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Research Article

In vitro and *In vivo* Antiplasmodial Activities and Cytotoxicities of *Clerodendrum violaceum* Leaf Extracts

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ABSTRACT

The antiplasmodial activity of hexane, ethyl acetate, and methanolic leaf extracts of *Clerodendrum violaceum* were tested *in vitro* using *Plasmodium falciparum* W2 strain blood parasites. HepG2 A16 hepatoma cells were used in the cytotoxicity experiments. Chloroquine-sensitive *Plasmodium berghei* NK 65-infected mice were examined for suppressive and curative efficacy. Seven (7) groups of five mice were employed for each extract: groups 1-5 were given 31.25, 62.5, 125, 250, and 500 mg/kg body weight of the various extracts. Animals in group 6 were given 20 mg/kg body weight of chloroquine, whereas those in group 7 were given an identical volume of 5% DMSO as a control (the vehicle used in dissolving the extracts). The drug and extracts were orally administered for four consecutive days. The different extracts were inactive *in vitro* and did not exhibit any toxicity against HepG2 cells *in vitro*. They did, however, provide some chemo-suppression *in vivo*; and the methanolic extract had the best antimalarial efficacy *in vivo*. In addition, the extracts increased the mean survival time of treated mice when compared to the control group. The findings of this study revealed that *Clerodendrum violaceum* leaf extracts exhibit antimalarial activity *in vivo*. As a result, it may be concluded that *Clerodendrum violaceum* leaves, particularly the methanolic extract, have promising antimalarial potential but may be hazardous in high dosages.

Keywords: *Clerodendrum violaceum*, *Plasmodium falciparum*, *Plasmodium berghei*, antiplasmodial.

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INTRODUCTION

Malaria is a life-threatening infectious disease caused by parasites belonging to the genus *Plasmodium* which are transmitted to vertebrate hosts through the bites of infected female *Anopheles* mosquitoes and although malaria especially that caused by *Plasmodium falciparum* can lead to potentially life-threatening infections, it is preventable and curable (WHO, 2020; WHO, 2021). However, despite giant strides made towards the control and treatment of malaria, it still remains an important cause of death and illness in children and adults in tropical countries with a mortality rate of over a million per year (WHO, 2021). Pregnant women are particularly vulnerable to malaria infections; thus increasing the risk of maternal-fetal complications, especially in areas where malaria is endemic. In Nigeria, 76% of the population lives in high malaria transmission areas (USAID, 2020). According to the 2020 World Malaria Report, the highest number of both global cases of and deaths from malaria was recorded in Nigeria (WHO, 2019).

The treatment and control of the disease have been complicated by the emergence of parasite resistance to commonly used antimalarial drugs (Noedl, *et al.*, 2009; Kuehn, 2021.). This necessitates a continuous effort to search for new drugs (Sam *et al.*, 2011, Kaushik *et al.*, 2015). In malaria-endemic countries, traditional medicinal plants are frequently used to treat malaria (Gessler *et al.*, 1995; Mensah and Gyasi, 2012). This is because plants commonly used in traditional medicine are thought to be safe owing to their long usage in folklore according to knowledge accumulated over centuries for the treatment and management of diseases (Jeruto *et al.*, 2015). The antimalarial potentials of compounds derived from plants are proven by examples such as quinine (obtained from *Cinchona* species) and Artemisinin (from *Artemisinia annua)* (Lee, 2002, Coleman *et al.*, 2004; Jeruto *et al.*, 2015). Several plants that are used to treat diseases traditionally have been tested and validated by scientific methods (Keay, 1989; Intisar *et al.*, 2014). Encouraging results from such studies have therefore prompted an upsurge in the study of herbal remedies in several parts of the world (Dalziel, 1997, Dahanukar*et al.*, 2000). In Africa, over 80% of the population still relies on herbal remedies for the treatment of many diseases, including malaria because they are easily affordable and widely accessible (Agbedahunsi, 2000, Adebayo and Krettli, 2011). One of such plants used in the traditional treatment of malaria is *Clerodendrum violaceum*.

Clerodendrum violaceum Gürke (*Verbenaceae*) is commonly called Clerodendrum in English and `Ewe isedun´ in Yoruba (Nigeria). A decoction of the leaves is used for the treatment of fever/malaria. It is therefore of interest to explore the antimalarial potentials of the *Clerodendrum violaceum* leaf extracts as a potential agent in the fight against malaria infection.

