

Evaluation of *Adansonia digitata* Stem Bark Extract and Derived Fractions for In Vivo Antimalarial Activity

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ABSTRACT

Background: Malaria is one of the most common major health problems responsible for the death of millions of children, pregnant women and adults. Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today. Plant resources that either treat or prevent parasite invasion desirable in developing countries are potential targets for research and development of alternative malaria drugs. **Objective:** This study investigated the suppressive and prophylactic potentials of extracts and some fractions of *Adansonia digitata* stem bark in *Plasmodium berghei* infected mice. **Methodology:** The albino mice were administered with two different doses (200mg/kg body weight and 400mg/kg body weight) of aqueous extract (AQ), methanolic extract (ME), chloroform fraction (CF) and ethylacetate fraction (EF) of *Adansonia digitata* stem bark for five consecutive days. 5mg/kg body weight dose per day of artemether-lumefantrine and 5mg/kg body weight dose per day of chloroquine was used as positive control while the negative control mice received only the vehicle (5% v/v tween 80). In the prophylactic groups, the mice were pretreated daily for five days before they were challenged with inoculums of 1×10^7 chloroquine-sensitive *P. berghei* infected erythrocyte intraperitoneally. **Results:** The results showed a dose dependent chemosuppression in the fractions and the extract treated groups. The 400mg/kg body weight was more effective with respect to the parasite clearance than the 200mg/kg body weight in all the groups. Both the 200mg/kg and 400mg/kg body weight dose of ethylacetate fraction (EF) exhibited the highest chemosuppression. The chemosuppression caused by Artemether-lumefantrine (AL) and Chloroquine (CQ) treated groups were significantly ($P < 0.05$) higher than the fractions and extract treated groups. The percentage parasitemia also decreased in this manner. There was a mutual delay in parasitemia with EF and ME. The packed cell volume (PCV) increased significantly ($P < 0.05$) in the AL and CQ, and 400mg/kg body weight dose EF and ME respectively and increased for the other fraction and extract used at 400mg/kg body weight dose compared with the control. **Conclusion:** This study showed that EF of *Adansonia digitata* stem bark has potent antimalarial property which could be of future importance in malaria management.

Keywords: antimalaria, *Plasmodium berghei*, *Adansonia digitata*, chemosuppression, chloroquine, artemetherlumefantrine.

INTRODUCTION

Malaria is a complex disease that varies widely in epidemiology and clinical manifestation in different parts of the world. This variability is the result of factors such as the species of malaria parasites that occur in a given area, their susceptibility to commonly used or available antimalarial drugs, the distribution and efficiency of mosquito vectors, climate and other environmental conditions and the behaviour and level of acquired immunity of the exposed human populations.

In 2010, malaria caused an estimated 660,000 deaths, mostly among African children. Globally, an estimated 3.3 billion people are at risk of being infected with malaria and developing disease, and 1.2 billion are at high risk (>1 in 1000 chance of getting malaria in a year). According to the latest estimates, 198 million cases of malaria occurred globally in 2013 and the disease led to 584 000 deaths. The burden is heaviest in the WHO African Region, where an estimated 90% of all malaria deaths occur, and in children aged under 5 years, who account for 78% of all deaths¹.

Clinical symptoms of malaria ranges from acute febrile illness with fever associated with chills, headache, and vomiting to deadly complications like severe anaemia,

respiratory distress in relation to metabolic acidosis, or cerebral malaria which can eventually lead to death. Treatment strategies of malaria aim to terminate the acute blood infection, cure the clinical symptoms, clear hypnozoites from the liver, prevent future relapses and the spread of infection².

Current practice in treating cases of malaria is based on the concept of combination therapy. Most especially, the artemisinin-based combination therapy (ACT) has been recommended because of reduced risk of treatment failure,

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reduced risk of developing resistance, enhanced usage convenience and reduced side effects³. Presently, the production, prescription and application of ACT in the treatment of uncomplicated malarial cases have significantly increased.

Drug resistance has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where the disease had been eradicated. Drug resistance has also played a significant role in the occurrence and severity of epidemics in some parts of the world. Population

movement has introduced resistant parasites to areas previously free of drug resistance⁴. This has prompted research towards the discovery and development of new, safe and affordable anti-malarial chemotherapies.

