ANTIMALARIAL ACTIVITY OF *CLERODENDRUM CAPITATUM* (WILLD) ETHANOL LEAF EXTRACT IN *PLASMODIUM BERGHEI* INFECTED MICE

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ABSTRACT

Malaria is a major global health problem that continues to affect a large number of people yearly especially those in developing countries including Nigeria. Medicinal plants still remain a vital source for the discovery and development of new antimalarial drugs. The plant *Clerodendrum capitatum* (Family - Verbenaceae), is a fast growing, erect, well branched, perennial under shrub which is traditionally used for the treatment of a plethora of diseases including cold, hyperpyrexia, malaria, asthma, diabetes mellitus, hypertension and inflammation. The aim of the study was to evaluate the antimalarial activity of the ethanol leaf extract of the plant. Phytochemical screening and acute toxicity studies were conducted using standard protocols. Antimalarial activity was investigated using suppressive, curative and prophylactic models in mice infected with *Plasmodium berghei*. Phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, cardiac glycosides, tannins, terpenes and saponins. The oral median lethal dose of the extract in mice was estimated to be greater than 5000 mg/kg. In the suppressive and curative tests, a significant ($p < 0.05$) dose dependent decrease in parasitemia levels occurred at all tested doses compared to the standard drug (Artesunate, 5 mg/kg). However, in the prophylactic test, significant ($p < 0.05$) reduction in parasitemia level occurred only at the 200 and 400 mg/kg doses. Oral administration of the extract at 100 and 200 mg/kg doses prolonged the mean survival time of treated mice compared to the distilled water treated group. The findings from this study suggest that the ethanol leaf extract of *Clerodendrum capitatum* possesses antimalarial activity.

KEYWORDS: **Antimalarial activity, *Clerodendrum capitatum*, Curative, Prophylactic, Suppressive.**

INTRODUCTION

Malaria control still poses a great burden for endemic countries with 228 million infections and 405,000 deaths cited in the latest World Malaria Report [1]. In Nigeria, malaria is a major cause of illness, death, and poverty with an estimated 50% of the adult population having at least one episode of malaria each year and children under five having 2–4 attacks annually [2]. The situation in Nigeria is worsened by the fact that malaria transmission occurs throughout the year with 76 percent of the population living in high transmission areas and 24 percent in low transmission areas [3]. Despite the successful reduction in malaria mortality achieved through wide promotion of the use of insecticide-treated nets and treatment with artemisinin derivatives, reports of artemisinin resistance remain a serious course of concern [4-6]. In addition, the therapeutic effectiveness and potency of artemisinin is threatened by several other factors including

short half-life, neurotoxicity and low solubility affecting their bioavailability [7]. Thus, newer and more efficacious drugs are urgently needed to combat this infectious disease.

Medicinal plants since time immemorial have been used in the treatment of malaria as evidenced by the discovery of artemisinin and quinine from *Artemisia annua* and *Cinchona pubescens* (or *officinalis*) respectively [8]. Many people living in the developing countries still rely on traditional remedies including the use of medicinal plants for the treatment of malaria [9-10]. This reliance on the use of medicinal plants has been attributed to their cultural acceptability, perceived safety and physical accessibility [11]. An ethnobotanical survey in Nigeria identified about 98 species of plants from different families which are used in traditional medicine singly or in combination to treat malaria and/or fever [12]. However, the antimalarial activity of many of the plants are yet to be scientifically investigated.

Clerodendrum capitatum (Willd) Schumach et. Thonn. (Verbenaceae) is an erect or scrambling shrub that is sometimes tree-like with occasionally thorny stems and white flowers in globose terminal heads [13]. The genus Clerodendrum is made up of about 500 species widely distributed throughout the world [14] which have been used in traditional medicine to treat various ailments including cold, hyperpyrexia, malaria, asthma, diabetes mellitus, hypertension, rheumatism, obesity, pain, dysentery, mammitis, toothache, anorexia, leprosy and inflammation [15-20]. A review of the pharmacological profile of various parts of the plant revealed various biological activities including antidiabetic and antilipdemic activity [19, 21], serotonergic and erectogenic activity [22-23], antimicrobial activity [24] and insecticidal activity [25-26]. The aim of the present study was to investigate the antimalarial activity of the ethanol leaf extract of *Clerodendrum capitatum* in *Plasmodium berghei* infected mice.