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MATERIALS AND METHODS

Chemicals and reagents: Chloroquine diphosphate salt was obtained from Sigma Chemical Company, St. Louis, Mo, USA, n-hexane, ethyl acetate and methanol was obtained from BDH Laboratory Supplies, Poole Dorset BH15 UK. All other reagents used were of analar grade and prepared in all glass distilled water.

Animals: Adult Swiss albino mice with an average weight of 20 ± 2.0 g were obtained from the animal breeding unit of the Department of Biochemistry, University of Jos, Plateau State. The mice were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad libitum.*

Parasites: The *in vitro* antiplasmodial tests were performed with blood parasites of *P. falciparum* W2 clone at the Laboratorio de Malaria, Instituto de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte MG, Brazil. The *in vivo* tests were carried out using a chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Oyo state.

Cell lines: HepG2 A16 cell line was also obtained from Laboratorio de Malaria, Instituto de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte MG, Brazil.

Plant material: Fresh leaves of *Clerodendrum violaceum* were collected in Oyo town, Oyo State, Nigeria and botanically authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. A specimen with voucher number FHI 109857 was deposited.

Plant extract preparation: Fresh leaves of the plant were dried in the shade at room temperature (25 ± 2 ºC) and pulverized to powder using an electric blender (Mazeda Mill, MT 4100, Japan). Four hundred and fifty gram (450 g) of the powder was exhaustively extracted with 4 L n-hexane, 4L ethyl acetate and 4 L absolute methanol for 72 h each successively. The extracts were filtered using Whatman filter paper No 1 and concentrated after each extraction period using a rotary evaporator. The concentrates were then exposed to air and allowed to evaporate at room temperature to dryness (Adebayo *et al.,* 2003).

Phytochemical screening: The extract was screened for phytochemical constituents as described by Sofowora (1980) and Evans (2005).

***In vitro* antiplasmodial studies:** The *in vitro* tests were performed with blood parasites of *Plasmodium falciparum* W2 clone. All parasites were kept in continuous culture at 37°C on Group A^+ human erythrocytes in complete culture medium using the candle jar method as described by Trager and Jensen (1976).The antimalarial effects of the extracts were measured through inhibition of parasite growth, by the [^{3}H]-hypoxanthine incorporation assay, as described by Desjardins *et al.* (1979) and Zalis *et al*. (1998); and Histidine Rich Protein II (HRP II) assay (Noedl and Wernsdorfer, 2003). Controls without extracts or with chloroquine used as the reference antimalarial drug, were run in parallel.

Cytotoxicity test: HepG2 A16 hepatoma cells were kept at 37 °C in complete culture medium; cell growth was determined using the tetrazolium based colorimetric assay, 3-(4,5 dimethyl thiazol)-2,5 diphenyltetrazolium bromide (MTT) assay, after incubation with the extracts. The method measures formazan produced by mitochondrial metabolic reduction of MTT (Denizot and Lang, 1986). The minimum lethal dose (MLD) that killed 50% of the cells was determined as reported by Madureira *et al.* (2002); each assay was performed twice. Based on the values of cytotoxicity (MLD_{50}) and antimalarial activity (IC_{50}) the selective index (SI) of activity was calculated using the formula:

$SI= MLD_{50}/IC_{50}$.

***In vivo* antimalarial test (4-day suppressive test):** The 4-day suppressive test against chloroquine sensitive *Plasmodium berghei* (NK 65) infection in mice, as described by Peters (1965), was used to test the efficacy of the different extracts of *Clerodendrum violaceum* leaf. The mice were inoculated from the same donor mouse. The percentage parasitaemia and the red blood cell count of the donor mouse was first determined using a haemocytometer and appropriate dilutions of the infected blood with isotonic saline were made. Each mouse was inoculated intraperitoneally on day 0 with 0.2 ml of infected blood containing about 1 x 10^7 *P. berghei* parasitized red blood cells. For each extract, thirty five mice were randomly divided into seven groups of five mice each and treated as follows: Groups 1-5 were administered 31.25, 62.5, 125, 250 and 500 mg/kg body weight of the different extracts. Group 6 was treated with 20 mg/kg body weight of chloroquine while animals in group 7 (control) were administered an equal volume of 5% DMSO (the vehicle used in dissolving the extracts). Treatment was started three hours after parasite inoculation and was given orally for four consecutive days. On the fourth day (day 4), thin blood smears were made from tail blood of each mouse. The films are fixed in methanol, stained with Giemsa and examined microscopically (Ryley and Peters 1970, Cheesebrough, 2004). The parasite count was recorded and the suppression of parasitaemia was expressed as percent for each dose by comparing the parasitaemia in the control group with the treated ones. Additional smears were taken on days 6 and 8 post inoculation. The mean survival time of the mice in each treatment group was monitored for 30 days.