A number of medications are available to prevent malaria in travelers to malaria-endemic countries. Antimalarial drugs taken for prophylaxis by travelers can delay the appearance of malaria symptoms by weeks or month long after the traveler has left the malaria-endemic area. Generally, these drugs are taken daily or weekly, at a dose that would be used for treatment of a person who had actually contracted the disease. The prophylactic preventive treatment aims to inhibit the parasitization of the red blood cells and consequent multiplication of *Plasmodium falciparum* after infection⁵. The use of prophylactic drugs is practically seldom for full-time residents of malaria-endemic areas, and their use is usually restricted to short-term visitors and travelers to malaria regions. This is due to the cost of purchasing the drugs, negative side effects from long term use, and because some effective antimalarial drugs are difficult to obtain outside of wealthy nations⁶. The use of prophylactic drugs where malaria-bearing mosquitoes are present may encourage the development of partial immunity⁷.

The roles of medicinal plants have become indispensable in the present age, even though one cannot completely overcome the dependence on the synthetic drugs. The medicinal plants and their derivatives have properties which make them safer and alternatives to commercial drugs in many countries. They are not only available at an affordable cost, but are widely distributed and can be propagated by the local population. Most of these plant derivatives from the leaf, stem or roots have cellular components with high potential antioxidants, which are commercially exploited as herbal medicines.

Adansonia digitata is recognized as an effective treatment for many diseases. It is indigenous in many African countries^{8,9}. Many parts of the plant, especially leaves, fruit pulp, seeds and bark fibers, have been used traditionally for medicinal and nutritional purposes^{9,10}. The bark has been sold commercially in Europe for the treatment of fever, particularly that caused by malaria¹¹. Earlier studies by^{12,13,14} suggested that *Adansonia digitata* has significant antimalarial properties. Its medicinal applications include treatment of intestinal and skin disorders and various uses as anti-inflammatory, antipyretic and analgesic agents¹⁵⁻¹⁸. The present study therefore aims at investigating the antimalarial potentials of extract and some fractions of *Adansonia digitata* stem bark in *Plasmodium berghei* infected mice.

MATERIAL AND METHODS

Plant material

The stem of *Adansonia digitata* (Bombacaceae) was collected from Ido-Ekiti, Ekiti State Nigeria. The plant was identified and authenticated by in the herbarium unit of Forest Research Institute of Nigeria (FRIN) with identification number FHI 109806.

Extraction of plant material

The stem bark peels were air-dried at room temperature to avoid possible degradation or denaturation of their putative compounds. The air-dried stem bark of *Adansonia digitata* was blended to powder using an electric blender. This was stored in a glass container. Blended air-dried stem bark was soaked in sufficient volume of methanol for 72 hours at room temperature. It was continually stirred after each 24 hours. After 72 hours, the mixture was then filtered and the filtrate was concentrated using rotary evaporator at 40°C. The concentrate was heated over a water bath to obtain a solvent free extract, which was stored in a refrigerator at 4°C.

Solvent partitioning

About 500mL of distilled water was added to 35g of the dried methanol extract to form a slurry, this was poured inside a separating funnel and washed repeatedly with chloroform until near exhaustion. The marc remaining was finally washed with ethylacetate until near exhaustion. The aqueous fraction was filtered to remove plant fibers. The obtained fractions: chloroform fraction (CF), ethylacetate fraction (EF) and aqueous fraction (AF) were concentrated under pressure using rotary evaporator at 40°C. The concentrate was heated over a water bath to obtain a solvent free extract, which was stored in a refrigerator at 4°C.

Experimental animals

Fifty five albino mice weighing between 18-20g were obtained from the animal house, Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were acclimatized for two weeks in the animal house and fed *ad libitum* on rat chow and water throughout the period of the experiment.

Parasites

The *Plasmodium berghei* was obtained from the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mouse in volumes of 0.2 ml was used to infect the experimental animals intra-peritoneally.

