In vivo Antimalarial Activity of Stem Bark of Dry Zone Cedar *Pseudocedrela kotschyi* (Meliaceae) in Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AD and DPY designed and carried out the study, performed the statistical analysis, and wrote the first draft of the manuscript. Author NNW wrote the protocol and proof red the manuscript. Author GSM managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Malaria infection remains the most devastating infectious parasitic disease responsible for the death and economic losses among half the world's population. The development of resistance to the present antimalarial drugs by *Plasmodium* species has necessitated the search for effective antimalarial drugs. The stem bark of dry zone cedar (*Pseudocedrela kotschyi)* Meliaceae is used locally for the treatment of fever, hence the choice of the plant to prove scientifically the traditional claim.
Aim: The aim of the study was to evaluate the antimalarial activity of the stem bark extracts of *P. kotschyi* in mice.
Methodology: One hundred and twenty grammes of the plant powder were successively extracted. A total of three hundred and sixty mice were used for the study, one hundred and twenty for each extract (ethanol, ethyl acetate and aqueous) and forty per each test (suppressive, curative and prophylactic). Male and female, albino mice were inoculated with drug sensitive NK 65 *Plasmodium berghei berghei.* In each test animals were divided into five groups, each consisted of eight animals and treated separately with one of the

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following: 50, 100 and 200 mg/kg extracts, chloroquine / pyrimethamine and normal saline. Blood films were prepared and examined.

Results: The ethanol, ethyl acetate and aqueous crude extracts of *P. kotschyi* at 200 mg/kg significantly (P<0.05) inhibited the parasitaemia by 39.43%, 26.99% and 28.36% respectively in the suppressive test. Ethanol and ethyl acetate crude extracts also showed significant (p<0.05) cure rate of 29.17% and 20.28% respectively. However there was no significant (p>0.05) reduction in parasitaemia load in the prophylactic tests, indicating that the plant is probably not a potential prophylactic subject.

Conclusion: The results of the study showed that *P. kotschyi* indeed has antiplasmodial property, which could be exploited in the search for malaria drugs in the present day scenerio.

1. INTRODUCTION

Malaria is entirely preventable and treatable vector-borne disease which account for an estimated 219 million cases globally, killing about 660,000 people, mostly children under five years of age. The burden is concentrated in 14 endemic countries, which account for an estimated 80% of malaria deaths [1]. Over 90% of the deaths attributable to the disease occur in African region and 86% of these deaths are among children [2].

Malaria is caused by a eukaryotic protist of the genus *Plasmodium.* There are four distinct species that infect humans: *P. falciparum*, *P. vivax, P.malariae* and *P.ovale*. A fifth species, *P. knowlesi*, is a zoonotic that causes malaria in macaques but can also infect humen [3].The parasites are naturally transmitted by the female Anopheles mosquito from the blood of an infected host to the blood of an uninfected.

The clinical features of malaria vary. The most characteristic symptom is the occurrence of paroxysm which include fever with temperatures of up to 40 - 41ºC at regular intervals – every 48 or 72 hours (tertian or quartan), alternating with good periods of no fever. This is preceded by headache, lassitude, loss of appetite, muscle pain and chills, resulting in uncontrollable shivering with teeth chattering. This is accompanied by thirst, nausea, vomiting [4]. Severe and complicated malaria causing renal failure, hypoglycemia, anemia, pulmonary oedema, shock and coma can have fatal consequences, leading to death. According to [5], malaria usually results in increased maternal mortality and morbidity and the delivery of premature infants or infants who have low weights for their gestational ages.
Malaria transmission can be reduced by preventing mosquito bites by distribution of in-expensive mosquito nets and insect repellants, or by mosquito control measures such as spraying insecticides inside houses and drainage of stagnant water where mosquitoes lay their eggs. A wide variety of antimalarial drugs are available to treat malaria. In the last few years, treatment of *P. falciparum* infections in endemic countries has been transformed by the use of combinations of drugs containing an artemisinin derivative. Severe malaria is treated with intravenous and intramuscular quinine or increasing, the artemisinin derivative artesunate [6]. Efforts to reduce the high malaria morbidity and mortality rates have been hampered by the development of resistance, particularly by *P. falciparum* to long-standing inexpensive conventional drugs such as chloroquine [7,8,9,10]. This set back in the drug control and eradication of malaria has necessitated the identification of new effective alternative drugs. Although many are under development, the challenge of producing a

widely available vaccine that provides a high level of protection for a sustained period is still to be met [11].

