



Antimalarial activity of fractions of aqueous extract of *Acacia nilotica* root

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ABSTRACT

Background: The problem of resistance of malarial parasites to available antimalarial drugs makes the development of new drugs imperative, with natural plant products providing an alternative source for discovering new drugs. **Aim:** To evaluate the antimalarial activity of eluted fractions of *Acacia nilotica* root extract and determine the phytochemicals responsible for its antimalarial activity. **Materials and Methods:** The extract was eluted successively in gradients of solvent mixture (hexane, ethyl acetate, and methanol) in multiples of 100 ml, and each fraction was collected separately. Eluates that showed similar thin layer chromatographic profiles and R_f values were combined to produce 4 main fractions (F-1, F-2, F-3, and F-4), which were tested separately for antimalarial activity using the curative test. Changes in body weight, temperature, and packed cell volume (PCV) were also recorded. **Results:** Fraction F-1 of *A. nilotica* at 50 and 100 mg/kg b/w produced significant and dose-dependent reduction in parasite count in *Plasmodium berghei* infected mice compared to the control, and also significantly increased the survival time of the mice compared to the control group. This fraction also ameliorated the malaria-induced anemia by improving PCV in treated mice. **Conclusion:** Antimalarial activity of extract of *A. nilotica* root is probably localized in the F-1 fraction of the extract, which was found to be rich in alkaloids and phenolics. Further study will provide information on the chemical properties of the active metabolites in this fraction.

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Received: February 10, 2016

Accepted: March 20, 2016

Published: April 01, 2016

KEY WORDS: *Acacia nilotica*, antimalarial, fractions, medicinal plant

INTRODUCTION

Malaria is a preventable and curable disease, yet it remains a devastating tropical disease, with a high infection and mortality statistics. It is the most prevalent parasitic disease and the most common cause of hospital visitation in Nigeria [1]. Globally, there are approximately 214 million malaria cases in 97 countries with ongoing malaria transmission and 80% of these cases were reported in the Sub-Saharan Africa. The disease caused approximately 438,000 deaths, 90% of which were in Sub-Saharan Africa, and 78% of these deaths occur in children under 5 years [2]. The main challenge to the effective management of diagnosed malaria cases is the resistance of the plasmodium parasite to commonly used antimalarial drugs, which results in the nonresolution of symptoms and in treatment failure [3,4].

Cases of resistance are currently extending to the artemisinin combination therapy with report of resistance to artemisinin drugs coming from Thailand, Vietnam, and Cambodia [2,5-7]. Furthermore, no new class of antimalarial drug has been introduced in clinical practice in the last 10 years [8,9]. This strongly supports the need for further research into new products that could complement the existing antimalarial, bearing in mind that it takes minimum of 10 years to develop a drug from discovery stage to completion of clinical trials [10]. The development of safe and effective anti-malarial preparations by simple procedures from locally grown medicinal plants, may offer new and complimentary drugs for malaria control, especially in remote geographical locations and in rural areas where modern antimalaria drugs are not readily available and malaria mortality is higher [11].

Acacia nilotica (Linn.) Willd. Ex Del. (also called Gum Arabic tree; Prickly tree, 'Gabaruwa' in *Hausa* and 'Igi Kasia/Booni' in *Yoruba*) is a scented thorny tree native to Africa (grows in Egypt, South Africa, Kenya, and Nigeria) and Indian subcontinents [12]. It is a nitrogen-fixing tree that grows to 14-17 m in height and 2-3 m in diameter.

African Zulu use the bark of *A. nilotica* to treat cough, diarrhea, dysentery, and leprosy [13]. The Massai (Kenya) use the bark and root decoction as aphrodisiac to cure impotence. The fruit is used to treat tuberculosis [14], whereas the powdered pods are consumed by Egyptians to treat diabetes mellitus [15]. In Northern Nigeria, the root is used for the treatment of malaria [16,17]. *A. nilotica* is rich in many secondary metabolites, such as tannins, terpenes, alkaloids, flavonoids, and phenolic [17], with known pharmacological properties, thus making it relevant in the treatment of various ailments.