Transfection and treatment

Early malaria infection/suppressive test Estimation of the suppressive effects of some fractions and extracts of *Adansonia digitata* stem bark was carried out according to the method described by Peters¹⁹. Adult albino mice weighing 18 – 22g were inoculated by intraperitoneal injection with standard inoculum of *P. berghei* with 1×10^7 infected erythrocytes. The animals were randomly divided into eleven groups of 5 mice per group and treated for five consecutive days with 200mg/kg and 400 mg/kg body weight orally for each of the fraction and the aqueous and methanol extracts. The AL (Artemether-Lumefantrine) and CQ (Chloroquine) groups received 5mg/kg body weight/day for five days, while the negative control received the vehicle (5% v/v tween 80) only. Blood samples were collected from the mice tails and smeared on to microscope slides to make both the thick and thin film. The blood films were first fixed in 100% methanol and then stained with Giemsa prepared with buffered water (pH 7.2). Parasitemia was examined microscopically (using x 100

immersion oil objective). The packed cell volume (PCV) was determined on day of infection and day five by the microhematocrit method. Percentage parasitemia and percentage clearance/chemosuppression were estimated.

Prophylactic test

Estimation of the Prophylactic effects of some fractions and extracts of *Adansonia digitata* stem bark was carried out according to the method described by Peters¹⁹. The animals were divided into eleven groups of five mice each and they were pretreated for five days using two different doses (200mg/kg body weight/day and 400mg/kg body weight/day) for each of the fraction and the aqueous and methanol extracts. The AL (ArtemetherLumefantrine) and CQ (Chloroquine) groups were equally pretreated once with 5mg/kg body weight/day for five days, while the negative control received the vehicle (5% v/v tween 80) only. After five days the mice were transfected intraperitoneally with an inoculum size of 1×10^7 of chloroquine sensitive strain of *plasmodium berghei* infected erythrocytes. After 72 hours blood samples were collected from the mice tails and smeared on to microscope slides to make both the thick and thin film. The blood films were first fixed in 100% methanol and then stained with Giemsa prepared with buffered water (pH 7.2). Parasitemia was examined microscopically (using x 100 immersion oil objective). Slides were collected for five consecutive days. The packed cell volume (PCV) was determined on day of infection and day five by the microhematocrit method. Percentage parasitemia and percentage clearance/chemosuppression were estimated.

%Parasitemia= (Total number of parasitized cells/Total number of cell) x 100%

%Clearance/Chemosuppression= [(Negative control parasitemia) – (Parasitemia with drug)]/Negative control parasitemia.

Statistical analysis

Results were expressed as mean \pm standard error of mean. The Duncan multiple range test and student t-test were used to analyze and compared the results at 95% confidence level. Values of $p < 0.05$ were considered significant.

RESULTS

Early infection/Suppressive test

Table.1 showed the effect of some fractions and extract of *Adansonia digitata* stem bark on early infection. The fractions (chloroform and ethylacetate) and the extract (aqueous and methanol) produced a dose dependent significant ($P < 0.05$) reduction in percentage parasitemia level. The reduction in parasitemia level of 400mg/kg body weight dose/day was significantly ($P < 0.05$) higher than the 200mg/kg body weight/day in all the groups. There was a daily increase in parasitemia level in the negative control group. The parasitemia level in the group treated with artemether-lumefantrine and chloroquine were significantly lowered than the fractions and extract treated group. The artemether-lumefantrine (AL) and chloroquine (CQ) treated group showed the highest chemosuppression/clearance and zero parasitemia was achieved on the third day which was maintained throughout the experiment.

Moreso, the extract and fractions produced a significant ($P < 0.05$) dose dependent chemosuppressive effect at the different doses employed. The fractions and extract treated group on day 5 at 400mg/kg body weight for AQ, ME, EF, and CF caused chemosuppression of 72.98 %, 80.28%, 81.66% and 70.41% respectively. The standard drug, AL and CQ caused 100% and 100% chemosuppressive respectively, which was higher than that of the fractions and extract treated group. Artemether-lumefantrine was found to be more effective than chloroquine.