Since malaria continues to exist with its great immeasurable devastating effects on human and animals and parasites showing resistance to the existing antimalarial drugs, there is a need to explore our abundant plant resources for the development of new drugs or vaccines for the treatment, prevention and management of the disease. The stem bark of *P. kotschyi* (Dry zone cedar) is used locally for the treatment of fever in Plateau State; hence the choice of the plant to prove scientifically the traditional claim. It is on this basis that the present study is carried out to determine the *in-vivo* antiplasmodial property of *P. kotschyi.* on *P. berghei berghei* in mice.

Plants continue to play a key role in health care systems, indeed it is estimated that up to 80% of the world populations utilize traditional medicines for the treatment of diseases. The majority of African populations use traditional medicine for their health care needs. The medicaments are often bought in places such as open markets and local stores [12, 13, 14]. *P. kotschyi* Harm (Meliaceae) is used for various purposes. [15] reported the inhibition of *P. falciparum* trophozoites transformation into schizonts by *P. kotschyi* root extract. [16] reported that the ethanolic stem bark extract of *P. kotschyi* reduced the trypanosomal load; prolonging the survival of *Trypanosoma brucei brucei* infected rats. The dichloromethane extract of *P. kotschyi* root demonstrated marked antiprotozoal activity against *Leishmania*, *Trypanosoma* and *Plasmodium* [17]. The ethanolic extract of the plant was reported active against *Haemonchus contortus* [18]. [19] in their *in vitro* evaluation of some plants used by the Karimojong (Uganda) pastoralists for ascaricidal activity reported that *P. kotschyi* had significant activity against *Ascaris suum*.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Ethical approval was obtained from the ethical committee of the Animal House, University of Jos, Jos, Nigeria. Animals were treated as approved by international standard for the treatment of experimental animals. Four weeks old Male and female albino mice, free from *Plasmodium* parasites, weighing 18-23g used in the study, were obtained from the animal house, University of Jos. They were fed on a standard rodent's diet consisting of crude protein, fat, calcium, available phosphate, vitamins, crude fibre and constant supply of water, in plastic cages measuring 25cm x 20cm x10cm with a metal cover for free passage of air, at room temperature of about 27ºC.

2.2 Parasite Species

Chloroquine sensitive *P. berghei berghei* NK 65 was used to test the plant extracts. The parasites were obtained from the National Institute for Pharmaceutical Research and Development (NIPRD) Idu-Abuja and the Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria by inoculation of uninfected mice with blood from *P. berghei* infected mice. The parasites were maintained by successive inoculations of parasite free mice every four or five days with 0.2 ml of the diluted blood from the infected mice using one (1) ml Syringe and needle.

2.3 Preparation of Plant Materials

The plant used for the study was *P. kotschyi* (Meliaceae). It was obtained from Tim along Mangu – Bwai road in Mangu Local Government Area, Plateau State, Nigeria and identified by taxonomists from the Federal College of Forestry Jos and Department of Plant Science and Technology, University of Jos, Jos. A voucher specimen was deposited at the the Federal College of Forestry, Jos and in the Department of Pharmacognosy, University of Jos, Jos with specimen numbers SF00108J and UJ/PGNS/HSP/0901 respectively. The stem parts of the plants above the soil were removed using a knife or axe. The stem were peeled and dried under shade as described by [20]. The dried materials were ground to powder using mortar and pestle.

2.4 Extraction of Plant Materials

This stem powder was successively (serially) extracted in a similar method described by [21, 22] starting with ethyl acetate, followed by ethanol and then water. 120 grammes of the powdered stem bark was measured and transferred into a flask and 2.5 liters of each solvent was added and caped with the cover in each step to macerate for 24 hours with intermittent agitations. The marc was always removed and dried to evaporate the solvents before re-introducing into the container for each subsequent extraction. The extracts were collected in a flask and concentrated to dryness using a rotary evaporator and water bath at 40 –60°C. The percentage yield for each sample was determined and crude extracts were stored in a refrigerator at 4ºC for further use.

