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Augustine Ini Lawrence Bassey
Department of Clinical Pharmacology and Therapeutics, Faculty of Clinical Sciences, University of Uyo, Uyo, Nigeria

Ette Okon Ettebong
Department of Clinical Pharmacology and Therapeutics, Faculty of Clinical Sciences, University of Uyo, Uyo, Nigeria

Paul Alozie Nwafor
Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria

Correspondence
Augustine Ini Lawrence Bassey
Department of Clinical Pharmacology and Therapeutics, Faculty of Clinical Sciences, University of Uyo, Uyo, Nigeria

Evaluation of anti-plasmodial, anti-pyretic and mean survival time of rodents treated with ethanolic extract of *Enantia chlorantha* stem bark

Augustine Ini Lawrence Bassey, Ette Okon Ettebong and Paul Alozie Nwafor

Abstract
Background and aim of study: *Enantia chlorantha* is a medicinal plant that is used all over Nigeria for treatment of malaria and other febrile illnesses. Anti-plasmodial effects of the extract and its different fractions (n-hexane, chloroform, ethyl acetate, methanol and aqueous) were tested at doses of 32.40 mg/kg, 64.80 mg/kg and 96.20 mg/kg in Chloroquine sensitive *Plasmodium berghei berghei* infected mice. The antipyretic effects were evaluated also.
Experimental: Parasitized blood donor of Swiss albino mice with at least 1.0 x 10^7 parasitized red blood cells was used. Suppressive, prophylactic and curative models were investigated. The mean survival time was observed. Evaluation of anti-plasmodial activity of the fractions using the 4-day test was done. The effect of extract on 2, 4-Dinitrophenol (DNP)-Induced pyrexia in adult albino rats (150-180 g) was also evaluated.
Results: Statistically significant dose-dependent reduction in parasitaemia was noted in all three models (p<0.001) when compared to control. The mean survival times were increased in a dose- dependent manner which was statistically significant (p<0.001). N-hexane, chloroform, ethyl acetate and methanol fractions exerted varying degrees of anti-plasmodial activity with methanol being the highest. The extract treated groups showed dose-dependent reduction in temperature that was statistically significant (p<0.05 – 0.001).
Conclusion: The above results provide support for the traditional use of *Enantia chlorantha* stem bark in treating febrile illnesses such as malaria in Akwa Ibom state. This extract shows a lot of promise and is a good candidate for evaluation as a potential new drug for malaria and other febrile and inflammatory conditions.

Key words: *Enantia chlorantha*, ethanol extract, malaria, rodents, pyrexia

1. Introduction
Enantia chlorantha is a fair sized ornamental forest tree that can reach heights of 30m. It grows in dense shade and may be recognized by its bright yellow slash and conspicuous black fruits (Vivien and Faura, 1985) [29]. It is located in the West African region and extends from southern Nigeria to Gabon, Zaire and Angola. It is commonly called African white wood, Moambe Jaune and Annikia chlorantha. Locally, the name varies from place to place. The Ibios of Akwa Ibom call it Uno eto, the Yoruba's call it Osupupa or dokita Igbo. The Edo people refer to it as Erenbav bogo while Ikale and Boki tribes refer to it as Osumolu and Kakerim respectively. The family is Annonaceae and the specie is chlorantha. There are over 40 different species that grow in Nigeria as is seen by the various names that it is called and the varied uses to which it is applied. Enantia chlorantha is used to treat a wide variety of conditions using its roots, stem bark, fruit and leaves. In Akwa Ibom it is used to treat malaria fever, typhoid fever, jaundice, dysentery, wounds, infections, high blood pressure and many other related illnesses. It has been used also as anti-viral, anti-candidal and for gastroenteritis (Moody *et al*, 1995) [18], (Gill and Akinwunmi, 1986) [9]. A decoction of the stem bark in illicit gin is usually taken to relieve painful and swollen joints, fever, headache and toothache. A literature search showed that the plant has been worked on by many investigators who have examined different aspects of the plant. We aim by this work to use other models in the hope that the mechanism of action of the plant may be inferred.

