

## In Vivo Anti-Plasmodial Effect of Ethanol and Aqueous Extracts of *Alchornea cordifolia*

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### Abstract

Ethanol and aqueous extracts of the leaves, stem bark and roots of *Alchornea cordifolia* were tested for *in vivo* anti-malarial activity in 27 Swiss albino mice infected with chloroquine resistant *Plasmodium berghei* NK 65. The plant extracts were administered to the animals intraperitoneally, at a dose of 100 mg/kg b.w.t., and compared with Artesunate (1.6 mg/kg) and Chloroquine (10 mg/kg) administered and an untreated control group. Ethanol yielded more extracts from the leaves and stem bark than water. Tannins, flavonoids, anthraquinones, alkaloids and glycosides were widely distributed in both ethanol and aqueous extracts of the plant. All animals administered the plant extracts and Artesunate gained weights in comparison with the chloroquine and untreated groups. The animals in the untreated and chloroquine treated groups died due to the infection at the end of days 5 and 10 respectively. The Artesunate group was eradicated of parasitemia by the 7<sup>th</sup> day. Comparison of the parasitemia levels of the plant's extracts treated groups between days 0 and 14, showed that while ethanol leaves, stem bark and root extracts reduced parasitemia by 72.22%, 50.00% and 20.00% respectively, those of aqueous extracts reduced parasitemia marginally by 0%, 9.50% and 19.17% respectively. The results indicate that ethanol extracted active phytochemicals more from the leaves and stem bark of the plant than water, and that the presence of these secondary metabolites might be responsible for the higher anti-malarial activity observed with the leaves and stem bark extracts. This confirms the folkloric preferential use of *A. cordifolia* leaves as an anti-malarial agent.

**Keywords:** Anti-malarial therapy; *Plasmodium berghei*; *Alchornea cordifolia*; Plant extracts; *In vivo* study

### Introduction

Malaria is one of the most important tropical diseases and the greatest cause of hospitalization and death among children aged 6 months to 5 years [1]. Malaria has great morbidity and mortality than any other infectious disease of the world [2]. The World Health Organisation reported that there were an estimated 246 million malaria cases distributed among 3.3 billion people at risk in 2006, causing at least a million deaths. Data from Nigeria further reveals that 92% of pregnant women and children under 5 years of age are very susceptible to malaria because of their low immunity and resistance [3]. Approximately 80% of malaria cases in the world are estimated to be in Africa where the disease is endemic [4].

Five species of the plasmodium parasites can infect humans. However, the most wide spread and virulent form of the disease is caused by *Plasmodium falciparum*, and is responsible for about 80% of all malaria cases, and about 90% of the deaths [5]. *Plasmodium vivax*, *P. ovale* and *P. malariae* have milder symptoms in humans and not generally fatal. A fifth specie, *P. knowlesi*, is a zoonosis that causes malaria in macaques and also in humans. The female anopheles mosquito transmits these parasites to humans. In Africa and other countries where malaria is endemic, traditional medicinal plants are frequently used to treat or cure malaria [6]. It is a fact that conventional anti-malarial drugs such as chloroquine, quinine and artemisinin derivatives originated from plants [7]. It is therefore important to investigate the anti-malarial activity of medicinal plants in order to determine their potentials as source of new anti-malarial agents [8].

*Alchornea cordifolia* known as Christmas Bush is a shrub or small tree found abundantly along the coastal area of the West African Sub-region. *Alchornea cordifolia* is used for treatment of a variety of diseases by traditional medical practitioners in Nigeria. Its different parts had been used to treat diarrhoea, wounds, sores, and cuts [9]. *A. cordifolia* is also reported to possess a multiplicity of biological effects. It is anti-

bacterial [10], spasmolytic [11], anti-inflammatory [12], anti-diarrhoeal [13], antioxidant [14] and antimicrobial [15] agent. These diverse pharmacological actions have been linked to several active principles isolated from the leaves, root and stem of *A. cordifolia*. In spite of the wide traditional use of *A. cordifolia*, very little is known about its anti-malarial effect. This study is therefore aimed at investigating the phytochemical composition and *in vivo* anti-malarial effect of ethanol and aqueous extracts of the leaves, stem bark and roots of the plant.

### Materials and Methods

#### Plant identification

The Plant *Alchornea cordifolia* used for this research was collected from the Botanical Garden of School of Agricultural Technology, Federal University Of Technology Owerri (FUTO) Nigeria. The plant was identified by Mr. Francis Iwunzeof the Department of Forestry and Wild life, School of Agricultural Technology, FUTO. The plant was authenticated by a plant taxonomist, Dr E.N Mbagwu of Imo State University Owerri. The plant was prepared and kept at the University herbarium with voucher number IMSU H524.