2.5 Experimental Design

A total of three hundred and sixty mice were used for the study, one hundred and twenty for each extract (ethanol, ethyl acetate and aqueous) and forty per each test (suppressive, curative and prophylactic). In each test, forty mice were randomly inoculated with 0.2 ml diluted blood containing 1.2×10^{7} parasitized red blood cells to study the effect of stem bark extract. The animals were divided into five groups, each consisting of eight animals. Three of the five groups were each administered one of the following: 50 mg/kg, 100 mg/kg or 200 mg/kg of the extract, one of the two remaining groups (control 1) received 0.2 ml of the vehicle (normal saline) while the other group (control 2) received 5 mg/kg of chloroquine phosphate or pyrimethamine. Every mouse was treated once daily with the dosage for four (in suppressive and prophylactic tests) or five (in curative test) consecutive days and the level of parasitaemia were examined.

2.6 Antimalarial Activity

2.6.1 4-Day suppressive test

The suppressive test was conducted in a similar method used by [23,24]. 40 Mice were divided into 5 groups of 8 animals each. Three of the 5 groups were each administered one of the following: 50 mg/kg, 100 mg/kg or 200 mg/kg of the extract. One of the remaining groups was administered 0.2 ml normal saline (control 1) while the other group received chloroquine phosphate (5 mg/kg) (control 2). Each mouse was inoculated on the first day (Do), intraperitoneally, with infected blood containing *P. berghei berghei* parasitized red blood cells. The mice were treated daily from day 0 (immediately after infection) to day 3. Thin smears fixed in methanol stained with Giemsa stain were prepared from the tail blood

of each mouse daily from day 4 to day 7, examined microscopically under oil immersion and the percentage parasitaemia was determined by counting the parasitized erythrocytes in at least ten random fields. The mean parasite count for each group was determined and the average percentage chemosuppression for each dose was calculated as [(A-B)/A], where A is the average percentage of parasitaemia in the normal saline group (control 1) and B is the average percentage of parasitaemia in the test groups.

2.6.2 Rane (Curative) test

This was conducted in a similar method adopted by [20,24]. Another set of 40 albino mice were infected with parasitized erythrocytes. Seventy-two hours after infection, the mice were divided into 5 groups of 8 animals each. Three of the 5 groups were each administered one of the following: 50 mg/kg, 100 mg/kg or 200 mg/kg of the extract. The fourth group (control 1) was given 0.2 ml normal saline while the last group (control 2) received 5 mg/kg of chloroquine phosphate. Each mouse was treated orally once daily with the dose for 5 consecutive days (D4 - D8) post inoculation during which the parasitaemia level was monitored daily.

2.6.3 Repository (Prophylactic) test

This was carried out according to the method of [25]. The mice were divided into 5 groups of 8 animals each. Three of the 5 groups were each administered one of the following: 50 mg/kg, 100 mg/kg or 200 mg/kg of the extract. The fourth group (control 1) was given 0.2ml normal saline while the last group (control 2) received 1.2 mg/kg of pyrimethamine for four consecutive days. On day four the mice were inoculated with *P. berghei berghei*. Seventy two hours later (D3), smears were made from the mice (D3-D7) and the parasitaemia levels assessed.

2.7 Inoculation of Animals

Inoculation of mice was done as carried out by [26,27]. The animal was held at the back and neck and the tail between fingers. The peritoneal region was cleaned with cotton wool moistened with alcohol and allowed to dry.

Each mouse received intraperitoneally a standard inoculum of about 1.2×10^{7} *P. berghei* infected erythrocytes on day zero (D_o). The injected site was cleaned with cotton wool moistened with alcohol after inoculation.

2.8 Administration of Extracts and Drugs

The extract and drugs employed in this study were administered orally with the aid of a cannula connected to one (1) ml syringe. The required dose was given according to the weight of the animal four hours after inoculation of parasites on D_0, then once daily for three more days ($D_1 – D_3$)

2.9 Evaluation of Parasitaemia

Thin blood films of infected and treated mice were prepared and examined for parasitaemia level under the microscope from day four through day seven ($D_4 – D_7$). The stained slides were mounted in oil immersion. Red cells were counted in 10 fields and the parasitized ones

were marked down on a paper, then the percentage parasitaemia in a group was calculated as:

$$\text{Percentage parasitaemia} = \frac{\text{Number of parasitised cells}}{\text{Total cells}} \times 100$$

The average percentage suppression for each dose of each extract was calculated in comparison to controls according to [20,26] as:

$$\text{Average \% suppression} = \frac{\text{Average \% parasitaemia in control} - \text{Average \% parasitaemia in treated group}}{\text{Average \% parasitaemia in control}} \times 100$$

2.10 Statistical Analysis

Data were analysed using one way analysis of variance (ANOVA) and students t-test coupled Least Significant Difference (LSD) to compare the parasitaemia of mice between doses and among treatment and control (normal saline) group.