2. Materials and Methods

2.1 Plant material

The plant *Enantia chlorantha* was collected in January 2012 in Uyo the capital city of Akwa Ibom State, Nigeria. It was identified and authenticated by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom state Nigeria. A voucher specimen (voucher number UUH 018/13) was deposited with the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

2.2 Extraction

The plant stem bark was washed and partially air dried for about 2 weeks at ambient temperature ($25^0C \pm 1^0C$). It was then ground to powder using pestle and mortar. The pulverized sample was divided into two parts. One part was cold macerated in 70 % ethanol at room temperature for 72 hr and then filtered. The filtrate was dried in a rotary evaporator at 40 ^0C. This extract is referred to as crude. The other part was successively and gradiently macerated for 72 hr at room temperature in the following solvents: n-hexane, chloroform, ethyl acetate, methanol and water to obtain different fractions. The crude extract and the fractions were stored in the freezer at -4^0C until required.

2.3 Acute toxicity study of the extract

The method of Lorke (1983) was used to determine the LD_{50} of the extract in Swiss albino mice. The extract was administered to three groups of mice containing three mice each at a dose range of 100-1000 mg/kg, (i.p). The animals were observed for physical signs of toxicity and the number deaths in each group within 24 h were recorded. The animals were fasted for 24 h prior to the experiment but allowed water *ad libitum*. The LD_{50} was calculated as the geometric mean of the maximum dose producing 0 % mortality (A) and the minimum dose producing 100 % mortality (B). $LD_{50} = \sqrt{AB}$

2.4 Phytochemical screening

The phytochemical screening of the extract was carried out according to the methods of [Odebiyi and Sofowora, 1978] [19], [Trease and Evans, 1996] [28]. The following bioactive compounds were screened for their presence: saponins, tannins, and alkaloids. Others were flavonoids, antraquinones, cardiac glycosides and reducing sugars.

2.5 Animal stock

Swiss albino wistar rats weighing 150-180 g of both sexes were used for the experiments. They were obtained from the Department of Pharmacology Animal House in the University of Uyo, Uyo. The animals were housed in standard plastic cages at room temperature and moisture under naturally illuminated environment of 12:12 h dark/light cycle. They were fed with pelleted feeds (Bendel Feeds) and allowed water *ad libitum.*

3. Evaluation of Anti-Malarial Activity

3.1 Parasite Inoculation

A parasitized blood donor of Swiss albino mice with at least 1.0 x 10^7 parasitized red blood cells was anaesthetized with chloroform and blood collected through a cardiac puncture using a sterile syringe and a heparinized bottle. Each mouse was given 0.2 ml of infected blood intraperitoneally. Each inoculum contained at least 1.0 x 10^7 Plasmodium berghei berghei infected erythrocyte per ml. This was prepared by determining both the percentage parasitaemia and the erythrocyte count of the donor mouse and diluting the blood with isotonic saline (Odetola and Basir, 1980) [20].

3.2 Determination of Suppressive Activity on Early Infection (4-day Test)

The suppressive activity of the extract was evaluated using the method of Knight and Peters (1980) [14]. An intraperitoneal inoculation of each mouse was done on the first day (day 0) using 0.2 ml of infected blood containing 1.0 x 10^7 Plasmodium berghei berghei parasitized erythrocytes. The animals were then randomly divided into six groups of 6 animals each. Ten minutes after the inoculum was given, mice in group 1 were administered with distilled water (10 ml/kg, p.o.). Groups 2-4 were administered with 32.40, 64.80 and 96.20 mg/kg, (i.p.) of the extract respectively. The fifth group received artesunate (5 mg/kg, i.p.) while group 6 was administered with artesunate (5 mg/kg, i.p.) and then 10 min later, the extract (64.40 mg/kg) was given. Administration of extract and drug was continued for 4 days (D0-D3) between 8.00am and 9.00am. On the fifth day (D4), a thin blood smear was taken from the tail of each mouse. The films then stained with Leishman's stain and examined under high power field. The percentage parasitaemia was determined by counting the number of parasitized red blood cells in a minimum of 500 erythrocytes in random high power fields of the microscope.

Percentage (%) parasitaemia =No. of parasitized RBC/Total no. of RBC counted x 100/1

Average Percentage (%) chemosuppression was calculated as A-B/A x 100.

Where A is the average parasitaemia in the negative control group and B is the average Percentage (%) parasitaemia in the test group.