MATERIALS AND METHODS

Experimental animals
Animals used in this study were Swiss albino mice of both sexes, weighing between 20 - 24 g and obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were accommodated in clean, plastic ventilated cages, exposed to normal light/dark cycle and allowed free access to standard pellets and water *ad libitum*. Handling of

the laboratory animals was in line with the Ahmadu Bello University Animal Use and Care Protocol.

Parasites
Rodent parasite (*Plasmodium berghei* NK - 65) Chloroquine sensitive strain was obtained from the National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. The parasite was maintained in the Departmental Animal House by intraperitoneal injection of a 0.2 mL standard inoculum containing approximately 1×10^7 of parasitized erythrocytes from a donor mice to uninfected mice.

Collection of plant material
The leaves of *Clerodendrum capitatum* were collected from Sabon Gari Local Government Area of Kaduna State, Nigeria and brought to the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria. The plant was identified and authenticated by a taxonomist – Mr Namadi Sanusi. The herbarium specimen was prepared and deposited with voucher number 902.

Plant extraction
The leaves were air dried under shade and then reduced into powder (1 kg) using a mortar and pestle. The powdered leaves were macerated with 2.5 L of 90% v/v aqueous ethanol for three days in a maceration jar with occasional shaking after which it was filtered. The filtrate was collected and concentrated over a water bath at 40 °C to obtain the crude extract. The dried ethanol leaf extract of *Clerodendrum capitatum* (ELECC) was placed in an air tight labelled plastic container and stored in a desiccator.

Phytochemical screening
Phytochemical screening was carried out on the extract to detect the presence of secondary metabolites using standard phytochemical methods as previously described [27].

Pharmacological evaluation

Acute toxicity test
Acute oral toxicity test for the crude extract was conducted on a group of five female mice following the Organization for Economic Cooperation and Development 425 guidelines [28]. Mice were deprived of food before commencing the study. Initially, one mouse was given the extract orally at a dose of 5000 mg/kg in distilled water (v/v) by oral gavage and observed for the first 30 min, intermittently for 4 h and then over 24 h. Later, the remaining four mice were given the same dose of the extract as the first mouse and were observed

continuously for the first 30 min, intermittently for 4 h over a period of 24 h, and daily for 14 days. Mice were observed for any changes in general behavior and physical signs of toxicity.

Experimental design

A total of 5 groups each containing six mice were used to evaluate antimalarial activity of the extract using the suppressive, curative and prophylactic models. The first and last groups served as negative and positive controls respectively, while the remaining three groups were administered with graded doses of the extract. All drug and extract administrations were through the oral route.

Preparation of standard inoculum

Blood was obtained from the donor mice with 20–30% parasitemia level through jugular vein puncture and then collected in a Petri dish containing 0.5% trisodium citrate. Physiological saline (0.9%) was used to dilute the blood based on the parasitemia level of the donor mice to obtain a blood suspension in which there were approximately 5×10^7 infected erythrocytes per milliliter. Each mouse was then injected intraperitoneally with 0.2 mL of blood suspension containing approximately 1×10^7 infected erythrocytes per milliliter [29-30].