3. RESULTS

The percentage yields of crude extracts of *P. kotschyi* stem bark after evaporation were 20.4 g (17%), 10.2g (8.5%), and 1.92 g (1.6 %) in ethanol, aqueous and ethyl acetate respectively. The *in-vivo* antimalarial activity of the ethanol extract of the suppressive test showed significant difference (P < 0.05 and 0.01) between the extract treated and the normal saline control 1 (Table 1). The administration of 200 mg/kg in the curative test produced a significant difference (P < 0.05) compared with the untreated group, while there was no statistical significant difference (P > 0.05) between the treated and the untreated control 1 group in the prophylactic test. The ethanol extract produced the highest inhibition in the suppressive test, followed by curative and prophylactic with 39.04 %, 29.17 % and 6.53 % respectively in groups given 200 mg/kg. The standard drugs produced a significant difference (P < 0.05) in all the tests compared with the normal saline group with percentage inhibition of 95.04 %, 93.54 % and 92.05 % in the suppressive, curative and prophylactic test respectively.

A significant difference (P < 0.05) between the extract treated and the untreated groups was observed only in the group given 200 mg/kg in the suppressive test (Table 2). The average percentage inhibition of parasitaemia of the extract treated groups were generally low with 28.36 %, 17.15 % and 21.97 % at 200 mg/kg in the suppressive, curative and prophylactic tests respectively, while the standard drugs had a higher inhibition of more than 90 %.

The ethyl acetate administration activity showed a significant difference (P < 0.05) between the groups treated at 200 mg/kg in the suppressive and curative tests (Table 3). The percentage inhibition at 200 mg/kg were 26.99, 20.28 and 14.40 in the suppressive, curative and prophylactic test respectively, while significant (P < 0.05) inhibition in groups given the standard drugs were greater than ninety percent.

**Table 1. Antimalarial activity of *P. kotschyi* ethanol stem bark extract in
P. berghei berghei infected mice**

Test	Dose(mg/kg)	Parasitaemia (%) mean ±S.E	% Inhibition
Suppressive	NS 0.2 ml	20.77 ± 2.11	-
	Ext. 50	15.08 ± 1.5*	27.40
	Ext. 100	13.70 ± 1.44*	34.04
	Ext. 200	12.58 ± 1.3*	39.43
	CQ 5	1.03 ± 0.17*	95.04
Curative	NS 0.2 ml	24 ± 2.5	-
	Ext. 50	22.5 ± 2.4	6.25
	Ext. 100	20.13 ± 2.12	16.13
	Ext. 200	17 ± 1.62 *	29.17
	CQ 5	1.55 ± 1.03*	93.54
Prophylactic	NS 0.2 ml	21.13 ± 2.14	-
	Ext. 50	20.68 ± 2.01	2.13
	Ext. 100	20.73 ± 2.08	1.89
	Ext. 200	19.75 ± 1.76	6.53
	PM 1.2	1.68 ± 0.23*	92.05

NS- Normal saline, Ext- Extract, CQ- Chloroquine, PM- Pyrimethamine.
SE- Standard error for 8 mice in each dose
*- P<0.05 compared to control group (student's t- test).

**Table 2. Antimalarial activity of *P. kotschyi* aqueous stem bark extract in
P. berghei berghei infected mice.**

Test	Dose(mg/kg)	Parasitaemia (%) mean ±S.E	% Inhibition
Suppressive	NS 0.2 ml	20.7 ± 2.06	-
	Ext. 50	18.68 ± 2.01	9.76
	Ext. 100	17.28 ± 1.89	16.52
	Ext. 200	14.83 ± 1.52(*)	28.36
	CQ 5	2.05 ± 0.26 (*)	90.10
Curative	NS 0.2 ml	23.03 ± 2.49	-
	Ext. 50	21.75 ± 2.42	5.56
	Ext. 100	20.88 ± 2.34	9.34
	Ext. 200	19.08 ± 1.94	17.15
	CQ 5	2.25 ± 0.13 (*)	90.23
Prophylactic	NS 0.2 ml	22.3 ± 2.36	-
	Ext. 50	19.5 ± 2.22	12.56
	Ext. 100	17.93 ± 2.07	19.60
	Ext. 200	17.4 ± 1.96	21.97
	PM 1.2	1.65 ± 0.15 (*)	92.60

