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In vitro antiplasmodial activity of aqueous extracts of *Ochna schweinfurthiana* leaf on *Plasmodium falciparum*

Babasoji P. Omoniwa[1*], Titilayo O. Johnson[2], Omolola Soji-Omoniwa[3], Istifanus Gurumtet[2] and Ruth A. Manzah[1]

[1]Department of Science Laboratory Technology; [2]Department of Biochemistry; University of Jos, Jos. Nigeria.
[3]Department of Biochemistry, University of Ilorin, Ilorin. Nigeria.

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Abstract
The *in vitro* antiplasmodial activities of cold and hot aqueous extracts of *Ochna schweinfurthiana* leaf was evaluated against *Plasmodium falciparum* at concentrations 10, 20, 40 and 80 µg/ml. Parasitaemia was observed after 24, 48 and 72 hours under a light microscope at X100 magnification. The antiplasmodial activity of the extracts were compared with a standard Artemisinin-based Combination Therapy (ACT), artemether/lumefantrine. Phytochemical analysis showed the presence of flavonoids (65.97 mg/100g), alkaloids (0.20 mg/100g), saponins (47.39 mg/100g) and tannins (0.53 mg/100g). There was a significant reduction ($P< 0.05$) in parasitemia of groups treated with the various doses of the extracts when compared to the control group. The reduction in parasitemia was however not as pronounced as that observed for the standard drug-treated groups. The 10, 20, 40 1nd 80 µg/ml doses of the extracts gave a percentage parasite inhibition of 79.77, 81.86, 83.38 and 86.42 (cold extract) and 76.26, 78.54, 82.72 and 85.06 (hot extract) respectively which are lower than 99.71, 99.81, 100.00 and 100.00 respectively observed for the standard drug after 72 hours. However, *in vitro* assay of plasmodium lactate dehydrogenase (pLDH) activity did not show any significant difference among the extract-treated groups and those treated with artemether/lumefantrine standard. Findings from this study show that hot and cold aqueous extracts of *Ochna schweinfurthiana* leaf possess high inhibitory activity against *Plasmodium falciparum in vitro* especially after 72 hours and justifies it folkloric use as an antimalarial.

Keywords: Antiplasmodial; *Ochna schweinfurthiana*; Extracts; Parasitemia; ACT

INTRODUCTION

Malaria is a major public health challenge in developing countries and in 2004 was arguably the number one cause of death in Nigeria accounting for 15% of hospital admission and about 50% out-patient consultation. In 2008, 109 countries, 45 of which are within the World Health Organization African region were reportedly endemic to malaria [1] putting 3.2 billion people (nearly half the world's population) at risk of malaria. In 2015 alone, 214 million new cases and 400,000 malarial-related deaths were reported. WHO [2] reported that Africa bears the heaviest burden and in 2015 accounted for approximately nine out of ten malaria cases and deaths worldwide with Nigeria and The Democratic Republic of Congo together accounting for over 35% of global malaria deaths [2].

Malaria eradication crusade has been faced with several challenges which include:

[*] Corresponding author. *E-mail*: sojiomoniwa@gmail.com *Tel*: +234 (0) 8037362620
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(1) Failure to implement the World Health Organization recommended control interventions in many malaria-endemic countries resulting to increase in malaria incidences, mortality and morbidity; (2) unavailability and high cost of antimalarial drugs to those that are mostly affected, especially in developing countries, [3,4]; and (3) *Plasmodium falciparum* malaria treatment failures with Artemisinin-based Combination Therapies (ACTs), which are the first line antimalarial drug for uncomplicated and severe malaria [5-7].

The development of reduced sensitivity to ACTs by *P. falciparum* and parasites recrudescence after treatment with ACTs have been reported and are the reasons for *Plasmodium falciparum* malaria treatment failures. Reduction of ACT's sensitivity to *P. falciparum* has been established by reports at the Thailand-Myanmar and Thailand-Cambodia borders [8,9]. It has also been reported that *P. falciparum* reducing sensitivity to ACT is spreading to Africa as well [10]. In the event of *P. falciparum* developing resistance to ACTs, there is no suitable replacement for ACTs. Therefore, the lack of new antimalarial drugs in the pipeline highlight the urgent need to search for new antimalarial drugs to replace the currently used or to expand the antimalarial drug arsenal.

Researchers, especially in Africa, have turned their attention to medicinal plants. The use of plants for medicinal purposes has existed since antiquity and is still important for primary health care today [11]. It has been reported [2] that more than 80% of people living in developing countries still depend on herbal medicines for the treatment of common diseases, including malaria. Medicinal plants produce phytochemicals during metabolism, which are pharmacologically active against many human diseases [12] and have been isolated and used for the synthesis of drugs [13].