3.3 Determination of Repository (Prophylactic) Activity

The method of Peters (1965) [22] was adopted for the test. Six groups of Swiss albino mice were randomly selected with six mice per group. Mice in group 1 were administered with distilled water (10 ml/kg, p.o.). Groups 2-4 were administered with 32.40, 64.80 and 96.20 mg/kg, (i.p.) of the extract respectively. Group 5 received pyrimethamine, 1.2 mg/kg and served as positive control. Group 6 received 1.2 mg/kg of pyrimethamine and middle doses of extract (64.80 mg/kg). The animals were pre-treated with the extract for three consecutive days (D0-D2). On the fourth day, the mice were inoculated intraperitoneally with 0.2 ml of infected blood containing 1.0 x 10^7 Plasmodium berghei berghei parasitized erythrocytes. Tail blood was taken and parasitaemia evaluated by thin blood smear and microscopy after 72 h. The percentage parasitaemia and average chemo suppression were calculated as shown in section (3.2) above.

3.4 Determination of Effect of Extract on Established Infection (Curative Test)

The method of Ryley and Peters (1970) [25] was used to determine schizonticidal activity of the extract in the presence of established infection. Thirty six mice were inoculated intraperitoneally with 1.0x10^7 *Plasmodium berghei berghei* infected erythrocytes on day 1 (D0). 72 h later, the animals were randomly selected and divided into 6 groups of 6 mice per group. Mice in group 1 were administered with distilled water (10 ml/kg, p.o.) as negative control. Groups 2-4 were administered with 32.40, 64.80 and 96.20 mg/kg, (i.p.) of the extract respectively. Group 5 received 5 mg/ kg of artesunate and served as positive control. Group 6 received artesunate (5 mg/kg). Ten minutes later, the extract (64.80 mg/kg) was

administered.
The extract was administered once daily for 5 days. Thin smears were made from tail blood and stained with Leishman's stain to monitor the level of parasitaemia. The mean survival time of each group was determined over a period of 30 days (D0-D29) by evaluating the average survival time for each group.

3.5 Evaluation of Anti-plasmodial Activity of the Fractions using the 4-day Test
Different fractions of Enantia chlorantha (n-hexane, chloroform, ethyl acetate, methanol and aqueous) were administered orally to the animals. The rats were divided into six groups of six rats per group. A single dose of the fractions (64.80 mg/kg) was administered to each group. Groups 1-3 were administered with n-hexane, chloroform, and ethyl acetate respectively while groups 4 and 5 were given methanol and aqueous fractions equally. Group 6 was given distilled water (10 ml/kg, p.o.).
After 30 min, 0.2 ml of infected blood containing about $1.0x10^7$ Plasmodium berghei berghei was administered intraperitoneally. The administration of fractions and distilled water was done for 4 days. Thin blood films were made on day 5 from tail blood and the level of parasitaemia evaluated by counting the number of parasitized erythrocytes per random high power field of not less than 500 red blood cells. The mean survival time was determined over a period of 30 days. The percentage chemo suppression was then calculated.

3.6. Evaluation of Anti-pyretic Activity of Extract in Rats
3.6.1 Effect of Extract on 2, 4-Dinitrophenol (DNP)-Induced Pyrexia in Rats
Adult albino rats (150-180 g) of both sexes were used for this experiment. The animals were fasted for 24 h but allowed access to water ad libitum. The basal rectal temperature was taken before administration of any drug. DNP (10 mg/kg) was given to all animals intraperitoneally (i.p). Hyperthermia was induced at least 30 min after the administration of DPN. Animals with increase in temperature were selected and randomized into 5 different groups of 6 rats per group. However, those that showed temperature greater than 20C were excluded. Group1 served as control and received 10ml/kg of distilled water orally. Groups 2, 3 and 4 were administered with 32.40, 64.80 and 96.20 mg/kg, (i.p.) respectively. Group 5 animals received ASA (100 mg/kg, p.o.). Rectal temperatures of all the groups were then taken at hourly intervals for 5 hr. (Winter *et al*., 1962; Blackhouse, Delporte, Negrete, Munoz, and Ruiz, 1994; Mbagwu *et al*., 2007) [30, 6, 17].