This research aims at evaluating the antimalarial activity of fractions obtained from crude aqueous extract of *A. nilotica* root and investigates the active antimalarial phytochemical in the crude extract.

MATERIALS AND METHODS

Plant Collection, Identification, and Authentication

Fresh root samples of *A. nilotica* were collected around 8.45 am in Bamburu-Chaza, Suleja, North-central part of Nigeria. They were identified and authenticated as *A. nilotica* root at the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja by a taxonomist, Mrs. Grace Ugbabe. Voucher specimen (NIPRD/H/6401) was prepared and deposited at the herbarium for referencing.

Experimental Animals

Swiss albino mice (*Mus musculus*, 25.00 ± 2 g) used for this study, were obtained from Animal Facility Center, NIPRD, Abuja. They were housed in well ventilated cages, fed with rat pellets and water *ad libitum*, and maintained under standard laboratory conditions (temperature of 25 ± 3°C and 12 h light/12 h dark cycle), in accordance with the guidelines for the care and use of laboratory animals by National Academy of Science (1996). Ethical approval for animal studies was obtained from the Animal Ethics Committee of NIPRD.

Malaria Parasites

Chloroquine-sensitive rodent *Plasmodium*; *P. berghei* NK 65 strain, was obtained from the National Institute for Medical Research, Lagos, Nigeria, and maintained alive by serial intraperitoneal passage in mice, every 5 days.

The reinfected mice were kept at the Animal Facility Center of NIPRD where this study was carried out.

Reagents and Chemicals

The chloroquine phosphate and silica gel (70-230 mesh) were obtained from Sigma-Aldrich Corporation, Missouri, 63103, USA. All other chemicals and reagents used were of analytical grade and prepared using distilled water.

Preparation of Aqueous Extract of *A. nilotica* root

The root sample of *A. nilotica* was air dried to constant weight and pulverized using grinding machine. The powder was stored in an airtight container and kept in a cool, dry place. Aqueous extraction was carried out following the cold maceration method. Four hundred grams of the powdered root of *A. nilotica* was soaked in 1 L of distilled water and kept for 24 h with intermittent shaking. The suspension was filtered after 24 h, with muslin cloth followed by filtration with Whatman filter paper (No.1). The filtrate was freeze-dried using AMSCO/FINN-AQUA GT2 Freeze dryer (Germany). This extraction procedure was carried out thrice to obtain sufficient quantity of extract for the entire study.

Column Fractionation of *A. nilotica*

Wet silica gel (70-230 mesh) was loaded into a column, and the crude extract was added on the upper layer [18]. The extract was eluted successively in gradients of solvent mixture (hexane, ethyl acetate, and methanol) in multiples of 100 ml and each fraction was collected separately. Eluates that showed similar thin layer chromatographic profiles and R_f values were combined to produce 4 main fractions, which were tested separately for antimalarial activity using the curative test.

Phytochemical Screening

The four fractions obtained were subjected to phytochemical screening following the procedures described by Sofowora [19], to determine the predominant secondary metabolite in each fraction.

Antimalarial Test

Parasite inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2 ml of infected blood (containing about 1×10^7 parasitized erythrocytes) obtained from a single donor mouse previously infected with chloroquine-sensitive *P. berghei* (containing 25.5% parasitemia). This was prepared by calculating the percentage parasitemia of donor mouse and diluting the blood with physiological saline so that 0.2 ml of diluted blood contained 1×10^7 infected erythrocytes [20].

Grouping and Drug Administration

P. berghei-infected mice were randomly divided into five groups of 6 mice per group. Thirty mice were used for the antimalarial test of each of the four fractions. Group 1 was administered

10 ml/kg body weight distilled water (negative control), Groups 2, 3, and 4 were administered 25, 50, and 100 mg/kg b.w of each fraction. Group 5 was administered 5 mg/kg b/w chloroquine (positive control). Fractions of *A. nilotica* and chloroquine drug used in this study were administered orally using stainless metal oral cannula. Doses administered were calculated from the value of median lethal dose. Same procedure as above was repeated for the antimalarial test for fractions F-2, F-3, and F-4.