Ochna schweinfurthiana F. Hoffin belongs to the family *Ochnaceae.* It is called *Ochna* in (English) was named after Dr. August Schweinfurth (1836-1925), a German botanical collector and taxonomist. *Ochna shcweinfurthiana* is a small tree or shrub that grows about 4m in height. It grows into a decorative shrub bearing bright-yellow flowers. It is found in Africa/savannah woodlands, from Guinea to North and Southern Nigeria, across central Africa to Sudan, Uganda, Zimbabwe and Mozambique. *Ochna shcweinfurthiana* has claimed efficacy in the treatment of several illnesses. The powdered bark is used as antimalarial and anthelminthic while the decoction of the root, leaves or bark is used in dressing wounds. In Northern Nigeria, the plant is said to be used for the treatment of measles, typhoid fever and fungal skin infections. The plant is reported to also have laxative, antiseptic, stimulant and febrifugal properties among others.

Result of preliminary phytochemical screening of the plant showed that it contains saponins, flavonoids, carbohydrates, steroids, glycosides and tannins [14].

The aim of this work was to evaluate the antiplasmodial properties of hot and cold aqueous leaf extracts of *Ochna schweinfurthiana* on *Plasmodium falciparum in vitro.*

EXPERIMENTAL

Plant material. *Ochna schweinfurthiana* leaf was collected from Old Lamingo, Jos, Plateau State, Nigeria. The plant was identified and authenticated at the Herbarium of the Department of Plant Science Technology, Faculty of Natural Sciences, University of Jos, Nigeria, where the specimen was deposited and the voucher number; UJH16000256 obtained.

Test organism. *Plasmodium falciparum* was obtained from infected blood sample of an

infected person at G-Medical and Diagnostic Centre, Jos, Plateau State, Nigeria

Chemicals and reagents. Malsat reagent (triton x-100, L-Lactate, Tris Buffer, APAD (3-acetyl pyridine adenine dinucleotide) and Nitro blue tetrazolium/phenazine ethosulfate (NBT/PES) were purchased from Santa Cruz Biotechnology Ltd, Heidelberg, Germany. RPMI 1640 was a product of Sigma-Aldrich Inc. (Germany). Other reagents were of analytical grade and were prepared in all glass distilled water.

Preparation of cold and hot water extracts of *Ochna schweinfurthiana* leaf. The leaves of *Ochna schweinfurthiana* were dried to constant weight in an oven at 45^0C. The dried leaves were pulverized using an electric blender. Sixty grams (60 g) of the powdered leaf was weighed into two beakers each and labelled A and B. The contents of A and B were then percolated with 500 ml of distilled water at room temperature and 500 ml of boiling distilled water respectively and stirred thoroughly. The mixtures were allowed stand until the content of beaker B had returned to room temperature. The beakers were then left to extract for 48 hours in a refrigerator. The mixtures were then filtered using a Whatman No.1 filter paper and filtrates freeze-dried. The extracts were reconstituted into the doses used in this research.

Phytochemical analyses. Qualitative phytochemical screening was carried out on the aqueous extracts of *Ochna schweinfurthiana* leaf. Alkaloids, tannins, phlobatannins, cardiac glycosides and anthraquinones were screened by the methods described by [15]. The method of [16] was employed detecting the presence of phenolics and flavonoids while saponins were detected using the method described by [17]. Some of the detected phytochemical were quantified using standard procedures.

***In vitro* antiplasmodial activity assessment.** The malaria parasites (*P. falciparum*) were hosted within erythrocytes (from O^+ donor) in 50ml of RPMI 1640 solution supplemented with 10 ml of 0.5% Albumax II. The broad-spectrum antibiotic, Gentamycin (0.1 ml). Already prepared RPMI 1640 culture media (100 µl) was pipetted into different wells followed by 50 µl of already prepared blood sample. Already prepared plant extracts (100 µl) of different concentrations were distinctly pipetted into these wells. The antiplasmodial assay was carried out at concentrations 10, 20, 40 and 80 µl/ml of the extracts and Reference drug. The control wells did not include the extracts. The microtitre plate was then incubated in an anaerobic jar at 37°C. Microscopy was done to observe the morphology of the parasites and to determine the average percentages of parasitaemia after 24, 48 and 72 hours. Thick/thin film smears of incubated samples were made on well labelled dry grease free slides by picking Red Blood Cells from each culture well at each interval of 24, 48 and 72 hours and allowed to air dry. Immediately smears are made, cultures are maintained by adding 50 µl of RPMI 1640 medium to avoid drying and ensure continuous and proper parasite growth.