Table 2. showed the packed cell volume (PCV) in the control and treatment groups. There was an improvement in PCV in a dose dependent manner. The packed cell volume in the negative control was significantly lowered at ($P < 0.05$) when compared with the fractions and extract treated groups. The PCV of AL and CQ treated groups improved significantly than the fractions and extract treated groups. *Prophylactic test*

Table.3 showed the prophylactic effect of fractions and extract of *Adansonia digitata* stem bark on *P. berghei* infected mice. The result obtained showed a delay in parasitemia in all the treatment groups compared to untreated control. The control group showed increase in parasitemia while the artemether-lumefantrine (AL) and chloroquine produced a daily reduction in parasitemia. There was a significantly ($P < 0.05$) decrease in percentage parasitemia at 400mg/kg body weight dose of the fractions (chloroform and ethylacetate) and extract (aqueous and methanol) than the 200mg/kg body weight dose.

The percentage chemosuppression of AL and CQ on day 1 were significantly ($P < 0.05$) higher than both the 200mg and 400mg dose of the fraction and extract. Also, zero parasitemia was achieved on the third day in AL and CQ treated groups and maintained throughout. As the experiment progressed, the activity of EF though lowered than the AL and CQ became significantly ($P < 0.05$) higher than CF, ME and AQ treated groups. This was noticeable both in percentage parasitemia and percentage chemosuppression. The methanolic extract (ME) is nearly as good as EF. There was a significant ($P < 0.05$) decrease

Table 1: Suppressive effects of *Adansonia digitata* stem bark extract and fractions on *P. berghei*-infected mice.

DAYS		1		3		5	
		%P	%C	%P	%C	%P	%C
AQ	200mg	3.27 ± 0.17 ^a	8.40 ± 0.00 ^d	2.77 ± 0.24 ^b	34.54 ± 0.00 ^d	2.10 ± 0.25	58.58 ± 0.00 ^d
	400mg	2.67 ± 0.32 ^{ab}	25.21 ± 0.00 ^b	2.07 ± 0.33	51.06 ± 0.00 ^b	1.37 ± 0.32	72.98 ± 0.00 ^b
ME	200mg	3.10 ± 0.21 ^a 0.00 ^d	13.17 ±	2.70 ± 0.35 ^a 0.00 ^d	36.17 ±	1.70 ± 0.12	66.47 ± 0.00 ^d
	400mg	2.43 ± 0.28 ^{ab}	31.93 ± 0.00 ^b	1.70 ± 0.15	59.81 ± 0.00 ^b	1.00 ± 0.12	80.28 ± 0.00 ^b
EF	200mg	3.00 ± 0.21 ^a	15.97 ± 0.00 ^d	2.43 ± 0.29 ^c	42.55 ± 0.00 ^c	1.63 ± 0.18	67.85 ± 0.00 ^d
	400mg	2.40 ± 0.25 ^{ab}	32.77 ± 0.00 ^b	1.50 ± 0.15 [#] 0.00 ^b	64.54 ±	0.93 ± 0.19 [#]	81.66 ± 0.00 ^b
CF	200mg	3.07 ± 0.23 ^a 0.00 ^d	14.01 ±	2.63 ± 0.32 ^a	37.83 ± 0.00 ^d	1.67 ± 0.15	67.06 ± 0.00 ^d
	400mg	2.77 ± 0.27 ^{ab}	22.41 ± 0.00 ^c	2.30 ± 0.15	45.63 ± 0.00 ^c	1.50 ± 0.15	70.41 ± 0.00 ^{cd}
AL	5mg	1.07 ± 0.15 ^b	70.31 ± 0.00 ^a	0.00 ± 0.00	100 ± 0.00 ^a	0.00 ± 0.00	100 ± 0.00 ^a
CQ	5mg	1.13 ± 0.12 ^b	68.35 ± 0.00 ^a	0.00 ± 0.00	100 ± 0.00 ^a	0.00 ± 0.00	100 ± 0.00 ^a
Contro	5%v/v	3.57 ± 0.07 ^a	-	4.23 ± 0.12 ^a	-	5.07 ± 0.12 ^a	-

200mg=200mg/kg body weight dose; 400mg= 400mg/kg body weight dose; 5mg= 5mg/kg body weight dose; 5%v/v= 5%v/v of tween-80; %P= percentage parasitemia; %C= percentage clearance. Each value is a mean of several determinations ± SE after five days of exposure to treatment.

