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Original Research Article

Antiplasmodial Activity of *Lophira lanceolata* Tiegh. (Ochnaceae) Leaf Methanol Fractions on *Plasmodium berghei* in Mice

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ABSTRACT

Malaria, which is one of the most important health problems in Nigeria continues to kill millions of people yearly as conventional drugs are inaccessible or unaffordable. This study therefore evaluated the antiplasmodial activity of the methanol extract and fractions of *Lophira lanceolata* (Ochnaceae) in the *in vivo* antimalarial suppressive (early infection), curative (established infection) and prophylactic (residual infection) in *Plasmodium berghei*-infected mice. The methanol extract of the leaf of *L. lanceolata* was fractionated sequentially by column chromatography using n-hexane, ethyl acetate, and methanol. These fractions were subjected to phytochemical analysis and *in vivo* antimalarial suppressive, curative and prophylactic tests performed in mice. The result showed that the 400 mg/kg dose of the n-hexane fraction exhibited a significant ($p < 0.05$) inhibition in established, early and residual infections (85%, 16%, 75%) respectively. The methanol fraction (MF) at 400 mg/kg evoked a significant ($p<0.05$) inhibition of parasitaemia on the residual infection (70%) and established infection (76%). Suppression of parasitaemia was not evident with MF at early infection. The ethyl acetate fraction (EF) (400 mg/kg) exhibited a significant ($p < 0.05$) suppression in established infection (93%), early infection (82%) and residual infection (95%) similar to artesunate (5 mg/kg) occurring at 86%, 46% and 46% respectively.
Taken together, the result of this study showed that the fractions of the methanol leaf extract of *L. lanceolata* possess promising antimalaria activity and there is potential for isolation of lead compounds.

Keywords: Antimalarial activity, *Lophira lanceolata*, Malaria, *Plasmodium berghei*.

Introduction

Malaria is arguably one of the most important diseases in the world with an estimated 350 -500 million clinical cases each year with a corresponding mortality rate of 2-3 million cases each year.[1]Approximately 3.2 billion people, almost half the world's population, are exposed to malaria risk and an estimated 214 million new cases of malaria and 438,000 deaths had been recorded worldwide.[2] More than 85% of malaria cases and 90% of malaria deaths occur in Sub-Saharan Africa, mainly in children under five years.[3]Malaria is a mosquito-borne infectious disease of humans caused by the protist of the genus, Plasmodium. The discovery of antimalarial lead compounds is more than ever, a priority due to the alarming spread of resistance to available drugs.[4] No new chemical class of antimalarials has been introduced into clinical practice since 1996 and there has recently been an increase in parasite strains with reduced sensitivity to the newest drugs.[5] Due to the limited availability and/or affordability of pharmaceutical medicines in the tropical countries, the majority of the population depends on traditional medical remedies.[6] The use of plants as sources of medicines for the treatment of infectious and non-infectious disease is an old human tradition[7] and the practice is now increasing due to increased global health challenges. Many species of plant have been traditionally used for the treatment of malaria.[8-11] Africa is blessed with vast amounts of medicinal plants which are consumed for the treatment of malaria. An ethnobotanical survey conducted in Sahel region of Burkina Faso reported that about 40 plant species were used by traditional healers in recipes for malaria treatment.[12]

Lophira lanceolata is a tree of the wooded savannah and occurs in many countries in West Africa like Nigeria, Cameroon, Mali and Benin. *L. lanceolata* is a multipurpose tree with edible seeds. All organs/parts of the plant have been recognized as being used either for food, medicinal, magico-mystic, wood or as a pesticide.[13] In traditional medicine, the oil is used to treat dermatitis, toothache and muscular tiredness while rubbing the skin with the oil prevents dryness.[14] The oil is mixed with porridge and given to children as a tonic. A decoction prepared from the roots as well as fresh/dried young leaves is drunk by women against menstrual pain, intestinal troubles, diarrhea, dysentery and malaria.[15,16] The bark of the roots and trunk is used against pulmonary diseases. The bark is also used to treat fevers and gastro-intestinal problems and in Southern Nigeria, the root bark is a remedy for yellow fever.[17] The infusion of the combination of the bark and leaves is an anti-trypanosome.[18]This study was aimed to investigate the antiplasmodial activity of the fractions of the methanol leaf extract of *Lophira lanceolata* using *in vivo* models in mice.