Percentage parasitaemia. Parasitaemia was expressed as percentage of total infected red blood cells over the total non-infected red blood cells (total number of red blood cells) were counted for each smear. The average parasitaemia of the smear replicates for each concentration was calculated. Enough fields were counted. The percentage parasitaemia in each field was calculated using the formula:

$$\%\ Parasitemia = \frac{\text{Number of infected Red Blood Cells X 100\%}}{\text{Total Red Blood Cells counted}}$$

Percentage inhibition of *P. falciparum*. The percentage inhibition of the parasites was calculated using the formula as follows:

$$\%\ Parasite\ Inhibition = \frac{(\%\ \text{Parasitemia of control} - \%\ \text{Parasitemia of test})\ \text{X 100\%}}{\%\ \text{Parasitemia of control}}$$

Median inhibition concentration (IC_{50}). The IC_{50} values, concentrations required to inhibit parasite growth by 50 %, were determined by

linear regression. A graph of Percentage Plasmodium Inhibition (on y-axis) was plotted against Extract Doses (on x-axis). The IC_{50} values were then intrapolated from the dose-response curves using the formula:

$$IC_{50} = a*X + b$$
$$\text{Therefore } (0.5 - b) / a = X$$

Where a and b represent the slope and intercept of the linear regression curve respectively.

***In vitro* evaluation of *Plasmodium* lactate dehydrogenase activity.** Plasmodium Lactate dehydrogenase assay was carried out in 96-well microtitre plates as described by [18] with some modifications [19]. Prepared RPMI 1640 culture media (100 µL) was added to the 96 well plate, after which 50 µL of malaria infected blood sample was added to the prepared culture media and finally 100 µL of plant extracts were added. The microtiter plate was incubated for 72 hours. 100 µL of Malstat reagent was added to a new microtitre plate thereafter, the culture in each well was mixed and 20 µL of the culture taken from each well and added to the corresponding well of the Malstat plate, 25 µL of NTB/PES were then added to each well, thereby initiating the lactate dehydrogenase reaction. Colour development of the LDH plate was monitored and the absorbance was taken at 650 nm with the aid of a plate reader after 45 minutes of incubation in the dark. Percentage pLDH activity and Percentage pLDH inhibition as follows:

$$\% \text{ activity} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Percentage inhibition (%) = 100 – Percentage activity

Statistical Analysis. Data are expressed as mean of five replicates± Standard Deviation (SD). The obtained data were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) software. All significant differences were determined by one-way analysis of variance (ANOVA) and Post Hoc multiple comparison was done using Duncan's multiple range test. Significance level was set at $p< 0.05$. IC_{50} was determined by linear regression analysis.

RESULTS

The results for qualitative and quantitative secondary metabolite analyses are presented in Tables 1 and 2. Alkaloids, flavonoids, tannins, saponins, balsams, phenols, terpenes and steroids were detected in the aqueous extracts of *Ochna schweinfurthiana* leaf while resins and anthraquinones were absent (Table 1). We were able to quantify only four of the secondary metabolites detected. Flavonoids had the highest concentration at 65.97 mg/100 g while alkaloids were lowest at 0.2 mg/100 g. Saponins and tannins had concentrations of 47.39 and 0.53 mg/100 g respectively (Table 2).

The *in vitro* effect of *Ochna schweinfurthiana* leaf aqueous extracts on the parasitemia of *Plasmodium falciparum* is presented in Table 3. After 24 hours, there was a significant increase in the parasite count of the control group when compared to the treated groups. Groups that received the standard drug (artemether-lumenfantrine) at different doses showed a drastic significant decrease ($p < 0.05$) in parasitemia when compared with the control and the extract-treated groups. The significant decrease ($p < 0.05$) in parasitemia observed in the extract-treated groups was dose-dependent. However, there was no significant difference ($p < 0.05$) in the parasitemia of groups treated with the 20 µg/ml dose of the cold aqueous extract and 80 µg/ml dose of hot aqueous extract. Similar results were obtained at 48 and 72 hours. Overall, the anti-plasmodial effects of the standard drug and the extracts was both dose and duration dependent.

Table 4 represents the *in vitro* effect of *Ochna schweinfurthiana* leaf aqueous extracts on percentage parasitemia of *Plasmodium falciparum.* The percentage parasitemia of the control group increased with time

progression. However, treatment with the standard drug and the extracts caused a significant decrease ($p < 0.05$) in the percentage parasitemia in all the treated groups in a dose-dependent manner. There was no significant difference ($p < 0.05$) in the percentage parasitemia of the groups treated with the 20 and 40 µg/ml of the cold aqueous extract after 24 hours.