3.6.2 Effect of Extract on D-amphetamine-induced Pyrexia in Rats
Adult albino rats of both sexes were used for this study. They were fasted for 24 hr and allowed access to water ad libitum. The basal rectal temperatures of the animals were taken. Amphetamine (5 mg/kg, i.p.) was given and hyperthermia developed 30 min after administration. Rectal temperatures were taken within 30min of receiving amphetamine. Those with 10C rise in temperature were selected for the study. Thereafter, they were randomized into six groups of 6 rats each. Group 1 was given 10 ml/kg, (p.o.). Groups 2-4 were administered the extract (32.40, 64.80 and 96.20 mg/kg, i.p.) respectively. Group 5 animals received middle dose of extract and 10 min later, acetyl salicylic acid (ASA, 100 mg/kg, p.o.) was administered. Group 6 was administered with ASA (100 mg/kg, p.o.). Rectal temperatures were then taken hourly for 5 h (Bamgbose and Noamesi, 1981; Blackhouse *et al*., 1994; Mbagwu *et al*., 2007) [5, 6, 17].

3.6.3 Effect of Extract on Yeast-induced Pyrexia in Rats
Adult albino rats (150-180 g) of both sexes were fasted for 24 h and were allowed access to water ad libitum. They were randomized into 6 groups of 6 animals each. The basal rectal temperatures were determined. Aqueous suspension of Brewer's yeast (10 ml/kg of 15 % w/v) was administered subcutaneously. Rectal temperatures were then taken at hourly intervals and animals with a 10^0C rise in temperature were selected for the experiment. The extract was administered at doses of 32.40, 64.80 and 96.20 mg/kg, (i.p.) to animals in groups 2-4 respectively. Group 1 received 10 ml/kg, (p.o.) of distilled water while group 6 was administered acetylsalicylic acid (100 mg/kg, i.p.). Group 5 animals received middle dose of extract and 10 min later, ASA (100 mg/kg, p.o.) was administered (Agrawal and Paridhavi, 2007) [2].

3.7 Statistical analysis
Results were expressed as multiple comparisons of mean ± SEM. Significance was determined using One-way Analysis of Variance (ANOVA) followed by Turkey-Kramer multiple comparison post test. A probability level of less than 5 % was considered significant.
4.0 Results/Discussions

4. Evaluation of Anti-plasmodial Activity
The result of suppressive activity of the extract is shown in Table1. The extract produced a dose-dependent chemo suppressive effect at the different doses employed in the study. The percentage chemo suppressions were 47.35, 56.37 and 76.60 % for 32.40, 64.80 and 96.20 mg/kg/day doses respectively. These effects were statistically significant (p<0.001) when compared to control. The effects of the various doses of the extract were incomparable to that of artesunate, 5 mg/kg .

4.1 Repository/Prophylactic Activity of Extract
The results of prophylactic activity of extract is as shown in Table 2.The extract exerted a dose-dependent repository activity at the various doses employed resulting in significant (p<0.001) reduction of parasitaemia in extract treated groups when compared to control. Chemo suppressive effects of 30.23, 49.05 and 49.05 % were respectively recorded for 32.40, 64.80 and 96.20 mg/kg/day doses. The chemo suppressions exerted by the different doses of the extract were not as high as that of the standard drug, pyrimethamine with chemo suppression of 79.46 %.

4.2 Effect of Extract on Established Infection in Mice (Curative Test)
The effect of extract on established infection of P. berghei is as shown in Figure 1. There was a dose–dependent reduction in parasitaemia in the extract-treated groups similar to that of the artesunate treated group. The reductions were statistically significant (p<0.001) when compared to control. The control group showed daily increase in parasitaemia. On Day7, the percentage chemo suppression for the groups were 77.72, 79.70, 85.07, 88.76 and 91.21 for 32.40 mg/kg, 64.80 mg/kg, 96.20 mg/kg, extract (64.80) plus artesunate (5 mg/kg),and artesunate groups respectively (figure 1).

4.3 Effect of Extract on Mean Survival Time

The result of mean survival time (MST) is shown in Table 3. The MST of the extract treated groups were significantly (p<0.01) longer than that of the control though less than that of the artesunate treated group. Artesunate treated group had MST value of 30.00 days.