Materials and Methods

Drugs, Reagents and Chemicals

Artesunate (Mekophar Chemical Pharmaceutical Joint Stock Company, Vietnam), n-hexane, methanol and ethyl acetate (JHD,

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Guandong Guanghasci-tech co, LTD, China). Giemsa stain (Joechem Chemicals Nsukka), Tween 80 (Guangha Chemical Co. China).

Plant collection and identification
The fresh leaves of L. lanceolata were collected from Nsukka, Enugu state, Nigeria in the month of May, 2016, identified and authenticated by Mr Alfred Ozioko of the International Centre for Ethnomedicine and Drug Development (Inter CED), Nsukka, Enugu state. Thereafter, a dried voucher specimen was preserved at the Pharmacognosy Hebarium, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka (specimen number: PCG/UNN/0311).

Preparation of plant extract and fraction
The leaves of *L. lanceolata* were air-dried at room temperature and ground into powder using a grinder (ADDIS, Nigeria). The powdered material (2370 g) was macerated with 4.5 L of 70% methanol for 72 h with constant shaking. The resultant mixture was filtered using Whatman (No. 1) filter paper and the filtrate was concentrated to dryness under vacuum at 40^{o}C using rotary evaporator. The methanol extract (200 g) was subjected to solvent-guided fractionation in a silica gel (60 - 120 mesh size) column (60 cm in length and 7.5 cm in diameter), successively eluted with n-hexane, ethyl acetate and methanol. The fractions were concentrated under reduced pressure in a rotary evaporator (40 - 50°C) to obtain the hexane fraction (HF; 41.5 g; 20.8% w/w), ethyl acetate fraction (EF; 51.8 g; 25.9% w/w) and methanol fraction (MF; 54.2 g; 27.1% w/w). These fractions and the remaining crude extract were subjected to in vivo pharmacological tests.

Phytochemical analysis of the extract
Phytochemical analysis was carried out according to the methods of Trease and Evans.[19]

Animals
Swiss albino mice (18-23 g) obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka were used. The animals were housed in the institutional facility under standard condition (25 ± 2^{o}C and 12 h light/dark cycle) and were maintained on standard livestock pellet (Vital feeds, Jos, Nigeria). The ethical approval/use and care of the animals were obtained from the Institutional Ethics committee of the Pharmacology and Toxicology Department, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka and in accordance with the guideline as approved by the European Union Directives for the Protection of Animals used for Experimental and other Scientific Purposes (EU Directive: 2010/63/EU) of 2010.

Parasite
Parasitized erythrocyte (*Plasmodium berghei*) was obtained from a donor infected mouse maintained at Animal Facility Centre, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The parasite was maintained by continuous re-infestation of mice.

Acute Toxicity Study
Acute toxicity of the extract was determined using Lorke's method.[20]
Antimalarial investigations

Inoculation of the mice
Each mouse used for the study was given standard intra peritoneal inoculums (0.2 mL) of 1.0x10^{7} *P. berghei* parasites.