Table 5 shows *in vitro Plasmodium falciparum* percentage inhibition by *Ochna schweinfurthiana* leaf aqueous extracts. There was a significant decrease ($p < 0.05$) in the percentage parasite inhibition of the extract-treated groups when compared with the Artemether-lumenfantrine-treated groups at 24, 48 and 72 hours. Among the extract-treated groups, the percentage parasite inhibition significantly increased ($p < 0.05$) with increase in dose and duration of treatment. Nevertheless, there was no significant difference ($p < 0.05$) in the percentage parasite inhibition of the groups treated with the 20 µg/ml dose of the cold aqueous extract and 80 µg/ml of hot aqueous extract after 24 hours and also in the groups treated with the 10 µg/ml dose of the cold aqueous extract and 80 µg/ml dose of the hot aqueous extract after 48 hours.

The IC_{50} concentrations of *Ochna schweinfurthiana* leaf aqueous extracts on *Plasmodium falciparum* is presented in Table 6. Artemether-lumenfantrine showed an IC_{50} of 0.29, 0.28 and 0.28 µg/ml after 24, 48 and 72 hours respectively. These values are much lower than those observed with the cold aqueous extract (0.75, 0.56 and 0.34) and the hot aqueous extract (1.02, 0.70 and 0.34). After 72 hours, the IC_{50} values for the cold and the hot aqueous extracts were comparable.

Table 7 and 8 represent the *in vitro* effect of *Ochna schweinfurthiana* leaf aqueous extracts on percentage plasmodium Lactate dehydrogenase (pLDH) activity and percentage plasmodium Lactate dehydrogenase activity inhibition respectively. There was no significance difference ($p < 0.05$) in the percentage pLDH activity and inhibition of the extracts-treated groups when compared with the artemether-lumenfantrine-treated groups.

Table 1: Secondary metabolites constituent of *Ochna schweinfurthiana* leaf

Secondary metabolites	Status
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Terpenes and steroids	+
Balsam	+
Phenol	+
Resins	-
Anthraquinones	-

Key: += Present, - = Absent

Table 2: Secondary metabolites concentration in *Ochna schweinfurthiana* leaf

Secondary metabolites	Concentration
Flavonoids	65.97 mg/100 g
Alkaloids	0.20 mg/100 g
Saponins	47.39 mg/100 g
Tannins	0.53 mg/100 g

Table 3: *In vitro* effect of *O. schweinfurthiana* leaf extracts on parasitemia (count) of *Plasmodium falciparum*

Treatment	Dose	24 h	48 h	72 h
Control	-	$196.00^{a} \pm 1.00$	$223.00^{a} \pm 1.00$	$351.00^{a} \pm 2.64$
Artemether-Lumefantrine (µg/ml)	10	$4.67^{b} \pm 0.58$	$2.67^{b} \pm 0.58$	$1.00^{b} \pm 0.00$
	20	$2.33^{c} \pm 0.58$	$1.33^{c} \pm 0.18$	$0.67^{c} \pm 0.13$
	40	$2.00^{c} \pm 0.00$	$0.67^{d} \pm 0.19$	$0.00^{d} \pm 0.00$
	80	$0.67^{d} \pm 0.18$	$0.00^{e} \pm 0.00$	$0.00^{d} \pm 0.00$
Cold Water Extract (µg/ml)	10	$140.67^{e} \pm 1.15$	$131.00^{f} \pm 1.00$	$71.00^{e} \pm 1.73$
	20	$135.67^{f} \pm 1.53$	$121.67^{g} \pm 0.58$	$63.67^{f} \pm 1.52$
	40	$127.33^{g} \pm 0.58$	$113.33^{h} \pm 0.58$	$58.33^{g} \pm 1.15$
	80	$113.33^{h} \pm 1.53$	$104.33^{i} \pm 1.53$	$47.67^{h} \pm 1.15$
Hot Water Extract (µg/ml)	10	$163.67^{i} \pm 1.15$	$158.67^{j} \pm 0.58$	$83.33^{i} \pm 1.53$
	20	$150.33^{j} \pm 0.58$	$141.33^{k} \pm 0.58$	$75.33^{j} \pm 0.58$
	40	$143.67^{k} \pm 1.15$	$125.33^{l} \pm 0.15$	$60.67^{k} \pm 0.58$
	80	$137.67^{f} \pm 0.58$	$118.00^{m} \pm 1.00$	$53.67^{l} \pm 2.08$

Values are Mean ± SD (n=5). Values with different superscripts on a column are significantly different ($p < 0.05$)