Curative test (Rane test): Evaluation of the curative potential of the different extracts of *Clerodendrum violaceum* leaf was carried out as described by Ryley and Peters (1970). Animals were grouped and treated as in the suppressive test, but treatment was delayed for 72 hours to allow for the

establishment of infection, and treatment was resumed once parasitaemia was confirmed by screening infected animals' tail blood for malaria parasites after fixing in methanol and staining with Giemsa. The treatment was also given orally once a day for four days in a row (D_4-D_7).

On day 8, blood was collected from the tail of each mouse and thin films were made on a microscope slide and examined microscopically to determine the parasitaemia. The mean survival time of the mice in each treatment group was also monitored for 30 days.

Statistical analysis

The group averages ± SD for each parameter were computed, and significant differences were evaluated using the SPSS-PC program package's Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at a confidence level of 5% (Version 22.0, SPSS Inc. Chicago).

RESULTS

Phytochemical tests: The different extracts of *Clerodendrum violaceum* leaf contain varying amounts of phytochemicals. The methanolic extract had the highest amounts of total phenols, total flavonoids, and alkaloids (Table 1).

***In vitro* antiplasmodial and cytotoxicity tests:** The extracts were not active against *Plasmodium falciparum* W2 strain *in vitro* neither did they exhibit any toxicity on HepG2 cells *in vitro* (Table 2).

Table 1:
Some Phytochemicals of *Clerodendrum violaceum* Leaf Extracts

Phytochemicals	Percentage composition (dry weight)		
	Hexane extract	**Ethyl acetate extract**	**Methanolic extract**
Tannins	0.04 ± 0.01	0.07 ± 0.02	0.17 ± 0.07
Total phenols	0.07 ± 0.01	0.09 ± 0.02	2.30 ± 0.09
Saponins	0.08 ± 0.02	0.11 ± 0.03	0.24 ± 0.07
Total flavonoids	0.10 ± 0.02	0.12 ± 0.04	2.96 ± 0.33
Steroids	0.07 ± 0.02	0.09 ± 0.03	0.05 ± 0.02
Phlobatannins	0.02 ± 0.01	0.04 ± 0.02	0.91 ± 0.03
Alkaloids	0.13 ± 0.07	0.24 ±0.04	2.98 ± 0.06
Triterpenes	0.08 ± 0.02	0.81 ± 0.03	0.09 ± 0.03
Glycosides	0.07 ± 0.03	0.04 ± 0.02	0.10 ± 0.05
Anthraquinones	0.03 ± 0.01	0.06 ± 0.03	0.09 ± 0.02

Values are means ± SD of 3 replicates

Antimalarial tests

Four-day suppressive test: Extracts that cause less than 30% chemosupression in *in vivo* antimalarial tests are considered inactive. Those having 30 to 50% are considered partially active while those that cause over 50% chemosupression are considered active.

The hexane extract of *Clerodendrum violaceum* leaf only exhibited partial antimalarial activity at the dose of 250 and 500 mg/kg body weight on day 6 post-inoculation (Table 3).