Table 2: Packed Cell Volume (PCV in percentage) of the suppressive treated groups.

Day	Of	Treatment	AQ	ME	EF	CF	Control	
Infection		200mg	45.33 ± 0.33	40.67 ± 1.76	44.33 ± 0.88	43.00 ± 1.00	45.67 ± 1.45	
		400mg	45.00 ± 2.08	45.67 ± 3.18	42.00 ± 2.52	45.00 ± 2.89		
		Artemether-Lumefantrine 5mg/kgbw =	46.33 ± 3.38					
		Chloroquine 5mg/kgbw =	43.33 ± 3.93					
Last day of Treatment		Treatment	AQ	ME	EF	CF	Control	
		200mg	30.67 ± 2.40	31.00 ± 0.58	30.00 ± 1.15	30.67 ± 1.76	14.00 ± 1.15 [*]	
		400mg	34.00 ± 1.00	36.00 ± 2.65	34.67 ± 1.76	34.00 ± 1.15		
		Artemether-Lumefantrine 5mg/kgbw =	44.00 ± 3.51 ^b					
		Chloroquine 5mg/kgbw =	42.33 ± 3.33					

**P. berghei* infected mice which did not receive any treatment except the vehicle. Each value is a mean of several determinations ± SE after five days of exposure to treatment.

in percentage parasitemia on day 5 when compared to AQ and CF. On day five at 400mg/kg body weight dose, AQ, ME, EF, and CF caused chemosuppression of 61.48%, 68.31%, 74.76% and 62.05% respectively while the standard drug AL and CQ caused 100% and 100% chemosuppression respectively. AL was observed to cause a delay in parasitemia than CQ.

Table.4 showed the packed cell volume of the prophylactic groups. The PCV improved in dose dependent manner. The AL and CQ treated group had the highest value compared with all other treated groups and negative control. The AQ had the lowest PCV both at 200mg/kg and 400mg/kg body weight. EF had the highest value for PCV and differed insignificantly from other treated groups.

DISCUSSION

One of the greatest challenges facing malaria control is the spread and intensification of parasite resistance to antimalarial drugs. The availability of limited number of effective drugs has led to increasing difficulties in the development of antimalarial drug policies and adequate disease management. Surveys have shown rates of treatment failure higher than 50 per cent for chloroquine in most affected regions, as well as poor efficacy of sulfadoxin-pyrimethamine in sub-saharan Africa and Southeast Asia²⁰.

The rationale for ACTs is that the short-acting but highly potent artemisinin delivers a rapid reduction in parasite biomass, with the remaining parasites being removed by the intrinsically less active but more slowly eliminated partner drug. The ACTs have an additional benefit of

dramatically reducing the production of gametocytes, the sexual stage of the parasite²¹.

ACTs have now become an intrinsic part of the global antimalarial treatment policy endorsed by the World Health Organization and are deployed in >80 malaria endemic countries. A variety of excellent alternatives are available, each with a slightly different profile of tolerability, adherence, availability, cost, efficacy, and

two (200mg/kg and 400mg/kg) doses while the AL (5mg/kg) and CQ (5mg/kg) recorded a zero parasitemia as from day 3. This observation also agrees with the submission of Kiseko *et al.*²³ who reported that the effect of standard antimalarial drugs, chloroquine (CQ) and artemether/lumefantrine (AL) exerted 100% suppression on the third day at 5mg/kg body weight against *Plasmodium berghei* infected mice. The higher efficacy of

Table 3: Prophylactic effects of *Adansonia digitata* stem bark extract and fractions on *P. berghei*-infected mice.