Table 4: *In vitro* effect of *O. schweinfurthiana* leaf extracts on % parasitemia of *Plasmodium falciparum*

Treatment	Dose	24 h	48 h	72 h
Control	-	$66.21^{a} \pm 0.34$	$76.11^{a} \pm 0.34$	$121.46^{a} \pm 0.92$
Artemether-Lumefantrine (µg/ml)	10	$1.58^{b} \pm 0.20$	$0.91^{b} \pm 0.20$	$0.35^{b} \pm 0.01$
	20	$0.79^{c} \pm 0.19$	$0.45^{c} \pm 0.07$	$0.23^{c} \pm 0.01$
	40	$0.68^{c} \pm 0.01$	$0.23^{d} \pm 0.01$	$0.00^{d} \pm 0.00$
	80	$0.23^{d} \pm 0.20$	$0.00^{e} \pm 0.00$	$0.00^{d} \pm 0.00$
Cold Water Extract (µg/ml)	10	$47.53^{e} \pm 0.40$	$44.71^{f} \pm 0.34$	$24.57^{e} \pm 0.60$
	20	$45.83^{f} \pm 0.52$	$41.71^{g} \pm 0.20$	$22.03^{f} \pm 0.53$
	40	$45.83^{f} \pm 0.19$	$38.68^{h} \pm 0.20$	$20.19^{g} \pm 0.40$
	80	$38.29^{g} \pm 0.51$	$35.61^{i} \pm 0.52$	$16.49^{g} \pm 0.40$
Hot Water Extract (µg/ml)	10	$55.29^{h} \pm 0.39$	$54.15^{j} \pm 0.20$	$28.83^{i} \pm 0.53$
	20	$50.79^{i} \pm 0.19$	$48.23^{k} \pm 0.20$	$26.07^{j} \pm 0.20$
	40	$48.54^{j} \pm 0.40$	$42.77^{l} \pm 0.39$	$14.62^{k} \pm 0.93$
	80	$45.84^{f} \pm 0.20$	$40.27^{m} \pm 0.30$	$18.57^{l} \pm 0.72$

Values are Mean ± SD (n=5). Values with different superscripts on a column are significantly different ($p < 0.05$)

Table 5: *In vitro Plasmodium falciparum* percentage inhibition by *Ochna schweinfurthiana* leaf aqueous extracts

Treatment	Dose	24 h	48 h	72 h
Artemether-Lumefantrine (µg/ml)	10	$97.48^{a} \pm 0.45$	$98.81^{a} \pm 0.26$	$99.71^{a} \pm 0.29$
	20	$98.81^{b} \pm 0.29$	$99.40^{b} \pm 0.25$	$99.81^{a} \pm 0.20$
	40	$99.31^{bc} \pm 0.57$	$99.70^{bc} \pm 0.26$	$100.00^{a} \pm 0.00$
	80	$99.66^{c} \pm 0.29$	$100.00^{c} \pm 0.00$	$100.00^{a} \pm 0.00$
Cold Water Extract (µg/ml)	10	$28.21^{d} \pm 0.35$	$41.26^{d} \pm 0.62$	$79.77^{b} \pm 0.56$
	20	$31.12^{e} \pm 0.95$	$45.43^{e} \pm 0.37$	$81.86^{c} \pm 0.37$
	40	$35.03^{f} \pm 0.44$	$49.18^{f} \pm 0.13$	$83.38^{d} \pm 0.32$
	80	$42.17^{g} \pm 0.49$	$53.22^{g} \pm 0.58$	$86.42^{e} \pm 0.23$
Hot Water Extract (µg/ml)	10	$16.49^{h} \pm 0.72$	$28.85^{h} \pm 0.56$	$76.26^{f} \pm 0.56$
	20	$23.29^{i} \pm 0.20$	$36.19^{j} \pm 1.05$	$78.54^{g} \pm 0.28$
	40	$26.69^{j} \pm 0.34$	$43.80^{j} \pm 0.57$	$82.72^{h} \pm 0.18$
	80	$30.77^{e} \pm 0.63$	$41.59^{d} \pm 1.54$	$85.06^{i} \pm 0.35$

Values are Mean ± SD (n=5). Values with different superscripts on a column are significantly different ($p < 0.05$)

Table 6: IC_{50} of *Ochna schweinfurthiana* leaf aqueous extracts on *Plasmodium falciparum*

Time (h)	Artemether-Lumefantrine (µg/ml)	Cold water extract (µg/ml)	Hot water extract (µg/ml)
24	0.29	0.75	1.02
48	0.28	0.56	0.70
72	0.28	0.34	0.34