Evaluation of Antimalarial Activity of Fractions of *A. nilotica*

The Rane test was used to evaluate the schizonticidal activity of the fractions of *A. nilotica* in established malaria infection.

The evaluation of the curative potential of fractions obtained from *A. nilotica* root against established infection was carried out as described by Ryley and Peters [21]. Thirty mice were inoculated as earlier described on day 0, and left untreated for 72 h. The mice were weighed and randomized into five groups of six mice each. Group 1 was administered 10 ml/kg body weight of distilled water (negative control); Groups 2, 3, and 4 received fraction of *A. nilotica* at doses of 25, 50, and 100 mg/kg body weight/day orally, respectively, whereas mice in Group 5 (positive control) received 5 mg/kg body weight/day of chloroquine orally for 4 days (D₄-D₇). On day-8, each mouse was tail-bled, and a thin blood film was made on a microscope slide.

The films were stained with Giemsa stain and examined microscopically to monitor the parasitemia level [22,23]. The mean survival time of the mice in each treatment group, monitored over a period of 30 days was calculated using the expression:

$$\text{Mean survival time} = \frac{\text{Sum of survival of every animal in each group in days}}{\text{The number of animals in each group}}$$

Determination of Percentage Parasitemia

Thin blood films were made from the tail blood of each infected mouse, stained with Giemsa and examined under the microscope at ×100 magnification (oil immersion), to determine the level of parasitemia. Percentage parasitemia was calculated using the formula below:

$$\text{Percentage Parasitemia} = \frac{\text{Parasitized RBC}}{\text{Parasitized RBC} + \text{Non - Parasitized RBC}} \times 100$$

RBC = Red blood cells.

Determination of Packed Cell Volume (PCV)

PCV of the mice was measured before infecting them with parasite and after treatment. PCV was done to determine the

effectiveness of the fractions in preventing hemolysis resulting from increasing parasitemia level associated with malaria. Heparinized capillary tubes were used for collection of blood from tail of each mouse. The capillary tubes were filled with blood up to 75% of their volume and sealed at the dry end with sealing clay. The tubes were placed in a microhematocrit centrifuge (Hawksley, England) with the sealed end outward and centrifuged for 5 min at 11,000 rpm [24]. The tubes were then taken out of the centrifuge and PCV was determined using a standard Micro-Hematocrit Reader (Hawksley, England).

Monitoring of Body Weight and Temperature Changes

Body weight of each mouse was measured before infection (day 0) and on day 8, using a sensitive digital weighing balance (OHAUS, USA). Rectal temperature was also measured with a digital thermometer before infection, and then daily. In order to evaluate the effect of the fractions on body weight and temperature, the fractions were administered to healthy (uninfected) mice at the doses of 25, 50, and 100 mg/kg b.w for 4 days.

Data Analysis

Graphpad prism Version 5.02 was used to analyze the data obtained and these were expressed as mean ± standard error of mean (SEM). The differences between means were compared using one-way Analysis of Variance, followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

RESULTS

Phytochemical Screening

The summary of the phytochemical constituents of fractions of *A. nilotica* root extract, shown in Table 1, revealed that the fractions gave positive reactions to phenolic, tannins, alkaloids, anthraquinones, flavonoids, terpenes, and sterols. Fraction F-1 showed high amount of alkaloids compared with other three fractions [Table 1].