In vivo antimalarial tests

Peters' 4-day suppressive test

The method as described by Peters et al., [31] was employed to evaluate the antiplasmodial activity of ethanol leaf extract of *Clerodendrum captitatum* against *P. berghei* infection. Thirty (30) mice were infected intraperitoneally with blood containing approximately 1×10^7 parasitized erythrocytes contained in 0.2 mL inoculum on the first day (D0). They were randomly divided into 5 groups of 6 mice each. Two hours post infection, the first and last test groups received distilled water (10 ml/kg) and the reference drug (Artesunate, 5 mg/kg) respectively orally, while the 3 test groups were also administered orally with the extract at doses of 100, 200, and 400 mg/kg respectively. Drug administration was repeated 24, 48, and 72 h post infection (D1 to D3). On D4 (day 5 post treatment), tail blood smears were prepared, stained with 10% Giemsa in phosphate buffer, pH 7.2 for 15 min and examined under microscope at x 100 objective lens. A total of four immersion fields were viewed and the number of parasites in each field was counted using a tally counter. The percentage (%) suppression of parasitemia was calculated by comparing the parasitemia present in infected controls with those of test mice using the formula below.

% Suppression =

$$\frac{\text{Average parasitemia in control} - \text{Average parasitemia in each treated group}}{\text{Average parasitemia in control}} \times 100$$

Curative test

Evaluation of the curative potential extract against established infection was carried out as described by Ryley and Peters, [32]. Forty (40) mice were infected intraperitoneally with standard inoculum of 1×10^7 *Plasmodium berghei* NK - 65 infected erythrocytes on the first day (D0) and left for 72 h before commencement of treatment. Blood smears were collected and examined microscopically on D3 to establish parasitemia level. Thirty (30) mice with established *P. berghei* infection were then selected and randomized into 5 groups of 6 mice each. The three test groups were administered with the extract orally at doses of 100, 200 and 400 mg/kg daily for 5 days (D3 – D7). Positive control group received Artesunate (5 mg/kg) daily while the negative control group received distilled water (10 ml/kg) orally. Blood smears were collected and examined microscopically on D8 to check for the parasitemia level. Percentage parasite suppression was calculated as previously described. Thereafter, animals were fed *ad libitum* and observed till day 28 ($D_0 – D_{27}$) post inoculation of parasite for death. Any death that occurred during this period was noted and used to determine the mean survival time during this period. The mean survival time (MST) was calculated using the formula below.

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

Prophylactic test

Prophylactic activity of the extract was tested using the residual infection test described by Peters, [33]. Thirty (30) mice were weighed, randomized into five groups and treated for 3 days (D1 – D3). The three test groups were administered with the extract orally at doses of 100, 200 and 400 mg/kg daily. Positive control group received pyrimethamine 1.2 mg/kg daily while the negative control group received distilled water 10 ml/kg orally. On the 4th day (D4), all mice were infected with the *Plasmodium berghei* by a single 0.2 mL intraperitoneal administration of the diluted infected blood, containing approximately 1×10^7 infected erythrocytes. Animals were left for 72 h and thin blood smears were made from the tail vein on a slide. The slides were dried and subsequently

stained with Giemsa stain and viewed under the microscope to examine for the presence of parasitemia. Percentage parasite suppression was then calculated as previously described.

Statistical analysis
Data obtained from all tests were analyzed with one-way ANOVA followed by Dunnett's post hoc test. Results were deemed statistically significant at p values < 0.05.

RESULTS

Oral acute toxicity study
Oral administration of the extract at dose of 5000 mg/kg produced no overt physical and behavioral signs of toxicity nor mortality.

Phytochemical constituents
Phytochemical evaluation of the plant extract revealed the presence of various phytoconstituents such as alkaloids, flavonoids, tannins, carbohydrates, cardiac glycosides, saponins, triterpenes and steroids (Table 1).

Evaluation of suppressive effect of *Clerodendrum capitatum* ethanol leaf extract in *Plasmodium berhgei* infected mice
A significant (p<0.05) dose dependent reduction in parasitemia level was noted on administration of the extract. Highest percentage chemosuppression was observed at the dose of 400 mg/kg (66.2%). The standard drug (Artesunate, 5mg/kg) produced marked (p<0.05) chemosuppression of 85.6%. (Table 2).

