) was similar to that obtained with the ART alone group (74.7 %). MLCP + ART did not enhance efficacy beyond ART alone as observed from the percentage chemosuppression. Interaction between plant-based drugs and ART from previous studies has reported antagonism[51-53] and synergism effects.[21, 54] Antagonistic effects may arise when compounds in plant extracts disrupt the pharmacodynamics or pharmacokinetics of ART, by impairing drug absorption, altering metabolism, or obstructing binding to target sites. Conversely, synergistic effects may occur when bioactive compounds in the plants enhance the therapeutic efficacy of ART through complementary mechanisms, such as strengthening the immune response or targeting viral replication via alternative pathways.[55] These contrasting effects underscore the complexity of herb-drug interactions and highlight the importance of thoroughly studying plant-based drugs to ensure safety and efficacy.

Prophylactic study
In the preventive (prophylactic) study, treatment with MLCP at 400 mg/kg significantly ($p < 0.05$) lowered parasitemia levels relative to the negative control. However, pyrimethamine reduced parasitemia levels more effectively (Table 4). All tested doses of the plant extract suppressed parasitemia proliferation by more than 30 %, indicating strong chemoprophylactic capability. The observed chemoprophylactic activity against *P. berghei* infection may be mediated by direct cytotoxicity targeting the parasites[56] and by modifying erythrocyte membrane integrity to obstruct parasite invasion.[57]

Table 4: Prophylactic activity of *C. procera* methanol leaf extract in mice infected with *P. berghei*

Treatment groups	Dose (mg/kg)	Average Parasitemia	Percentage chemosuppression (%)
NS	10 mL/kg	19.64 ± 0.55	0
MLCP	100	13.40 ± 0.19	31.8
MLCP	200	13.40 ± 0.17	45.2
MLCP	400	10.76 ± 0.59*	54.8
PYR	1.2	4.32 ± 0.89*	78.0

Data are presented as mean ± SEM; n = 5 per group. Comparisons were made using one-way ANOVA with Dunnett's post hoc test. *$p < 0.05$ indicates a significant difference versus the NS (normal saline) control. MLCP = Methanol Leaf Extract of *Calotropis procera*; PYR = Pyrimethamine; all treatments administered orally.

Effect of coadministration of *C. procera* methanol leaf extract and pyrimethamine on parasitemia in a prophylactic malaria model

Coadministration of MLCP + PYR (standard prophylactic drug) produced a better

chemoprophylactic effect (78.7 %) compared to MLCP alone (54. 8%) (Figure 2). However, PYR alone produced chemoprophylactic activity of 78.0 %. This suggests no additive or synergistic antimalarial effect occurred when MLCP was combined with pyrimethamine. Thus, the combination is of no therapeutic advantage as there was no enhanced chemosuppression observed. Research on extracts exhibiting additive or synergistic effects with orthodox antimalarial drugs presents opportunities for the standardization and formulation of antimalarial medicinal plant-based combination therapies.

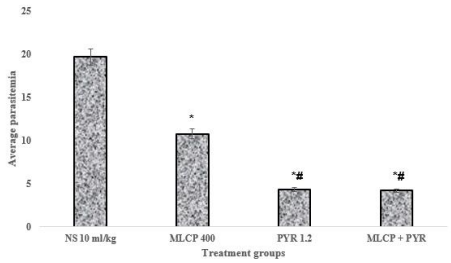

Figure 2: Prophylactic effect of coadministration of methanol leaf extract of *C. procera* with pyrimethamine in mice infected with *P. berghei*
Data are presented as mean ± SEM; n = 5 per group. Comparisons were made using one-way ANOVA followed by Tukey's post-hoc test; * = $p < 0.05$ versus NS control; # = compared to CQ; NS = Normal Saline; MLCP = Methanol Leaf Extract of *Calotropis procera*; PYR = Pyrimethamine; all treatments administered orally.

CONCLUSION

Combining methanol leaf extract from *Calotropis procera* with chloroquine showed increased antimalarial activity against experimental infection of mice with *Plasmodium berghei*. However, the coadministration of MLCP with artesunate and pyrimethamine did not improve antimalarial activity. Further mechanistic and clinical studies should be conducted to investigate the therapeutic benefit of the MLCP + CQ combination for possible development as an adjunct antimalarial therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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