#### Plant extraction processing

The apparently healthy parts of the plant (leaves, root and stem

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bark) were harvested in large quantities and air-dried for about 3 weeks to a constant weight under shade in the laboratory. The dried samples were ground into powdered form using an electric grinder (Saisho 200W) and stored separately.

Using maceration method, 100 g of each powdered sample was soaked separately in 600 ml each of distilled water and ethanol of analytical grade respectively, for 72 hour. Each sample solution was filtered using Whatman No.1 filter paper. The aqueous and ethanol filtrates were separately concentrated using water-bath at 45°C. All the extracts were weighed and then stored in well stoppered containers and kept in a refrigerator at 4°C until used.

**Animals:** Twenty-seven swiss albino mice weighing 15-20 g used for this study were obtained from University of Nigeria, Nsukka. The mice were acclimatized to laboratory conditions for a period of 14 days before the commencement of this study. The animals were fed with standard mouse feed (Vital finisher, Nigeria) and clean drinking water *ad libitum*. The mice were weighed divided into 9 groups of 3 mice each. The groups consisted of 3 ethanol extract/treatment groups, 3 aqueous extract/treatment groups, artesunate standard control, chloroquine standard control and untreated control groups.

### Culture of the chloroquine resistant *Plasmodium berghei* parasites

The chloroquine resistant *Plasmodium berghei* (NK 65) used was obtained from Department of Veterinary Pathology, University of Nigeria, Nsukka. The parasite was maintained by sub-passaging into healthy mice via an intraperitoneal route as earlier described [5,16,17]. Briefly, one millilitre of *P. berghei* infected blood was diluted with 10 ml of phosphate buffer saline (PBS) pH 7.2. The dilution was such that each 0.2 ml had approximately  $10 \times 10^7$  infected red cells/parasites per kg of body weight. Infection of each mouse was effected with a single intraperitoneal inoculum of 0.1 ml of diluted infected blood. Parasitemia was confirmed in the animals after 24 h of infection by making a smear of the blood on a microscopic glass slide, staining with Giemsa and viewing under the microscope.

### *In vivo* treatment of the infected albino mice

Tests were performed using a 4-day curative standard test using the chloroquine resistant *Plasmodium berghei* NK 65 [5,16-18].

Twenty four hours (24 h) after infecting the mice with the malaria parasites and parasitemia confirmed, the plant's ethanol and aqueous extracts were administered to the test groups at a dose level of 100 mg/kg body weight (b.w.t) for a total period of 4 days. The dose level of 100 mg/kg b.w.t of the extract was adopted based on a preliminary study carried out in mice [19]. Artesunate and chloroquine standards were administered once a day to the standard control groups at the standard dose of 1.6 and 10 mg/kg b.w.t respectively for 4 days [19]. The untreated control group mice were not treated but given the vehicle (normal saline). All drug administration was done through the intraperitoneal route [5].

### Estimation of parasitemia

Parasitemia was monitored in all the groups starting from day 0 to day 14 using thick and thin blood films made from blood obtained from the tail vein of mice [5,18]. The smear was air-dried, stained with 10% Giemsa at pH 7.2 for 15 mins, washed under running tap and allowed to dry. After which it was examined under the microscope with x100 objective lens to access the level of parasitemia. Percentage parasitemia was calculated according to the method outlined by Iwalewa et al. [20,21] as:

$$\text{Percentage parasitemia} = \frac{\text{No of parasitemia in treated}}{\text{No of parasitemia in control}} \times \frac{100}{1}$$

This is normally assumed to be,

$$\frac{\text{No of parasitemia in treated}}{500} \times \frac{100}{1}$$

### Phytochemical studies

Various parts of *Alchornea cordifolia* extracted with ethanol and water were screened for phytochemicals using standard procedures [7,22].

### Statistical analysis

Data generated were expressed as mean  $\pm$  standard deviation. Chi-square, One-way analysis of variance (ANOVA) and post-hoc Tukey tests were used to analyze the data with the aid of Graph Pad Prism version 5.3. Differences between means at  $p \leq 0.05$  were considered statistically significant.

### Results

#### Yield of extracts

The yield of ethanol showed that more extracts were obtained from leaves followed by stem bark and root respectively (Table 1). For aqueous extract, same applies, more extracts were from the leaves followed by stem bark and root respectively (Table 1). Generally, ethanol yielded more extracts than the aqueous solvent, except for the root where aqueous solvent yielded more.

#### Phytochemistry

Phytochemical analysis of the ethanol and aqueous extracts of different parts of *A. cordifolia* showed the presence of some active ingredients like tannins, saponins, flavonoids, terpenoids, steroids and alkaloids (Table 2).