Table 2:
In vitro Antiplasmodial Activities and Cytotoxicities of *Clerodendrum violaceum* Leaf Extracts

	Activity against *P. falciparum* W2(IC_{50} µg/ml)					
Compounds	Hypoxanthine	HRPII	Mean	MLD_{50} (µg/ml)	**SI**	Conclusion
EA	> 50	> 50	> 50	>1000	-	inactive
M	> 50	> 50	> 50	>1000	-	inactive
H	37	> 50	> 50	>1000	-	inactive

Values are means ± SD of two experiments each done in triplicate.
EA= Ethyl acetate extract, M= methanolic extract, H= hexane extract.
MLD_{50}- Minimum lethal dose, IC_{50}: Half maximal Inhibitory concentration, SI = MLD_{50}/IC_{50}.
EA= Ethyl acetate extract, M= methanolic extract, H= hexane extract.
MLD_{50}- Minimum lethal dose, IC_{50}: Half maximal Inhibitory concentration, SI = MLD_{50}/IC_{50}.

Table 3:
Effects of Hexane Extract of *Clerodendrum Violaceum* Leaf on Parasitaemia in *Plasmodium berghei* (NK 65)-Infected Mice (Suppressive Test)

Treatment	Parasitaemia (%) (% Reduction)		
	4º	**6º**	**8º**
Control	7.87	8.93	11.67
31.25 mg/kg	7.72 (1.91)	7.70 (13.77)	10.74 (7.97)
62.5 mg/kg	7.44 (5.46)	7.43 (16.79)	10.73 (8.03)
125 mg/kg	7.35 (6.61)	7.33 (17.92)	10.49 (10.11)
250 mg/kg	6.04 (23.25)	6.00 (31.47)	8.42 (27.85)
500 mg/kg	5.85 (25.67)	5.48 (34.60)	8.39 (28.11)
Chloroquine (20 mg/kg)	0.19 (97.59)	0.12 (98.65)	0.05 (99.57)

Values are means of 5 replicates. º- Days post-infection; b.wt. – body weight.

Table 4:
Effects of Ethyl Acetate Extract of *Clerodendrum violaceum* Leaf on Parasitaemia in *Plasmodium berghei* (NK 65)-Infected Mice (Suppressive Test)

Treatment	Parasitaemia (%) (% Reduction)		
	4º	**6º**	**8º**
Control	7.87	8.93	11.67
31.25 mg/kg	7.24 (8.00)	7.17 (19.71)	7.19 (38.39)
62.5 mg/kg	5.98 (24.02)	5.90 (33.93)	5.93 (49.19)
125 mg/kg	5.03 (36.09)	4.98 (44.23)	5.05 (56.73)
250 mg/kg	2.95 (62.52)	2.85 (68.09)	2.91 (75.06)
500 mg/kg	2.86 (63.66)	2.73 (69.43)	2.84 (75.66)
Chloroquine (20 mg/kg)	0.19 (97.59)	0.12 (98.65)	0.05 (99.57)

Values are means of 5 replicates. º- Days post-infection; b.wt. – body weight.

On day 4 post-inoculation, ethyl acetate extract exhibited antimalarial activity at the doses of 125, 250, and 500 mg/kg body weight while on day 6 post-inoculation it exhibited antimalarial activity at all doses except 31.25 mg/kg body weight. Moreover, on day 8 post-inoculation, it was active at all the doses administered (Table 4).

Methanolic extract of *Clerodendrum violaceum* leaf exhibited antimalarial activity at all doses administered on days 6 and 8 post-inoculation but only at the doses of 125, 250, and 500 mg/kg body weight on day 4 post-inoculation (Table 5).

Table 5:
Effects of Methanolic Extract of *Clerodendrum violaceum* Leaf on Parasitaemia in *Plasmodium berghei* (NK 65)-Infected Mice (Suppressive Test).

Treatment	Parasitaemia (%) (% Reduction)		
	4º	6º	8º
Control	7.87	8.93	11.67
31.25 mg/kg	6.33 (19.57)	6.17 (30.91)	6.28 (46.19)
62.5 mg/kg	5.73 (27.19)	5.42 (39.31)	5.69 (51.24)
125 mg/kg	4.51 (42.69)	4.28 (52.07)	4.46 (61.78)
250 mg/kg	1.62 (79.42)	1.47 (83.54)	1.45 (87.57)
500 mg/kg	1.47 (81.32)	1.45 (83.76)	1.42 (87.83)
Chloroquine (20 mg/kg	0.19 (97.59)	0.12 (98.65)	0.05 (99.57)

Values are means of 5 replicates. º- Days post-infection; b.wt. – body weight.