Table 1: Suppressive activity of extract in *Plasmodium berghei berghei* infected mice

Drug Extract	Dose mg/kg	Parasitaemia	% Chemosupprression
Distilled water	10 ml	55.00 + 1.06	___
Extract	32.40	39.00 + 1.06[a]	29.09
Extract	64.80	32.33 ± 0.56 [a]	41.21
Extract	96.20	22.00 ± 0.68[a]	60.00
Extract+ Artusenate	64.80 + 5.0	7.50 ± 0.76 [a]	86.36
Artesunate	5.0	6.00 ± 0.58 [a]	89.09

Values are expressed as mean ±SEM Significance related to control: [a]p<0.001(n=6).

Table 2: Repository activity of extract

Drug/Extract	Dose (mg/kg)	Parasitaemia	% Chemosuppression
Distilled water	10 ml	87.66 ± 0.84	_
Extract	32.40	61.66 ± 0.95[a]	30.23
Extract	64.80	44.66 ± 0.88 [a]	49.05
Extract	96.20	44.66 ± 0.88 [a]	49.05
Pyrimethamine +	1.20	29.85 ± 0.95 [a]	65.97
Extract Pyrimethamine	1.20 + 64.80	18.00 ± 0.82 [a]	79.46

Values are expressed as mean ± SEM
Significance relative to control: [a]p<0.001(n=6).

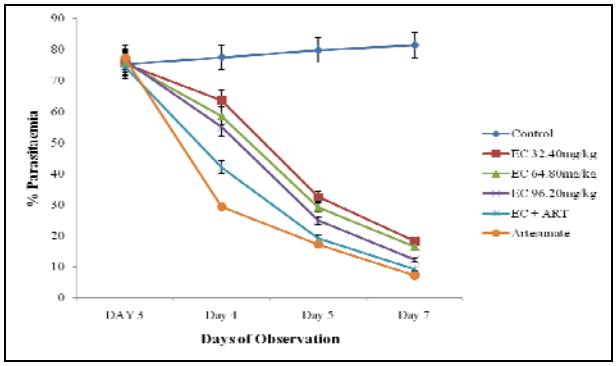

Fig 1: Curative effect of extract on *Plasmodium berghei berghei* parasitized mice

Table 3: Mean survival time (MST) of mice receiving varying doses of extract.

Drug/Extract	Dose (mg/kg)	MST (Days)
Distilled water	10ml	8.33 ±2.58
Extract	32.40	15.80 ± 2.50[a]
Extract	64.80	20.50 ±1.41 [a]
Extract	96.20	24.57 ± 2.25 [a]
Artesunate	5.00	30.00 ± 0.00 [a]
Artesunate + Extract	5.00 + 64.80	28.35 ± 0.57 [a]

Values are expressed as mean ±SEM
Significance relative to control: [a]p<0.001(n=6).

4.4 Anti-plasmodial Activity of Fractions of Extract

The results of suppressive activity of the various fractions of the extract are shown in Table 4. N-hexane, chloroform, ethyl acetate and methanol fractions exerted degrees of antiplasmodial activity. The chemosuppressive activities of the fractions were 61.24, 73.68, 77.01 and 77.45 % n-hexane, chloroform, ethyl acetate and methanol respectively. However, methanol fraction had the highest activity.

Table 4: Anti-plasmodial activities of extract fractions (4-day Test)

Drug/Extract	Dose (mg/kg)	Parasitaemia	% Chemosuppression
distilled water	10 ml	45.80 ± 0.71	_
n-hexane	64.80	17.75 ±0.65[a]	61.24
chloroform	64.80	12.05 ± 0.74 [a]	73.68
ethyl acetate	64.80	10.35 ± 1.82 [a]	77.01
methanol	64.80	10.15 ± 0.92 [a]	77.45

Values are expressed as mean ± S.E.M.
Significance relative to control: [a]p<0.001: n=6

4.5 Anti-pyretic Activity

4.5.1 Effect of Extract on Dinitrophenol-Induced Pyrexia in Rats

The anti-pyretic effect of the extract on 2, 4 dinitrophenol (DNP)-induced pyrexia is as shown in Table 5. Administration of the extract (32.40, 64.80 and 96.20 mg/kg) in the presence of the pyrogen caused a significant (p<0.05 – 0.001) reduction in the temperatures of the extract treated rats when compared with the control. The anti-pyretic effect was dose-dependent though not as high as that of the standard drug, ASA (100 mg/kg).