Antimalarial Activity of Fractions of *A. nilotica*; (F-1, F-2, F-3, and F-4)

Fractions F-1 and F-2 caused significant and dose-dependent reduction in the mean parasite count at doses of 50 and

Table 1: Phytochemical constituents of fractions (F-1, F-2, F-3, and F-4) of *Acacia nilotica* root

Phytochemicals	F-1	F-2	F-3	F-4
Phenolics	+	+	-	-
Tannins	-	+	+	-
Alkaloids	++	-	-	-
Saponins	-	+	+	-
Anthraquinones	-	-	+	+
Flavonoids	-	+	-	-
Terpenes	-	-	-	+
Sterols	-	-	-	+

-Not detected; + Present; ++ High concentration

100 mg/kg b/w when compared with the control, although the reduction was less than chloroquine used as a standard drug [Table 2]. However, F-3 and F-4 did not produce significant reduction in parasite count at all the doses administered [Table 3]. Survival time was also prolonged by fraction 1 and 2, with fraction 1 producing a significantly prolonged survival time that was comparable to chloroquine [Table 2], unlike F-3 and F-4 that did not prolong the survival time.

Fraction F-1 produced moderate weight gain and increased PCV at the dose of 100 mg/kg b/w; however, it did not prevent temperature lowering effects of malaria infection in mice [Table 4]. Fraction F-2 did not prevent weight loss, reduction in rectal temperature, and PCV in the treated mice [Table 5].

DISCUSSION

P. berghei NK 65 was used in this study for inoculation and to predict antimalarial treatment outcome because of its ability to produce rodent model of malaria similar to human malaria infection [25,26]. Chloroquine, though no longer a first-line drug in the treatment of malaria, is used as control drug in this study because the *Plasmodium* parasite used for inoculation in this study is a chloroquine-sensitive strain. The Rane's test is commonly used in antimalarial screening for evaluating the curative capability of extracts/drugs on established infections. This screening test was previously employed for the antiplasmodial screening of the crude extract of *A. nilotica* [17]. This study was carried out *in-vivo*, in order to factor in the

Table 2: Curative effect of fractions F-1 and F-2 of *Acacia nilotica* in *Plasmodium berghei* infected mice

Treatment dose mg/kg b.w	Parasitemia count				Mean survival time (days)
	Pretreatment (D-3)	Posttreatment (D-8)	Posttreatment (D-14)	Posttreatment (D-21)	
F-1					
D/water 10	16.50±1.85	29.50±1.45	D	D	8±1.00
25	17.00±0.73	13.70±0.37*	18.00±0.65*	D	15±1.50*
50	17.50±0.85	6.70±0.35*	7.00±0.45*	D	18±1.00*
100	18.00±0.65	5.80±0.37*	4.50±0.75*	4.00±0.55*	21±1.00*
Chloroquine 5	17.50±0.75	2.50±0.25*	2.00±0.35*	1.50±0.35*	28±2.00*
F-2					
D/water 10	16.50±1.83	28.50±1.04	D	D	8±1.50
25	16.00±1.75	15.50±0.48*	25.00±1.55*	D	14±1.00
50	17.00±1.85	7.20±0.86*	9.50±0.75*	D	17±1.00*
100	18.00±1.65	6.50±0.75*	9.20±0.55*	D	17±1.00*
Chloroquine 5	17.50±1.75	2.50±0.35*	2.00±0.35*	1.50±0.25*	28±2.00*

D-3, 8, 14, and 21=Days 3, 8, 14, and 21. *Significantly different from control at $P<0.05$. $n=5\pm$ SEM. D: Death, SEM: Standard error of mean

Table 3: Curative effect of fractions F-3 and F-4 of *Acacia nilotica* in *Plasmodium berghei* infected mice

Treatment dose mg/kg b.w	Parasitemia count				Mean survival time (days)
	Pretreatment (D-3)	Posttreatment (D-8)	Posttreatment (D-14)	Posttreatment (D-21)	
F-3					
D/water 10	16.00±0.83	28.50±1.04	D	D	8±2.00
25	18.00±0.73	27.70±0.86	D	D	9±1.50
50	18.00±0.95	24.20±0.71	D	D	12±1.00
100	17.00±0.75	20.50±0.48	24.50±1.45	D	14±1.50
Chloroquine 5	17.00±0.75	3.00±0.25*	2.50±0.35*	2.00±0.25*	28±2.00*
F-4					
10	16.00±1.83	29.50±1.04	D	D	8±2.05
25	16.00±1.83	26.70±1.86	D	D	9±1.65
50	18.00±1.95	25.20±1.71	D	D	10±1.34
100	17.00±1.75	24.00±1.48	D	D	11±1.50
Chloroquine 5	17.00±1.85	3.00±0.35*	2.50±0.25*	2.00±0.25*	28±2.10*