Evaluation of curative effect of ethanol leaf extract of *Clerodendrum capitatum* in *Plasmodium berghei* infected mice
The ethanol leaf extract of *Clerodendrum capitatum* produced a significant (p<0.05) dose dependent reduction in parasitemia level with highest percentage chemosuppresion at 400 mg/kg (62.2%) compared with the standard drug Artesunate (84.3%) (Table 3).

Effect of ethanol leaf extract of *Clerodendrum capitatum* on mean survival time in *Plasmodium berghei* infected mice
Administration of the ethanol leaf extract of *Clerodendrum capitatum* produced a significant (p<0.05) increase in mean survival time. However, this was not dose dependent. Mice administered with the standard drug (Artesunate, 5mg/kg) survived for a longer period compared to the extract treated animals (Table 4).

Evaluation of the prophylactic effect of ethanol leaf extract of *Clerodendrum capitatum* in *Plasmodium berghei* infected mice
The ethanol leaf extract of *Clerodendrum capitatum* produced a dose dependent significant reduction in parasitemia level with the highest percentage chemosuppression observed at a dose of 200 mg/kg. The standard drug (Pyrimethamine, 1.2 mg/kg) also produced marked chemosuppression (Table 5).

DISCUSSION
The present study evaluated the antimalarial activity of *Clerodendrum capitatum* using the suppressive, curative and prophylactic models in *Plasmodium berghei* infected mice. Preliminary phytochemical screening of the extract revealed the presence of secondary metabolites including; alkaloids, flavonoids, cardiac glycosides, tannins, terpenes and saponins. Similar phytochemicals have been found and reported previously in another study [34]. A number of phytochemical constituents like alkaloids, terpenes, coumarins and flavonoids have been shown to be potential antiplasmodial agents [35]. Thus, the presence of these phytochemical constituents may account for the antiplasmodial activity of the plant.

Oral acute toxicity study of *Clerodendrum capitatum* extract revealed no mortality up to a dose of 5000 mg/kg body weight indicating the safety of the extract [28].

The Peters 4-day suppressive test is a standard test commonly used for antimalarial drug screening in early *Plasmodium berghei* infection, and the determination of percentage inhibition of parasitemia is the most reliable parameter [33]. This test is the first line primary screen for *in vivo* testing of potential antiplasmodial compounds [36]. In the present study, the ethanol leaf extract of *Clerodendrum capitatum* produced a significant dose dependent parasite suppressive effect. According to Muthaura, [37], parasite suppressive effects of plant extracts might be through indirect boosting of the immune system or by inhibition of other target pathways which are yet to be fully realized. Thus, the parasite suppressive effect of the extract may be through a similar mechanism or yet to be identified pathway.

In the curative test, the extract did not eradicate parasites completely on the seventh day of the study. Malaria infection is associated with an increase in many inflammatory mediators which may enhance cell to cell interaction

Table 1: Phytochemical constituents present in the ethanol leaf extract of *Clerodendrum capitatum*.

Phytochemical constituent	Inference
Carbohydrates	+
Anthraquinones	-
Cardiac glycosides	+
Tannins	+
Flavonoids	+
Alkaloids	+
Triterpenes and steroids	+
Saponins	+

Key: + = Present   - = Absent

Table 2: Suppressive effect of ethanol leaf extract of *Clerodendrum capitatum* in *Plasmodium berghei* infected mice

Treatment groups	Dose (mg/kg)	Average parasitemia	Percentage chemosuppression (%)
DW	10 ml/kg	30.17 ± 1.83	-
ELECC	100	15.50 ± 1.38*	48.6
ELECC	200	14.38 ± 3.05*	52.3
ELECC	400	10.47 ± 2.71*	65.3
ART	5	4.33 ± 0.36*	85.6

n = 6; * = $p<0.5$; DW = Distilled water; ELECC = Ethanol Leaf Extract of *Clerodendrum capitatum*; ART = Artesunate.