Mean survival time (MST)

Treatment with various extracts of *Clerodendrum violaceum* leaf increased the mean survival time of treated animals compared to that of the untreated control (Table 6).

Table 6:
Effects of *Clerodendrum violaceum* Leaf Extracts on Mean Survival Time (MST) of *Plasmodium berghei* (NK 65)-Infected Mice (Suppressive Test)

Treatment	MST (days)		
	Hexane extract	Ethyl acetate extract	Methanolic extract
Control	10.80 ±1.32	10.80 ± 1.32	10.80 ± 1.32
31.25 mg/kg	11.00 ± 1.58	13.60 ± 2.14	15.80 ± 2.39
62.5 mg/kg	11.20 ± 1.59	15.40 ± 2.13	17.40 ± 1.99
125 mg/kg	11.60 ± 1.75	17.90 ± 2.36	19.40 ± 1.91
250 mg/kg	11.80 ± 1.88	20.60 ± 1.72	25.40 ± 1.21
500 mg/kg	12.00 ± 1.79	19.20 ± 1.40	20.40 ± 1.01
Chloroquine (20 mg/kg)	28.80 ± 0.19	28.80 ± 0.19	28.80 ± 0.19

Values are means ± SD of 5 replicates

Curative test: The hexane extract of *Clerodendrum violaceum* leaf did not exhibit any antimalarial activity on days 8, 9 and 10 post-inoculation but exhibited partial antimalarial activity at all doses on day 11 and partial activity at all doses on day 14 except 500 mg/kg which was active (Table 7).

The ethyl acetate extract of *Clerodendrum violaceum* leaf did not exhibit any antimalarial activity on days 8, and 9 post-inoculation and only exhibited partial antimalarial activity at the dose of 500 mg/kg body weight on day 10 and at the doses of 31.25, 62.5 and 125 mg/kg body weight on day 11 but was active at the doses of 250 and 500 mg/kg body weight on the same day. However, it was active at all doses on day 14 (Table 8).

Table 7:
Effects of Hexane Extract of *Clerodendrum violaceum* Leaf on Parasitaemia in *Plasmodium berghei* (NK 65)-Infected Mice (Curative Test)

Treatment	Parasitaemia (%) (% Reduction)				
	8º	9º	10º	11º	14º
Control	9.34	11.41	13.61	17.11	31.30
31.25 mg/kg b.wt	9.47 (< 0)	11.15 (2.28)	11.37 (16.46)	11.23 (34.36)	21.24 (32.14)
62.5 mg/kg b.wt	9.41 (< 0)	11.10 (2.72)	11.29 (17.05)	11.11 (35.07)	19.13 (37.92)
125 mg/kg b.wt	9.27 (0.75)	10.86 (4.82)	11.19 (17.78)	10.88 (36.41)	18.92 (39.55)
250 mg/kg b.wt	9.28 (0.64)	10.58 (7.27)	11.03 (18.96)	10.73 (37.29)	16.86 (46.13)
500 mg/kg b.wt	9.26 (0.86)	10.26 (10.08)	10.58 (22.26)	10.26 (40.04)	15.17 (51.53)
Chloroquine (20 mg/kg b.wt)	7.25 (22.38)	6.07 (46.80)	4.67 (65.69)	2.89 (83.11)	0.97 (96.90)

Table 8:
Effects of Ethyl Acetate Extract of *Clerodendrum violaceum* Leaf on Parasitaemia in Plasmodium berghei (NK 65)-Infected Mice (Curative Test)

Treatment	Parasitaemia (%) (% Reduction)				
	8º	9º	10º	11º	14º
Control	9.34	11.41	13.61	17.11	31.30
31.25 mg/kg b.wt	9.26 (0.86)	10.23 (10.34)	10.93 (19.69)	9.73 (43.13)	9.39 (70.00)
62.5 mg/kg b.wt	8.97 (3.96)	10.14 (11.13)	10.76 (20.94)	9.23 (46.05)	9.05 (71.09)
125 mg/kg b.wt	8.94 (4.28)	10.05 (11.92)	10.25 (24.69)	9.22 (46.11)	8.21 (73.77)
250 mg/kg b.wt	8.12 (13.06)	9.44 (17.27)	9.87 (27.48)	8.12 (52.54)	7.81 (75.04)
500 mg/kg b.wt	7.95 (14.88)	9.13 (19.98)	9.39 (31.00)	7.27 (57.51)	7.13 (77.22)
Chloroquine (20 mg/kg b.wt)	7.25 (22.38)	6.07 (46.80)	4.67 (65.69)	2.89 (83.11)	0.97 (96.90)