4.5.2 Effect of Extract onAmphetamine–induced Pyrexia in Rats

Table 6 shows the effect of the extract on amphetamine-induced pyrexia. The extract exerted a significant (p<0.05–0.001) dose-dependent anti-pyretic effect especially in fourth and fifth hours when compared to control.

4.5.3 Effect of Extract on Yeast-Induced Pyrexia in Rats

The result of the effect of the extract against yeast-induced pyrexia is as shown in Table 7. The extract caused considerable and sustained reductions in temperature change in rats treated with the extract. These reductions were statistically significant (p<0.05–0.001) relative to control.

Table 5: Effect of extract on dinitrophenol-induced pyrexia in rats

Treatment/ Dose (mg/kg)	Time Interval				
	Initial Temperature ◦c	Temp (◦c) 30 min after Induction	60 min	90 min	120 min
Control	36.94± 0.05	38.35 ± 0.16	38.19 ± 0.17	38.25 ± 0.16	38.17 ± 0.17
Extract 32.40	36.96± 0.06	38.25 ± 0.16	38.00 ± 0.19	37.56 ± 0.12 [c]	37.23 ± 0.08 [a]
64.80	36.94± 0.05[a]	38.25 ± 0.16[a]	37.73±0.06[a]	37.20 ± 0.07 [a]	37.03±0.06[a]
96.20	37.01± 0.04[a]	38.46 ± 0.13[a]	37.62±0.08[a]	37.25 ± 0.10 [a]	37.00± 0.07 [a]
ASA 100	36.96±0.06b	38.25 ± 0.16[a]	37.35±0.19[b]	37.03 ±0.02[a]	36.36±0.05[b]
ASA 100+ 64.80 mg/kg	36.91±0.12[a]	38.24 ± 0.17[b]	37.50±0.10[a]	37.10±0.02[a]	36.86±0.05[a]

Data are expressed as mean ± SEM.
Significant at [a]P<0.05, [b]P<0.01 when compared to control (n = 6).
ASA = Acetyl salicylic Acid

Table 6: Effect of extract on amphetamine-induced pyrexia in rats

Treatment/ Dose (mg/kg)	Time Intervals (h)							
	Basal Temperature	0	0.5	1	2	3	4	5
Control	36.10± 1.70	37.86 ± 0.80	38.04 ± 0.08	38.28 ± 0.08	38.43 ± 0.08	38.45 ± 0.06	38.40 ± 0.10	37.80 ± 0.10
Extract 32.40	36.48± 1.80[b]	37.55 ± 0.08[a]	37.58 ± 0.04[a]	37.57 ± 0.03[a]	37.40±0.04 [a]	36.85±0.07 [b]	36.65±0.07 [b]	36.65 ± 0.03 [b]
64.80	36.60± 0.80[b]	37.60± 0.07[b]	37.50 ± 0.10 [a]	37.20 ± 0.05 [b]	36.85±0.10 [b]	36.70±0.05[b]	36.60±0.05 [b]	36.48 ± 0.05 [b]
96.20	36.15±0.60[b]	37.48 ± 0.04[b]	37.30± 0.05 [b]	37.15± 0.04 [b]	36.80±0.05 [b]	36.50±0.05 [b]	36.35±0.04 [b]	36.20± 0.05 [b]
ASA 100	36.58±0.80[c]	37.55 ± 0.05[b]	37.50 ± 0.05[b]	37.30±0.04[b]	36.85±0.05[b]	36.60±0.05[b]	36.40±0.05[b]	36.20 ± 0.05[b]

Data are expressed as mean ± SEM.
Significant at [a]p<0.05, [b]p<0.01, [c]p<0.001 when compared to control (n = 6).
ASA = Acetyl salicylic Acid