Suppressive test
Suppressive activity of the n-hexane fraction (HF), methanol fraction (MF) and ethyl acetate fraction (EF) (4-day test) was performed as described by Knight and Peters.[21]Thirty-two Swiss albino mice of either sex weighing (18 to 23 g) were inoculated by intra-peritoneal (i.p) injection with 0.2 mL infected erythrocytes. The mice were divided into eight groups of four per group and administered with the fractions, control and standard drugs as scheduled below for four consecutive days.
Groups 1 and 2 received 3% Tween 80 (0.2 mL/kg) and Artesunate (5 mg/kg), respectively.
Groups 3 and 4 received 200 and 400 mg/kg of EF, respectively.
Groups 5 and 6 received 200 and 400 mg/kg of MF, respectively.
Groups 7 and 8 received 200 and 400 mg/kg of HF, respectively. All samples were administered once daily per oral for 4 days.
On day 5 of the study, thick and thin films were prepared with the blood collected from the tail of each mouse. The films were fixed for 3 minutes with absolute methanol and stained with Giemsa (2.5%) and parasitaemia was determined by counting the number of infected and uninfected red blood cells in six different fields. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice.
Inhibition of parasitaemia (%) was calculated using the relationship:

Inhibition of parasitaemia (%) = 100{1-(PT/PC)

Where; PT = parasitaemia of treated group, PC = parasitemia of control group.

Curative test
The investigation was done as described by Ryley and Peters.[22] Thirty-two mice were selected and intra-peritoneally injected with 1 x 10^{7} *Plasmodium berghei* infected erythrocyte on the first day. 72 hours after, the mice were arranged into eight groups of four per group and treated.
Groups 1 and 2 received 3% Tween 80 (0.2 mL/kg) and Artesunate (5 mg/kg), respectively.
Groups 3 and 4 received 200 and 400 mg/kg of EF, respectively.
Groups 5 and 6 received 200 and 400 mg/kg of MF, respectively.
Groups 7 and 8 received 200 and 400 mg/kg of HF, respectively, once daily *p.o*.
Treatment continued until the fifth day when thick and thin films were prepared with blood collected from the tail of each mouse. The films were fixed for 3 minutes with absolute methanol stained with Giemsa (2.5%) and parasitaemia was determined by microscopic examination in six different fields.

Prophylactic test
This investigation was performed according to the method of Peter.[23] Thirty-two healthy mice were randomly selected. The mice were arranged into eight groups of four per group and treated.
Groups 1 and 2 of each set of animals received 3% Tween 80 (0.2 mL/kg) and Artesunate (5 mg/kg) daily by the oral route, while groups 3 and 4 of each set received, daily oral doses of EF (200 and 400 mg/kg), MF (200 and 400 mg/kg) and HF (200 and 400 mg/kg), respectively once daily dosing for four days (day 1 - day 4). On day 5, all the mice were inoculated with the parasite. After 72 hours, parasitaemia in each mouse was determined microscopically and percentage inhibition of parasitemia determined. Inhibition of parasitaemia (%) was calculated using the relationship:

Inhibition of parasitaemia (%) = 100{1-(PT/PC):

Where; PT = parasitaemia of treated group, PC = parasitemia of control group.

Statistical Analysis
Results and the data analyzed were presented as Mean ± SEM, using one-way ANOVA in Graph pad prism 5 (Graph pad software Inc, San Diego, CA) and subjected to Dunnets multiple comparison test. Differences between means of treated and control group accepted significant at $p < 0.05$.

Results and Discussions

Phytochemical results
Preliminary phytochemical test indicates that the EF gave a positive reaction for tannins, resins, flavonoids, terpenoids, fat and oils, steroids. HF gave a positive reaction for flavonoids, steroids and terpenoids compounds while MF gave a positive reaction for tannins, flavonoids, saponins and steroids (As shown in Table 1).

Suppressive antiplasmodial effect of L. lanceolate
Ethyl acetate fraction exhibited a dose-dependent suppression of parasitemia with 82% suppression at 400 mg/kg. The suppression by

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EF (400 mg/kg) was significantly ($p < 0.05$) higher than that produced by artesunate (46%). The n-hexane fraction showed a decrease in parasitaemia for early infection at 16% for 400 mg/kg although this was not significant ($p>0.05$). Artesunate had 46% parasitemia suppression with significance ($p < 0.05$) observed only as compared to the negative control. The MF at 200 and 400 mg/kg show no inhibition of parasitaemia when compared to the standard antimalarial drug, artesunate (As shown in Table 2).