Table 3: Curative effect of ethanol leaf extract of *Clerodendrum capitatum* in *Plasmodium berghei* infected mice

Treatment groups	Dose (mg/kg)	Average parasitemia	Percentage chemosuppression (%)
DW	10 ml/kg	28.67 ± 2.15	-
ELECC	100	18.83 ± 1.47*	34.3
ELECC	200	14.67 ± 1.12*	48.8
ELECC	400	10.83 ± 1.25*	62.2
ART	5	4.50 ± 0.36*	84.3

n = 6; * = $p<0.5$; DW = Distilled water; ELECC = Ethanol Leaf Extract of *Clerodendrum capitatum*; ART = Artesunate.

Table 4: Effect of ethanol leaf extract of *Clerodanrum capitatum* on mean survival time in *Plasmodium berghei* infected mice

Treatment groups	Dose (mg/kg)	MST (days)
DW	10ml /kg	17.6 ± 2.15
ELECC	100	19.6 ± 1.47
ELECC	200	20.1 ± 1.11
ELECC	400	18.7 ± 1.24
ART	5	21.0 ± 1.74

n = 6; * = p<0.5; DW = Distilled water; ELECC = Ethanol Leaf Extract of *Clerodandrum capitatum*; ART = Artesunate.

Table 5: Prophylactic effect of ethanol leaf extract of *Clerodanrum capitatum* in *Plasmodium berghei* infected mice

Treatment groups	Dose (mg/kg)	Average parasitemia	Percentage chemosuppression (%)
DW	10ml/kg	26.18 ± 1.82	-
ELECC	100	20.57 ± 0.59	21.5
ELECC	200	13.17 ± 1.19*	49.7
ELECC	400	14.82 ± 2.03*	43.4
PYR	1.2	5.17 ± 1.46*	80.3

n = 6; * = p<0.5; DW = Distilled water; ELECC = Ethanol Leaf Extract of *Clerodandrum capitatum*; PYR = Pyrimethamine.

(cytoadherence), cell stimulation through malaria-derived antigens and host-derived factors like cytokines [38]. The cytokines are in turn responsible for the feverish symptoms that occur in the host [39]. The curative antiplasmodial properties of *Clerodendrum capitatum* may thus be due to the inhibition of the production and/or release of these inflammatory mediators associated with malaria. The mean survival time was prolonged in the extract treated groups compared to the distilled water treated group. In antimalarial studies, a test compound that has the ability to extend mean survival time beyond 12 days is regarded as having good parasite suppressing activity [40]. The extract could thus be regarded as having good parasite suppressive activity, as the mean survival time at all doses tested was extended beyond 12 days. This also suggests that the extract was able to cause a reduction in the overall pathological effect of the parasite in mice with suppression of parasitemia [41].

The prophylactic test evaluates the ability of extract to prevent parasite growth probably by inhibiting proliferation of parasites due to direct cytotoxic effect [42] and/or modulation of erythrocytes membranes to prevent parasite invasion [43]. The ability of the extract to reduce parasitemia levels compared to the distilled water treated group suggests that the extract possesses chemoprophylactic activity acting via any of the mechanisms previously mentioned.

In vivo antiplasmodial activity can be classified as moderate, good, and very good if an extract displays percentage parasitemia suppression equal to or greater than 50% at a dose of 500, 250 and 100 mg/kg body weight per day, respectively [44]. Based on this classification, the ethanol leaf extract of *Clerodendrum capitatum* showed moderate *in vivo* antiplasmodial activity.

CONCLUSION
The results of this study suggest that the leaves of *Clerodendrum capitatum* possesses significant *in vivo* antimalarial activity against *Plasmodium berghei* infection in mice. These findings thus justify and confirm the ethno botanical usage of this plant in the treatment of malaria.

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