Table 7: Effect of extract on brewer's yeast-induced pyrexia in rats

Treatment/ Dose (mg/kg)	Time Interval (h)						
	Basal Temperature	0.5	1	2	3	4	5
Control	36.50± 0.17	37.73 ± 0.25	36.47 ± 0.08	36.33 ± 0.01	36.43 ± 0.02	36.66 ± 0.13	36.87 ± 0.01
Extract 32.40	36.00± 0.16[a]	37.57 ± 0.01[c]	34.40 ± 0.01 [c]	34.93 ± 0.24 [c]	34.13 ± 0.02 [c]	33.23 ± 0.02 [c]	32.00 ± 0.18 [c]
64.80	35.83± 0.11[a]	37.23 ± 0.03[c]	34.53 ± 0.02 [c]	33.63 ± 0.03 [c]	33.13±0.05[c]	33.07 ± 0.27 [c]	32.90 ± 0.03 [c]
96.20	36.67±0.06[c]	37.33± 0.02[c]	33.73 ± 0.02 [c]	32.93 ± 0.02 [c]	32.83± 0.03 [c]	31.83± 0.02 [c]	31.47± 0.01 [c]
ASA 100	37.70±0.20c	38.37 ± 0.03[b]	35.93 ± 0.01[a]	35.03±0.02[c]	35.00±0.28[c]	34.13±0.02[c]	33.00 ± 0.03[c]
ASA 100+ 64.80 mg/kg	36.43±0.45[b]	37.07 ± 0.02[b]	34.43±0.14[b]	34.40±0.24[c]	34.33±0.20[c]	33.50±0.03[c]	32.03 ± 0.04[c]

Data are expressed as mean ± SEM.
Significant at [a]p<0.05, [b]p<0.01, [c]p<0.001 when compared to control (n = 6).
ASA = Acetyl salicylic Acid

5. Discussion

The anti-plasmodial evaluations of the extract showed a significant activity in suppressive, repository and curative models. These effects were comparable to those of the standard drugs employed in this investigation namely artesunate and pyrimethamine. Secondary metabolites such as alkaloids and terpenes like sesquiterpenes and monoterpenes have been implicated in anti-plasmodial activity of plants (Philipson and Wright, 1991) [23]; (Christensen and Kharazmi, 2001) [8]. The extract also contains protoberberine and berberine alkaloids which have been reported to be responsible for the anti-malarial activity of this plant (Kimbi *et al.,* 1996) [13]; (Tan *et al*., 2000) [26]; (Tan *et al.,* 2002) [27]. That the extract contains the above secondary metabolites suggest that the anti-plasmodial activity exhibited by the plant may in part be due to these active ingredients. The result of this study corroborates well with that of Kimbi *et al.* (1996) [13]; Tan *et al*. (2000) [26]; Tan *et al.* (2002) [27] and (Ogbonna, Sokari, and Agomoh, 2008) [21], who reported significant anti-plasmodial activity in the root of this plant. Artemisinin, sesquiterpene endoperoxide is reported to exert anti-plasmodial activity by generating reactive oxygen species

which alkylate with the heme protein resulting in the death of the parasite (Robert, Benoit-vical, Dechy-cabaret, and Meunier, 2001) [24]. Sesquiterpenes and monoterpenes like limonene have been implicated in endoperoxidation leading to plasmocidal activity (Hatzakis, Opesenica, Solaja, and Stratakis, 2007) [11]. Chloroform, ethyl acetate, n-hexane and methanolic fractions had comparative activity indicating possible localization of the active ingredients in these fractions. This study has demonstrated that the extract possess anti-malarial activity which justifies its use in traditional medicine.

On anti-pyretic activity, the extract inhibited significantly dinitrophenol, amphetamine and yeast-induced pyrexia. Dinitrophenol induces hyperthermia by uncoupling oxidative phosphorylation causing release of calcium from mitochondrial stores and also prevents calcium reuptake. This results in increased level of intracellular calcium, muscle contraction and hyperthermia (Kumar, Baker, and Seger, 2002) [15]. Yeast induces pyrexia by increasing the synthesis of prostaglandins (Al-Ghamdi, 2001) [3]. The extract may in part reduced pyrexia by reducing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-2 or by enhancement of the production of the body's own anti-pyretic substances such as vasopressin and arginine (Chandrasekharan, 2002) [7]. The hypothermic activity of the extract could have also been mediated by vasodilatation of superficial blood vessels leading to increased dissipation of heat following resetting of hypothalamic temperature control center. Diterpenes such as those present in the leaf of Croton zambesicus have been reported to possess vaso-relaxant activity (Baccelli *et al*., 2005) [4]. Therefore, the temperature lowering activity of the extract may not be unconnected with the inhibition of one or combination of the mechanisms mentioned above. This finding corroborates an earlier report by Adesokan *et al*. (2008) [1] who reported the anti-pyretic activity of the extract against brewer's yeast.

6. References

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