Table 7: *In vitro* effect of *O. schweinfurthiana* leaf extracts on % plasmodium lactate dehydrogenase activity

Treatment	Dose	%
Artemether-Lumefantrine (µg/ml)	10	$56.27^a \pm 9.18$
	20	$48.00^a \pm 4.63$
	40	$51.62^a \pm 5.81$
	80	$50.42^a \pm 2.64$
Cold Water Extract (µg/ml)	10	$47.08^a \pm 3.47$
	20	$47.00^a \pm 4.00$
	40	$42.77^a \pm 2.00$
	80	$43.59^a \pm 6.00$
Hot Water Extract (µg/ml)	10	$48.17^a \pm 3.08$
	20	$48.76^a \pm 7.75$
	40	$50.55^a \pm 7.56$
	80	$47.12^a \pm 6.45$

Values are Mean ± SD (n=5). Values with different superscripts are significantly different ($p < 0.05$)

Table 8: *In vitro* % inhibition of plasmodium lactate dehydrogenase activity by *O. schweinfurthiana* leaf extracts

Treatment	Dose	%
Artemether-Lumefantrine (µg/ml)	10	$43.78^a \pm 9.27$
	20	$52.01^a \pm 4.63$
	40	$48.38^a \pm 5.81$
	80	$49.58^a \pm 3.00$
Cold Water Extract (µg/ml)	10	$52.75^a \pm 4.38$
	20	$53.00^a \pm 4.00$
	40	$57.23^a \pm 2.00$
	80	$56.41^a \pm 9.91$
Hot Water Extract (µg/ml)	10	$51.83^a \pm 3.08$
	20	$51.24^a \pm 5.45$
	40	$49.45^a \pm 7.56$
	80	$52.88^a \pm 8.45$

Values are Mean ± SD (n=5). Values with different superscripts are significantly different ($p < 0.05$)

DISCUSSION

The therapeutic effects of plants have been attributed to the presence of secondary metabolites [20]. Many plants with antiplasmodial/antimalarial effect have been reported to contain these secondary metabolites [21]. Three known alkaloids (i.e. (+)-*N*-methylisococlaurine, atherosperminine and 2-hydroxyathersperminine) isolated from the bark of *Cryptocarya nigra* (Lauraceae) have been reported to possess encouraging antiplasmodial activity having strongly inhibited *in vitro* growth of a chloroquine-resistant strain of *Plasmodium falciparum* (K1 strain) [22]. In another study by [23], twenty-four bisbenzyl isoquinoline alkaloids were screened for antiplasmodial activity by use of *in vitro* microtests. Eight of the alkaloids had antiplasmodial activity, with a 50 % inhibitory concentration (IC_{50}) of less than 1µM against a multidrug-resistant strain of *Plasmodium falciparum* while a further eleven showed antiplasmodial activity with IC_{50} between 1 and 10 µM. Likewise, Flavonoids from *Morus mesozygia* and *Erythrina burttii* and saponins from *Glinus*

oppositifolius have also been reported to possess antiplasmodial activity [24-26]. The antiplasmodial activity observed in *Ochna schweinfurthiana* leaf aqueous extracts may be ascribed to the presence of any of alkaloids, flavonoids, tannins, saponins, balsams, phenols, terpenes and steroids.

The results obtained for the effect of the extracts on parasitemia (as well as percentage parasitemia and parasite inhibition) revealed that the cold aqueous extract has a higher antiplasmodial activity than the hot aqueous extract. We can infer from our findings that *Ochna schweinfurthiana* leaf aqueous extracts possess antiplasmodial action with the cold aqueous extract being more effective at inhibiting *Plasmodium falciparum* proliferation than the hot aqueous extract. The antiplasmodial activity of *Ochna schweinfurthiana* leaf aqueous extracts observed in this study aligns with an earlier report by [27] who evaluated the inhibitory effect of the plant leaf extract on *Plasmodium berghei in vivo*.

According to World Health Organization guidelines, antimalarial activity of plant extracts are grouped into: (i). High activity (IC_{50} < 5 μg/ml), (ii). Promising activity (IC_{50} = 5-15 μg/ml), (iii). Moderate activity (IC_{50} = 15-50 μg/ml) and (iv). Inactivity (IC_{50} > 50 μg/ml) [28]. Our findings show that both aqueous extracts possess high activity against *Plasmodium falciparum* with the cold aqueous extract displaying a higher activity than the hot aqueous extract after 24 and 48 hours. Their activities were comparable after 72 hours. However, both extracts displayed lower activities than the standard drug (artemether-lumefantrine).