Curative Antiplasmodial effect of L. lanceolata

The n-hexane fraction showed significant($p<0.05$) dose-dependent decrease in parasitaemia for established infection at 74% for 200 mg/kg and 85% for the 400 mg/kg daily dose and Artesunate had 86% parasitemia suppression which was significant ($p < 0.05$) when compared to the negative control. EF exhibited a dose-dependent, significant decrease in parasitemia (81% and 93%) at 200 and 400 mg/kg, respectively comparable to that of artesunate (86%). The MF at 200 and 400 mg/kg produced a significant $p < 0.05$ dose-dependent inhibition of parasitaemia at 73% and 76%, respectively when compared to the standard, Artesunate (5 mg/kg, 86%), (As shown in Table 3).

Table 1: Phytochemical Analysis of *Lophira lanceolata*.

	Fractions		
Phytoconstituents	**MF**	**EF**	**HF**
Flavonoids	+	+	+
Anthracene glycosides	_	_	_
Alkaloids	_	_	_
Saponins	+	_	_
Tannins	+	+	_
Resins	+	+	+
Proteins	_	_	_
Carbohydrate	+	_	_
Reducing sugars	+	_	_
Glycosides	+	_	_
Fat and oil	_	+	+
Steroids	+	+	+
Terpenoids	_	+	+
Acidic compounds	Neutral	Neutral	Neutral
Starch	_	_	_
Cardiac glycosides	_	_	_

Key: - absent;+present

Table 2: Suppressive antiplasmodial effect of *L. lanceolata fraction.*

Treatment	**Dose (mg/kg)**	**% Parasitemia**	**Inhibition**
Tween 80	5 mL/kg	5.17	-
EF	200	7.67*	No inhibition
	400	2.83**	82
MF	200	10.17	No inhibition
	400	7.00	No inhibition
HF	200	29.20***	No inhibition
	400	4.33	16.1
Artesunate	5	3.55**	45.7

(n=6).* $p < 0.05$, **$p<0.01$, ***$p < 0.001$.

Residual Antiplasmodial effect of L. lanceolate

The prophylactic antiplasmodial activity of the EF was dose-dependent, with a significant decrease in parasitemia (72%, and 95%) at 200 and 400 mg/kg, respectively which was significantly ($p< 0.05$) higher than that produced by Artesunate (46%). The n-hexane fraction showed a significant ($p<0.05$) dose-dependent decrease in parasitaemia for residual infection at 67% for 200 mg/kg and 75% for the 400 mg/kg. Artesunate, the standard drug, showed 46% parasitemia suppression which was significant ($p < 0.05$) as compared to the negative control. MF at 200 and 400 mg/kg produced a significant ($p < 0.05$) dose dependent inhibition of parasitaemia at 11% and 70% respectively when compared to the standard, Artesunate (5 mg/kg, 46%), (As shown in Table 4).

Plasmodium berghei is a protozoan parasite which causes malaria in certain rodents and it is routinely used to screen antimalarial agents because of the similarity of its symptoms and life cycle to the Plasmodium which causes human malaria.[24]

Acute toxicity screening indicates that the oral acute toxicity (LD_{50}) of methanol extract of *L. lanceolata* was found to be greater than 5,000 mg/kg.[25] Plants or plant products with LD_{50} values higher than 2000-3000 mg/kg are generally considered to be free of any toxicity.[26] This, therefore suggests that the extract might be safe for a single dose; however toxic and chronic effects due to repeated exposure/administration has not been investigated. Determination of percentage inhibition of parasite growth is regarded as the most dependable parameter in antimalarial drug discovery.[27,28] A mean parasitemia level $\leq$ 90% to that of mock-treated control animals usually indicates that the test compound is active in standard screening studies.[29] The n-hexane fraction was shown to possess significant curative and prophylactic activity. The inhibition obtained in the 4-day residual test (prophylactic) was dose dependent and comparable to the standard drug; Artesunate. However, there was negative inhibition in the suppressive test at the initial dose of 200 mg/kg which suggests an increase in parasitaemia. The presence or amounts of bioactive compounds in plants are influenced by several factors including seasons, environment, plant part used, intra-species variations and plant age [30] and this may explain the discrepancies in activity observed in the suppressive model.