NS- Normal saline, Ext- Extract, CQ- Chloroquine, PM- Pyrimethamine.
SE- Standard error for 8 mice in each dose.
*P<0.05 compared to control group (student's t- test)

**Table 3. Antimalarial activity of *P. kotschyi* ethyl acetate stem bark extract
in *P. berghei berghei* infected mice**

Test	Dose(mg/kg)	Parasitaemia (%) mean±SE	% Inhibition
Suppressive	NS 0.2 ml	25.88 ± 2.41	-
	Ext. 50	24.28 ± 2.28	6.18
	Ext. 100	22.3 ± 1.80	13.83
	Ext. 200	20.38 ± 1.51 (*)	26.99
	CQ 5	2.1 ± 0.27 (*)	91.89
Curative	NS 0.2 ml	24.9 ± 2.43	-
	Ext. 50	25.15 ± 2.40	1.00
	Ext. 100	24.38 ± 2.23	2.09
	Ext. 200	19.85 ± 1.68 (*)	20.28
	CQ 5	2.45 ± 0.37 (*)	90.16
Prophylactic	NS 0.2 ml	24.45 ± 2.33	-
	Ext. 50	24.13 ± 2.41	1.31
	Ext. 100	23.40 ± 2.27	4.29
	Ext. 200	20.93 ± 1.95	14.40
	PM 1.2	2.03 ± 0.28 (*)	91.70

NS- Normal saline, Ext- Extract, CQ- Chloroquine, PM- Pyrimethamine.
SE- Standard error for 8 mice in each dose
$P < 0.05$ compared to control group (student's t- test).

4. DISCUSSION

The study revealed that the ethanolic extract of the plant reduced parasitaemia by 39.43% when tested at an oral dose of 200 mg/kg, indicating that the effective dose of each extract was greater than 200 mg/kg. Reduction in parasitaemia was more evident in the groups administered with ethanol extract than in those tested with the aqueous and ethyl acetate extracts. The highest antimalarial activity produced by ethanol than water extract is consistent with [28] who reported highest *in vitro* antiplasmodial activity in organic solvents of *Cassia arereh*. However, water is the extraction solvent mostly use in traditional medicine in endemic areas. The extract produced dose –dependent relationship, with the higher doses resulting in greater reduction in the parasite density/ load than the lower doses. The highest antimalarial suppression of 39.43% observed in this study is low compared with 69.46% reported by [29], 81.36% [30], 66.67% [31] and 79.5% [32]. The low parasitaemia recorded in this study may be due to low dose which could be increased to give higher inhibition rate and at the same time considering the toxicity of the extract. However, [33] demonstrated that the antimalarial activity of a compound and its toxicity are not directly related. The low dose effect is confirmed by the report of [20] that inhibition of *P. berghei* by the extracts of *M. morindoides, P. niruri and C. occidentalis* at 200mg/kg was less than at 800mg/kg. However it is in line with the finding of [34] that lethality of *Ae aegypti and C. quinquefaciatus* larvae were more pronounced with increasing concentration of *Lantana camara*.

The standard drug 5 and 1.2 mg/kg (Chloroquine phosphate and pyrimethamine respectively) on the other hand significantly (P. <0.05) inhibited parasitaemia level by more than 90%. The low parasitaemia in these groups was due to the inhibition of the enzyme, dihydrofolate reductase involved in biosynthesis of folic acid an essential precursor in the formation of nucleic acids and also due to the action of the drug (Chloroquine phosphate/pyrimethamine) on the parasite lysosomal membrane.

[24] reported that *Vernonia amygdalina*, aqueous leaf extract at dose of 125mg/kg had the inherent ability to work in favour of the restoration of chloroquine efficacy as a prophylactic and chemotherapeutic agent against chloroquine sensitive and resistant *Plasmodium berghei* infection in mice.

5. CONCLUSION

The result of this study showed that the stem bark extract of *P. kotschyi* is not a prophylactic remedy against *Plasmodium* parasites since various solvent extracts had no significant (P > 0.05) prophylactic effect. However, it could probably be a potential chemotherapeutic agent which is evidenced in the suppressive and curative tests. The result of this study indicates that *P. kotschyi* stem bark extracts have antimalarial potentials, therefore the plant should be extensively investigated to isolate and identify their antimalarial active constituents. The present study has shown scientifically for the first time that *P.kotschyi* stem bark indeed has antimalarial property, so these findings support the traditional uses of the plant and this can be explored in the field of drug production for malaria.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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