Parasite lactate dehydrogenase (pLDH) activity is used to measure the sustainability or viability of plasmodium parasites [29]. The main source of plasmodium ATP during asexual intraerythrocytic cycle is glycolysis, which translates to glucose uptake that is 30-100 times higher than those of uninfected erythrocytes [30-31]. This is reflected by a very high expression of glycolytic enzymes in plasmodium at this stage than that observed in uninfected erythrocytes [32]. A very large percentage of the uptaken glucose by plasmodium is converted to lactate by lactate dehydrogenase since there seem not to be the expression of the enzymes of the Kreb's cycle [33]. The pyruvate-lactate reaction re-oxidizes NADH to NAD^+, which allows the glyceraldehyde 3-phosphate dehydrogenase-catalysed reaction of glycolysis to proceed. The extracts were able to inhibit plasmodium lactate dehydrogenase activity in a manner comparable to the standard drug Artemether-Lumefantrine and may be one of the mechanisms by which they elicit their antiplasmodial action.

Conclusion. Findings from this study shows that cold and hot aqueous extracts of *Ochna schweinfurthiana* leaf possess high inhibitory activity against *plasmodium falciparum in vitro.* Further research therefore is recommended to discover the bioactive principle(s) responsible for the observed antiplasmodial activity.

REFERENCES

1. World Health Organisation (2008). Global malaria control and elimination. Report of a Technical Review. *WHO Publications*, Geneva, Switzerland.

2. World Health Organization (2016). Report on eliminating malaria.

3. Mutabingwa, T. K. (2005). Artemisinin-based combination therapies (ACTs): Best hope for malaria treatment but inaccessible to the needy! *Acta tropica*, *95*(3), 305-315.

4. Matowe, L., and Adeyi, O. (2010). The quest for universal access to effective malaria treatment: how can the AMFm contribute? *Malaria Journal*, *9*(1), 274.

5. Noedl, H., Se, Y., Schaecher, K., Smith, B. L., Socheat, D., and Fukuda, M. M. (2008). Evidence of artemisinin-resistant malaria in western

Cambodia. *New England Journal of Medicine*, *359*(24), 2619-2620.

6. Rogers, W., Sem, R., Tero, T., Chim, P., Lim, P., Muth, S., Wongsrichanalai, C. (2009). Failure of artesunate-mefloquine combination therapy for uncomplicated *Plasmodium falciparum* malaria in Southern Cambodia. *Malaria Journal*, *8*(1), 10.

7. Beshir, K. B., Sutherland, C. J., Sawa, P., Drakeley, C. J., Okell, L., Mweresa, C. K *et al*,(2013). Residual *Plasmodium falciparum* parasitaemia in Kenyan children after artemisinin-combination therapy is associated with increased transmission to mosquitoes and parasite recurrence. *Journal of Infectious Diseases*, 208(12), 2017-2024.

8. Dondorp, A. M., Yeung, S., White, L., Nguon, C., Day, N. P., Socheat, D. and von Seidlein, L. (2010). Artemisinin resistance: Current status and scenarios for containment. *Nat Rev Microbiol* 8: 272 –280.

9. Lin, J. T., Juliano, J. J. and Wongsrichanalai, C. (2010). Drug-Resistant Malaria: The Era of ACT. Curr Infect Dis Rep, 12:165–173

10. Mita, T., Tanabe, K., and Kita, K. (2009). Spread and evolution of *Plasmodium falciparum* drug resistance. *Parasitology International*, *58*(3), 201-209.

11. Balunas, M. J., and Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life Sciences*, 78(5), 431-441.

12. Rates, S. M. K. (2001). Plants as source of drugs. *Toxicon*, *39*(5), 603-613.

13. Ginsburg, H., and Deharo, E. (2011). A call for using natural compounds in the development of new antimalarial treatments-an introduction. *Malaria Journal*, 10(Suppl 1), S1.

14. Abdullahi, M.I., Iliya. I.A., Haruna., K., Sule, M.I., Musa, A.M., Abdullahi, M.S.(2010) Preliminary phytochemical and antimicrobial investigations of leaf extracts of *Ochna schweinfurthiana (Ochnaceae)*, *Afr J Pharm Pharmacol*,4: 83-86.

15. Evans, W. C. (1989) Trease and Evans' pharmacognosy, 13th Edition, Bailliere Tindall, London.

16. Odebiyi, A. and Sofowora, A. E. (1978). Phytochemical Screening of Nigerian Medicinal Plants. Part III. *Lloydia*, 41: 234-246.