DAYS		1		3		5	
		%P	%C	%P	%C	%P	%C
AQ	200mg	3.50 ± 0.24 ^a	6.67 ± 0.00 ^c	2.90 ± 0.18 ^b	27.50 ± 0.00 ^c	2.53 ± 0.03 ^b	51.99 ± 0.00 ^c
	400mg	2.78 ± 0.35 ^{ab}	25.87 ± 0.00 ^d	2.40 ± 0.25 ^{bc}	40.00 ±	2.03 ± 0.12 ^{bc}	61.48 ± 0.00 ^{bc}
ME	200mg	3.30 ± 0.16 ^a	12.00 ± 0.00 ^c	2.52 ± 0.35 ^b	37.00 ± 0.00 ^c	2.07 ± 0.19 ^b	60.72 ± 0.00 ^c
	400mg	2.40 ± 0.51 ^{ab}	36.00 ± 0.00 ^d	1.80 ± 0.15 ^{bc}	55.00 ± 0.00 ^{bc}	1.67 ± 0.59 ^{bc}	68.31 ± 0.00 ^{bc}
EF	200mg	3.18 ± 0.10 ^a	15.20 ± 0.00 ^c	2.25 ± 0.47 ^b	43.75 ± 0.00 ^c	1.77 ± 0.29 ^{bc}	66.22 ± 0.00 ^c
	400mg	2.12 ± 0.35 ^{ab}	43.47 ± 0.00 ^c	1.50 ± 0.25 ^{bc}	62.50 ± 0.00 ^b	1.33 ± 0.17 ^c	74.76 ± 0.00 ^b
CF	200mg	3.24 ± 0.17 ^a	13.60 ± 0.00 ^c	2.28 ± 0.21 ^b	43.00 ± 0.00 ^c	1.87 ± 0.39 ^{bc}	64.52 ± 0.00 ^c
	400mg	2.87 ± 0.50 ^{ab}	25.33 ± 0.00 ^c	2.28 ± 0.15 ^b	43.00 ± 0.00 ^c	2.00 ± 0.25 ^b	62.05 ± 0.00 ^c
AL	5mg	0.68 ± 0.07 ^b	81.87 ± 0.00 ^a	0.00 ± 0.00	100 ± 0.00 ^a	0.00 ± 0.00	100 ± 0.00 ^a
CQ	5mg	1.00 ± 0.12 ^b	73.33 ± 0.00 ^b	0.00 ± 0.00	100 ± 0.00 ^a	0.00 ± 0.00	100 ± 0.00 ^a
Contro	5%v/v	3.75 ± 0.28 ^a	-	4.00 ± 0.07 ^a	-	5.27 ± 0.19 ^a	-

200mg=200mg/kg body weight dose; 400mg= 400mg/kg body weight dose; 5mg= 5mg/kg body weight dose; 5%v/v= 5%v/v of tween-80; %P= percentage parasitemia; %C= percentage clearance.

Each value is a mean of several determinations ± SE after five days of exposure to treatment.

effectiveness²². Of the newer combinations, artemetherlumefantrine (AL) and dihydroartemisinin-piperazine (DP) are prime examples: both coformulations have been well tolerated with excellent efficacy in almost all studies conducted.

The use of artemisinin combination therapy is however limited due to its high cost and accessibility. Medicinal plants are usually considered to be promising candidates as alternative and rich source of new drugs. The work of Ramadan *et al.*,¹⁵ on the non-toxicity *Adansonia digitata* explains why various parts of plant, especially leaves, fruit pulp, seeds and bark fibers, have been used traditionally for medicinal and nutritional purposes^{9,10}. The trends of parasitemia among the extracts and fractions of *Adansonia digitata* stem bark treatment groups and negative control appeared to demonstrate the antimalarial potential of the plant. Parasitemia in the negative control was higher than all the treatment groups. This showed that all the treatment had effect on the growth of *Plasmodium berghei* parasites in mice which agrees with the earlier work of^{12,13,14}.

The extracts and the solvent fractions of the stem bark of *Adansonia digitata* used exerted significant (P< 0.05) dose dependent reduction in percentage parasitemia level at the

artemether lumefantrine and chloroquine observed could be as a result of slow absorption and poor bioavailability of the extract.