It is also evident that the 400 mg/kg of the EF across the three models showed better activity than the standard drug, Artesunate. These experiments also showed that the ethyl acetate, was the best solvent for the extraction of the antimalarial principle(s) present in the plant. Prophylactic agents act on the parasitic forms (liver tissue forms) of the Plasmodium prior to the invasion of the red blood cell;[31] it is intended to inhibit the development of parasitaemia. Both MF and EF showed a significant effect on the residual parasitaemia infection. Curative agents produce a cure by acting on the asexual forms of the parasite in the blood, which is responsible for initiating malaria attack [31]. All the fractions reduced parasitaemia in the curative model. The mechanism of action for its curative antiplasmodial activity could either be by causing parasite red blood cell oxidation[32] or by inhibiting protein synthesis,[33] or by some other unknown mechanism. The prophylactic and curative activity may also be as a result of flavonoids present in the fraction.[34-36] The parasitemia suppression data showed that parasite clearances were significantly pronounced on day three (curative, day 0 - 3). These may be attributed to high blood levels of the fractions due to continued dosing for four days.

Suppressive agents act on the sexual form of the parasite in the blood and so prevent the diffusion and migration of these forms in the blood. These agents are intended to prevent parasitaemia and clinical symptoms, suggesting the potential to elicit suppressive cure and this action was observed in the ethyl acetate fraction but not HF or MF.

The data obtained in the present study suggests that the leaf of *L. lanceolata* possess antiplasmodial properties. EF exhibited a more significant antiplasmodial property than artesunate. The plant leaf extracts of *L. lanceolata* contains some active principles that can be useful in the treatment of malaria.

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Table 3: Curative antiplasmodial effect of *L. lanceolata fractions*.

Treatment	Dose (mg/kg)	Mean % Parasitemia			
		Day 0	Day 1	Day 2	Day 3
Tween 80	5 mL/kg	28.21	27.10	36.60	40.10
EF	200	26.50	10.17(61.9)	8.00(78.2)	7.66(80.5)
	400	29.33	9.83(63.1)	5.67(84.5)	2.83(92.8)
HF	200	28.33	14.17(48.2)	9.67(73.5)	10.33(73.6)**
	400	29.67	13.00(52.4)	10.50(71.2)	6.00(84.68)**
MF	200	27.20	22.30(18.3)	12.00(67.0)	11.3(72.9)
	400	27.80	12.00(56.0)	9.50(74.0)	10.2(75.7)*
Artesunate	5	22.50	7.22(73.9)	3.68(89.9)	5.44(86.0)

(n=6).* p< 0.05, **p<0.01, ***p<0.001, values in bracket represent % inhibition

Table 4: Prophylactic antiplasmodial effect of *L. lanceolata fraction.*

Treatment	Dose (mg/kg)	% Parasitemia	Inhibition
Tween 80	5 mL/kg	17.17	-
EF	200	4.80**	72
	400	0.83***	95
MF	200	15.33	10.7
	400	5.20**	69.7
HF	200	5.67**	66.9
	400	4.33**	74.8
Artesunate	5	5.50**	45.7

(n=6).* p< 0.05, **p<0.01, ***p<0.001

Conclusion

This research provides a scientific basis and the rationale for the use of *L. lanceolata* leaf in the management of malaria in some parts of Nigeria by traditional healers. Fractions of *L. lanceolata* should be further investigated for lead compounds which may potential candidates with therapeutic antimalarial activity.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

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

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