17. Kokate, C.K., (1994). Practical Pharmacognosy. Vallabh Prakashan, New Delhi, pp. 107–113.

18. Desjardins, R. E., Canfield, C. J., Haynes, J. D., and Chulay, J. D (1979). Quantitative assessment of antimalarial activity in vitro by a semi-automated microdilution technique. *Antimicrobial Agents and Chemotherapy*. 16 (6):710–718.

19. Ngemenya, M. N., Akam, T. M., Yong, J. N. et al. (2006). Antiplasmodial activities of some products from *Turreanthus africanus* (Meliaceae). *African Journal of Health Sciences*. 13 (1-2):33–39.

20. Yakubu, M. T. and Omoniwa, B. P. (2012). Effects of Aqueous Stem Extract of Massularia acuminata on Some Liver Function Indices of Male Rats. Iranian Journal of Toxicology. 6 (18): 716-722.

21. Lawal, B., Shittu, K. O., Kabiru, Y. A., Jigam, A. A., Umar, M. B., Berinyuy, B. E. and Alozieuwa U. B. (2015).Potential antimalarials from African natural products: A review. Journal of Intercultural Ethnopharmacology. 4(4): 318-343.

22. Ayu, A. N., Azeana, Z., Jamaludin, M. and Khalijah, A. (2013). Antiplasmodial Alkaloids from the Bark of *Cryptocarya nigra* (Lauraceae). *Molecules*, *18*, 8009-8017.

23. Marshall, S. J., Russell, P. F., Wright, C. W., Anderson, M. M., Phillipson, D. J., Kirby, G. C., Warhurst, D. C. and Schiff, P. L. Jr. (1994). *In Vitro* Antiplasmodial, Antiamoebic, and Cytotoxic Activities of a Series of Bisbenzylisoquinoline Alkaloids. Antimicrobial Agents and Chemotherapy, 38 (1): 96-103.

24. Traore, F., Faure, R., Ollivier, E., Gasquet, M., Azas, N., Debrauwer, L., keita, A., Timon-David, P. and Balansard, G. (2000). Structure and Antiprotozoal Activity of Triterpenoid Saponins from *Glinus oppositifolius*. *Planta Med*. 66: 368-371.

25. Yenesew, A., Hoseah M. Akala, H. M., Twinomuhwezi, H., Chepkirui, C., Irungu, B. N., Eyase, F. L., Kamatenesi-Mugisha, M., Bernard T. Kiremire, B. T., Johnson, J. D. and Norman C. Waters, N. C. (2012). The antiplasmodial and radical scavenging activities of flavonoids of *Erythrina burttii*. *Acta Tropica* 123: 123– 127.

26. Zelefack, F., Guilet, D., Valentin, A., Fongang, R. C. S., Kom, B., Chevalley, S., Ngouela, S. A., Tsamo, E., Fabre, N. and Dijoux-Franca, M. (2012).Antiplasmodial and cytotoxic activities of flavonoids and arylbenzofuran derivatives from *Morus mesozygia. Greener Journal of Biological Sciences*, 2 (2): 020-024.

27. Ibrahim, Z. Y. Y. Abdullahi, M. I., Musa, A. M., Maje, I. M., Yusuf, A. J. and Aliyu, I. M. (2015). Acute Toxicity Profile and *Plasmodium berghei* Inhibitory Activity of Ochna schweinfurthiana (F.

Hoffm) Ochnaceae Leaf Extract in Laboratory Animals. International Journal of Pharma Sciences and Research. 6(10): 1302-1306.

28. World Health Organization (2011). Working to Overcome the Global Impact of Neglected Tropical Diseases: First WHO Report on Neglected Tropical diseases: Update;2011

29. Makler, M. T., Ries, J. M., Williams, J. A., Bancroft, J. E., Piper, R. C., Gibbins, B. L., et al., (1995). Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. American Journal of Tropical Medicine and Hygiene 48, 739–741.

30. Jensen, M. D., Conley, M. and Helstowski, L. D. (1983). Culture of *Plasmodium falciparum*: the role of pH, glucose, and lactate. *Journal of Parasitology*. 69: 1060–1067.

31. Lang-Unnasch, N. and Murphy, A. D. (1998). Metabolic changes of the malaria parasite during the transition from the human to the mosquito host. *Annual Review of Microbiology*. 52: 561–590.

32. Roth Jr., E. (1990). *Plasmodium falciparum* carbohydrate metabolism: a connection between host cell and parasite. *Blood Cells.* 16: 453–460.

33. Homewood, C. A. and Neame, K. D. (1983). Conversion of glucose of lactate by intraerythrocytic *Plasmodium berghei*. *Annals of Tropical Medicine and Parasitology*. 77: 127–129.