The results of this study showed that there was a significant (P< 0.05) reduction and delay in parasitemia in the extracts and the fractions used. The results indicate that the extract of *Adansonia digitata* used has blood schizonticidal activity. The antimalarial activity of *Adansonia digitata* stem bark observed in this study could be linked with the traditional use of the plant in the management of the disease in Nigeria. Although the antimalarial mechanism of action of this extract has not yet been elucidated, suggested mechanisms of action for some antimalarial compounds isolated from plants include inhibition of hemozoin polymerization in the parasite^{24,25,26} inhibition of an essential enzyme, *Plasmodium falciparum* lactate dehydrogenase (pfLDH) responsible for generation of energy within the parasite through glycolysis²⁷; intercalation with the parasite DNA²⁸ or inhibition of protein synthesis²⁹. The antimalarial potentials of this plant could have been through either of these mechanisms or by some other unknown mechanism.

The extracts and fractions used in this study produced decrease in percentage parasitemia and increase

chemosuppression/clearance. Pretreatment of all groups increased the antiplasmodial activity of the extracts used possibly through a build up in the putative compounds in the animals prior to transfection, thus producing higher activity.

Studies have also shown the antimalarial activity of alkaloids and flavonoids in plants^{30,31}. The observed antimalarial potential in the extract treated group may be attributed to the presence of various secondary metabolites. The PCV values obtained in the result showed an improvement over therapeutic treatment. AL and CQ had the highest values while chloroform fraction has the

Table 4: Packed Cell Volume (PCV in percentage) of the prophylactic treated groups.

Day of Infection	Treatment	AQ	ME	EF	CF	±	Control
Day of Infection	200mg	46.20 ± 2.22	1.80 ± 1.69	47.20 ± 1.69	45.20	± 1.53	45.80 ± 2.78
	400mg	43.50 ± 2.18	45.00 ± 2.35	47.33 ± 1.58	47.40	± 2.50	
	Artemether-Lumefantrine 5mg/kgbw = 48.20 ± 2.44						
	Chloroquine 5mg/kgbw = 46.00 ± 1.79						
Last day of Treatment	200mg	22.60 ± 1.36	24.00 ± 1.92	22.60 ± 1.33	22.80	± 0.97	*Control 15.20 ± 1.77
	400mg	33.00 ± 1.47	37.00 ± 3.11	39.40 ± 1.08	36.80	± 2.54	
	Artemether-Lumefantrine 5mg/kgbw = 44.20 ± 2.08						
	Chloroquine 5mg/kgbw = 47.20 ± 1.77						

**P. berghei* infected mice which did not receive any treatment except the vehicle. Each value is a mean of several determinations ± SE after five days of exposure to treatment.

highest value among the extracts and fractions used at 400mg/kg body weight. The observed antimalarial activity is consistent with its traditional use in malaria management.

Further purification of the putative ethylacetate fraction could make it a promising candidate both in the antiplasmodial activity and a better PCV booster in malarial treatment. An inverse relationship exists between PCV and density of malaria³². The density and/or proliferation rate of parasites is analogous to the 'state' of multicellular organisms. During infections, numerous factors, such as competition with unrelated genotypes, other species, drug treatment, immune responses, RBC resource availability and host nutritional status can all change dramatically and impact upon parasite proliferation in the host. Thus, in-host environmental factors that negatively affect proliferation can be considered as 'stressors' which impact on the 'state' of parasites.

The decrease of parasitemia density and the increase PCV reflect the development of naturally acquired immunity against malaria. The mean PCV difference between treated and untreated groups suggests that malaria is a greater contributor to anemia³³.

CONCLUSION

The stem bark extract of *Adansonia digitata* exhibited a significant suppressive and prophylactic effect against *Plasmodium berghei* infected mice as demonstrated by the reduction in the level of parasitaemia dose dependently. The results of this study showed that ethylacetate fraction has the highest activity suggesting the presence of certain bioactive compounds in it. This effect may be attributed to the presence of alkaloids, terpenes and flavonoids that have been implicated in antiplasmodial activity. It is evident based on these findings that *Adansonia digitata* possess promising and potent antimalarial effect which justifies its

usage in folk medicine for the management of malaria. Further work is suggested to isolate, identify and characterize the active principle(s) from this plant.

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