D-3, 8, 14, and 21=Days 3, 8, 14, and 21. *Significantly different from control at $P<0.05$. $n=5\pm$ SEM. D: Death, SEM: Standard error of mean

Table 4: Temperature, weight, and PCV of *Plasmodium berghei*-infected mice treated with fraction F-1 of *Acacia nilotica*

Treatment dose (mg/kg b.w)	Temperature (°C)		% change	Weight (g)		% change	PCV (%)		% change
	D ₀	D ₈		D ₀	D ₈		D ₀	D ₈	
D/water 10	37.9±0.10	35.6±0.15	-6.1	18.6±1.55	16.5±1.35	-11.3	52.6±1.25	45.0±0.95	-14.4
25	37.8±0.11	37.4±0.08*	-1.0	18.2±1.75	17.5±1.55	-3.8	52.5±1.15	50.5±0.95	-3.8
50	37.7±0.05	37.5±0.10*	-0.5	19.2±1.45	19.0±1.25*	-1.0	51.5±1.05	51.7±1.15*	0.4
100	37.8±0.12	37.7±0.10*	-0.3	19.0±0.85	19.2±0.95*	1.0	51.6±1.35	52.5±1.65*	1.7
Chloroquine 5	37.9±0.13	38.0±0.12*	0.3	18.8±1.25	19.5±0.85*	3.7	52.8±1.55	53.9±1.45*	2.1

*Significantly different from control at $P<0.05$; $n=5\pm$ SEM; D₀ and D₈: Days 0 and 8, SEM: Standard error of mean, PCV: Packed cell volume, SEM: Standard error of mean

Table 5: Temperature, weight, and PCV of *Plasmodium berghei*-infected mice treated with fraction F-2 of *Acacia nilotica*

Treatment dose (mg/kg b.w)	Temperature (°C)		% change	Weight (g)		% change	PCV (%)		% change
	D ₀	D ₈		D ₀	D ₈		D ₀	D ₈	
D/water 10	38.0±.25	35.4±.15	-6.8	19.1±.95	17.0±.35	-11.0	51.3±.35	45.2±	-11.9
25	37.9±.15	37.1±.25	-2.1	18.8±.20	17.4±.15	-7.4	52.2±.15	46.5±.25	-10.9
50	37.8±.10	37.2±.20*	-1.8	19.0±.35	18.0±.35	-5.3	52.1±.45	45.0±.55	-13.6
100	37.9±.35	37.6±.25*	-0.8	19.4±.20	18.4±.15	-5.1	51.4±.25	44.8±.35	-12.8
Chloroquine 5	38.0±.20	38.0±.15*	0.0	18.9±.95	21.1±.25*	1.1	52.5±.55	53.5±.45*	2.0

*Significantly different from control at $P < 0.05$; $n = 5 \pm \text{SEM}$; D₀ and D₈: Days 0 and 8, PCV: Packed cell volume, SEM: Standard error of mean

possible effects of prodrug and involvement of immune system in the eradication of infection in a living host.