Values are means of 4 replicates. º- Days post-infection; b.wt. – body weight

Table 9:
Effects of Methanolic Extract of Clerodendrum violaceum Leaf on Parasitaemia in Plasmodium berghei NK 65)-Infected Mice (Curative Test)

Treatment	Parasitaemia (%) (% Reduction)				
	8º	9º	10º	11º	14º
Control	9.34	11.41	13.61	17.11	31.30
31.25 mg/kg	9.15 (2.03)	9.76 (14.46)	10.18 (25.20)	9.47 (44.65)	6.15 (80.35)
62.5 mg/kg	9.06 (2.99)	9.56 (16.21)	10.05 (26.16)	8.94 (47.75)	6.00 (80.80)
125 mg/kg	8.97 (3.96)	9.43 (17.35)	9.79 (28.07)	8.04 (53.00)	5.09 (83.73)
250 mg/kg	8.75 (6.32)	9.19 (19.46)	9.30 (31.67)	7.00 (59.01)	4.08 (86.96)
500 mg/kg	8.26 (11.56)	9.08 (27.20)	8.97 (34.09)	6.01 (64.87)	3.98 (87.28)
Chloroquine (20 mg/kg)	7.25 (22.38)	6.07 (46.80)	4.67 (65.69)	2.89 (83.11)	0.97 (96.90)

Values are means of 4 replicates. º- Days post-infection; b.wt. – body weight

The methanolic extract of *Clerodendrum violaceum* leaf did not exhibit any antimalarial activity on days 8 and 9 post-inoculation and only exhibited partial antimalarial activity at the dose of 250 and 500 mg/kg body weight on day 10. It also exhibited partial activity at the doses of 31.25 and 62.5 mg/kg body weight on day 11 but was active at the doses of 125, 250 and 500 mg/kg body weight on day 11. However, it was active at all doses on day 14 (Table 9).

Mean survival time (MST): Treatment with various extracts of *Clerodendrum violaceum* leaf increased the mean survival time of treated animals compared to untreated control (Table 10).

Table 10:
Effects of *Clerodendrum violaceum* Leaf Extracts on Mean Survival Time (MST) of *Plasmodium berghei* (NK 65)-Infected Mice (Curative Test)

Treatment	MST (days)		
	Hexane extract	Ethyl acetate extract	Methanolic extract
Control	11.07 ± 4.82	11.07 ± 4.82	11.07 ± 4.82
31.25 mg/kg	17.00 ± 3.56	18.40 ± 4.77	20.40 ± 5.50
62.5 mg/kg	17.60 ± 3.05	19.30 ± 4.89	22.40 ± 5.50
125 mg/kg	17.00 ± 3.67	20.80 ± 4.97	23.40 ± 4.22
250 mg/kg	17.80 ± 3.56	21.34 ± 4.00	26.80 ± 2.12
500 mg/kg	17.00 ± 3.32	21.00 ± 4.47	19.80 ± 3.00
Chloroquine (20 mg/kg)	28.00 ± 2.17	28.00 ± 2.17	28.00±2.17