From the result of the antimalarial activity of the fractions in Table 2, fraction F-1 produced significant and dose-dependent antimalarial activity which was comparable to that of chloroquine, the control drug used. At the highest dose of 100 mg/kg b/w, F-1 was able to reduce parasite count from 18.0 to 4.0 (77.7% inhibition) compared to the standard drug which reduced the parasite count from 17.5 to 1.5 (91.4% inhibition). In the infected and untreated group, parasite count increased from 16.5 to 29.5. The percentage inhibition of the F-1, which is the closest (among the four fractions) to the standard drug, suggests that the active antimalarial compound in the crude extract of *A. nilotica* is localized in the F-1 fraction. Alkaloids and phenolic found in F-1 may possibly, individually or synergistically, account for its significant antimalarial activity, when compared with other three fractions. Many alkaloids derived from plants such as quinine and quinidine have been demonstrated to exhibit antimalarial activity [27,28]. Although fraction F-2 contain phenolic which may also account for some antimalarial activity observed (albeit lower than fraction F-1), the presence of saponin (phytodetergent) in this fraction, may account for the destruction of erythrocyte membrane manifesting as reduced PCV [29], which may also be responsible for the reduction in survival time of the F-2 infected mice [Table 2].

Anemia, change in body (rectal) temperature, and reduction in body weight are the general features of malarial infection in rodent (mice) and human malarial infection [30]. A potent antimalarial is expected to ameliorate anemia, prevent body weight loss, and stabilize temperature in infected mice with increasing parasitemia.

PCV was measured in this study to determine the effectiveness of fractions of *A. nilotica* in preventing malaria-induced hemolysis alongside its antimalarial activity. Malaria-induced hemolysis, either in human or rodents, may be due to rapid destruction/clearance of infected erythrocytes and/or sequestration of infected erythrocytes [30]. Fraction F-1 at doses of 50 and 100 mg/kg b/w produced significant increase in PCV [Table 4] when compared with the control, which suggests that this fraction of the extract could ameliorate anemia associated with malaria infection. The ability of this fraction to reverse reduction in PCV may be due to rapid clearance of parasite from infected erythrocytes before hemolysis occur or via enhanced erythropoiesis [29]. There was no reduction in PCV of mice

at all the doses of fraction F-2 administered [Table 5]. The inability of fraction F-2 to improve the PCV of mice may be due to the presence of saponin in this fraction. Saponin is a phytodetergent known to destroy cell membrane by inducing cholesterol liberation from the cell membrane [31], resulting in erythrocyte hemolysis as manifested in the reduced PCV after administration of fraction F-2.

There was reduced rectal temperature with increasing parasitemia in the infected mice, in all the doses administered. The reduced rectal temperature could be attributed to reduced basal metabolic rate (observed during ongoing infection) or hypothermic effects of parasite multiplication. The highest dose of fraction F-1 at 100 mg/kg b.w, showed the best temperature stabilizing effect with the least reduction in rectal temperature in mice [Table 4]. Fraction F-2 could not stabilize the body temperature with increasing parasitemia as rectal temperature was significantly lower at all the doses of F-2 administered [Table 5].

Weight loss was recorded in all the doses of fractions F-1 and F-2 of *A. nilotica* administered to the mice except at 100 mg/kg b/w of F-2. This could either be due to reduced feed intake which may be attributed to appetite suppressing component of the extract such as saponin and tannins [32].

Our previous study showed that the crude aqueous extract of *A. nilotica* possess antimalarial activity [17]. Although the active antimalarial component of *A. nilotica* is not yet identified, the antimalarial activity of fractions F-1 of *A. nilotica* could be due to high proportion of alkaloid found in this fraction or synergistic effect of the alkaloids with the phenolic component. These phytochemicals have been documented to possess varying degree of antimalarial activity in plant extracts [10,16,32].

CONCLUSION

The results of this study showed that fraction F-1 of *A. nilotica* has significant antimalarial activity. This fraction was also able to improve the PCV of treated mice either by prevention of malaria-induced hemolysis or by enhancing erythropoiesis. However, F-1 could not reverse the reduced body temperature and weight loss associated with rodent malaria. The active antimalarial component of fraction F-1 of *A. nilotica* may be due to the alkaloid alone or combination of the alkaloid and the phenolic acting synergistically. This result also validate the antiplasmodial activity reported on the crude extract and the

use of the *A. nilotica* root extract for treatment of malaria by local communities in Northern Nigeria.

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Source of Support: Nil, Conflict of Interest: None declared.