Values are means ± SD of 5 replicates

DISCUSSION

In the determination of *in vitro* antiplasmodial activity, only extracts with IC_{50} values less than 25 µg/ml are generally considered active. The *in vitro* antiplasmodial test in this study revealed that all the extracts had IC_{50} values higher than that and MLD_{50} values greater than 1000 µg/ml which was the highest concentration used (Table 2). Consequently, the selectivity index which would have determined the most active extract *in vitro* could not be calculated meaning none of the extracts were active. However, results obtained in the 4-day suppressive and curative tests revealed that the methanolic extract exhibited the highest antimalarial activity followed by the ethyl acetate extract. Park *et al.* (1998) reported that first generation peroxide compounds fenozan, tetroxane, and tricyclic trioxanes which are all derivatives of the lactol dihydroartemisin, which is a major metabolite for each of the derivatives may be the most important chemical species for antimalarial activity *in vivo*. The *in vivo* antimalarial activity exhibited by the extracts may be through a similar mechanism involving bio activation and production of active metabolites. The difference in the response of infected animals to treatment with the various extracts suggests that the active compounds in the extract maybe more abundant in the methanolic extract compared to other extracts. The results obtained in the curative test produced a similar pattern of chemo-suppression but the response was slower than in the suppressive test but increased significantly on day 10 for all extracts. The delayed activity highlights the fact that the parasitaemia had increased to a certain extent before treatment was commenced. Reduction in parasitaemia was dose-dependent for all extracts; however, the animals treated with 500 mg/kg body weight methanolic and ethyl acetate extracts had a lower mean survival time compared to the groups treated with 250 mg/kg body weight despite the fact that the former group had the highest parasite clearance. This suggests toxicity of the extracts at 500 mg/kg body weight.

Although the mechanism of antimalarial action of these extracts have not been elucidated, antimalarial effects of medicinal plants have been attributed to some of their active phytochemical constituents (Sofowora, 1980; Ayoola *et al.*, 2008; Alli *et al.*, 2011; Bekono *et al.*, 2020). Some of these phytochemicals such as alkaloids, phenolics, flavonoids, tannins, and saponins etc. were detected in varying concentrations in all the extracts. The methanolic extract contained very high concentrations of alkaloids, flavonoids and total phenols. Alli *et al.* (2011) attributed the antimalarial activity of *Acacia nilotica* to its appreciable quantities of alkaloids, phenolics and tannins. Alkaloids have been reported to be a major constituent of the *Clerodendrum* species (Bashwira and Hootele, 1988) and methanolic leaf extracts of *Clerodendrum myricoides*, *Clerodendrum trichotomum* and *Clerodendrum inerme* have been shown to exhibit antimalarial effect (Muthaura *et al., 2007:* Tekalign *et al.*, 2010). Antimalarial activity has also been attributed to flavonoids, tannins, alkaloids, steroids and triterpenes present in the methanolic leaf and root extracts of *Sphenocentrum jollyanum pierre* (Olorunnisola and Afolayan 2011) Chinwuba *et al.* (2015) reported the antimalarial activity of *Citrus sinensis* and attributed it to flavonoids and alkaloids present in the

ethanolic stem extract of the plant. Akuodor *et al.* (2015) also attributed the antimalarial activity of *Pseudocera kotschyi* to alkaloids, tannins, flavonoids, and terpenoids present in the ethanolic leaf extract of the plant. Similar phytochemicals were found to exhibit antimalarial activity in leaf extracts and fractions of *Persea Americana* and *Dacroydes edulis* (Uzor *et al.,* 2021).

Flavonoids and phenols have been suggested to act as primary antioxidants or free radical scavengers that can counteract oxidative damage induced by the malaria parasite; this may assist in reducing the complications induced by oxidative stress resulting from the infection (David *et al.,* 2004). Moreover, many plants containing alkaloids and flavonoids have diuretic, antispasmodic, anti-inflammatory and analgesic effects (Owoyele *et al.,* 2002; Kaewdana *et al.,* 2021; Laryea and Bourquaye, 2021). Agents with such activity were reported to provide relief to malaria patients (Addae-Kyereme *et al.,* 2001). Balogun *et al.* (2014) reported the augmentation of the antioxidant system by extracts of *Clerodendrum violaceum* leaf as a means of ameliorating the deleterious effects of reactive oxygen species (ROS) produced *in vivo* during malaria infection through antioxidant species present in the extracts.

Thus, *Clerodendrum violaceum* leaf extracts may exert the antimalarial effect observed *in vivo* in this study by direct parasite clearance by the various phytochemicals present in the different extracts. The higher antimalarial activity demonstrated by the methanolic extract of the leaf of *Clerodendrum violaceum* may be due to the higher concentrations of these phytochemicals which may act through diverse mechanisms.

In conclusion, the results obtained in this study show that *Clerodendrum violaceum* leaf extracts play a role in malaria management by direct parasite clearance. Some phytochemical constituents present in the leaf extracts may also help to ameliorate some conditions associated with malaria infection. There is, thus, a need for further investigation to isolate its active principles and assess its safety

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