#### Body weight of experimental animals

The body weight of the animals infected with *P. berghei* in all the groups and treated with ethanol and aqueous extracts including the artesunate standard control group showed an increase in their body weights (Figure 1). The untreated and chloroquine control groups showed a decrease in their body weights (Figure 1).

#### Level of parasitemia

The negative untreated control group infected with *P. berghei* and the group treated with chloroquine became weak after day 6 and later died. For the infected mice treated with artesunate (1.6 mg/kg body weight), the parasite cleared on the day 6 of treatment and the animals survived. However, when the infected animals treated with crude ethanol extract of the root and leaf extract of *Alchornea cordifolia* were compared on day 5, there was a great reduction in parasitemia level from  $(1.20 \pm 0.16$  to  $1.00 \pm 0.16)$  and  $(1.86 \pm 0.80$  to  $0.70 \pm 0.10)$

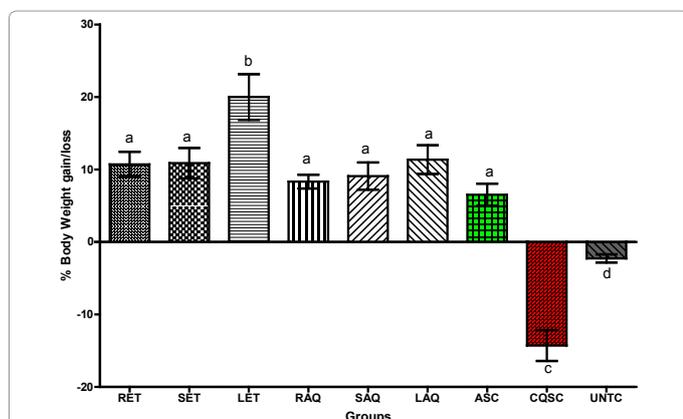
Samples	Solvent (ml)	Sample weight (g)	Weight of Extract (g)	
			Ethanol	Water
Leaves	600	100	20	14
Stem bark	600	100	9	7
Root	600	100	3	5

**Table 1:** Yield of crude extracts from different parts of *A. cordifolia* using ethanol and aqueous solvents.

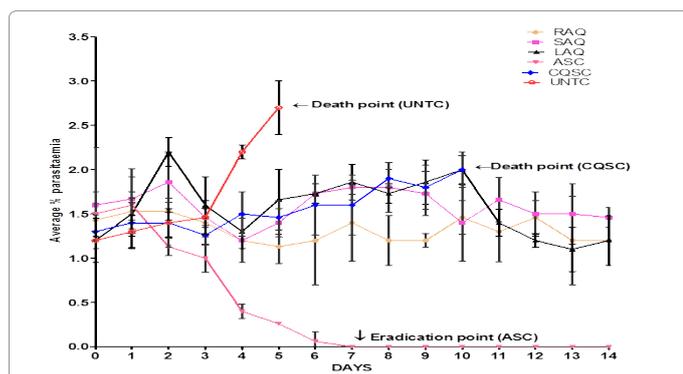
Test	Specific test	Result/Inference					
		Aqueous Leaves	Aqueous Root	Aqueous Stem	Ethanol leaves	Ethanol Root	Ethanol Stem bark
Tannins	Test with ferric chloride solution	+	+	+	+	+	+
Phlobatannins	Test with dilute hydrochloric acid	-	+	-	-	-	-
Flavonoid	Shinoda's test	+	+	+	+	+	+
Saponin	Frothing test	+	+	+	-	-	-
Anthraquinones	Borntrager's test	+	+	+	+	+	+
Terpene	Copper acetate test	+	+	-	-	-	+
Alkaloid	General test for alkaloids	+	+	-	+	+	+
Test for glycosides	Salkowskii test	+	+	+	+	+	+

+ = Present: - = Absent

**Table 2:** Results of qualitative phytochemical screening of aqueous and ethanol extracts of different parts of *A. cordifolia*.



**Figure 1:** Percentage body weight gain/loss of the parasitized experimental mice. Bars bearing different letters are statistically significant ( $p < 0.05$ ). RET, Ethanol root extract; SET, Ethanol stem extract; LET, Ethanol leaves extract; RAQ, Aqueous root extract; SAQ, aqueous stem extract; LAQ, aqueous leaves extract; ASC, Artesunate Standard Control; CQSC, Chloroquine standard control; UNTC, Untreated control.



**Figure 2:** *In vivo* effect of crude aqueous extract of different parts of *A. cordifolia* on chloroquine resistant *Plasmodium berghei* NK 65. RAQ, Aqueous root extract; SAQ, aqueous stem extract; LAQ, aqueous leave extract; ASC, Artesunate Standard Control; CQSC, Chloroquine standard control; UNTC, Untreated control.

respectively as compared to ( $1.20 \pm 0.25$  to  $2.70 \pm 0.30$ ) and ( $1.30 \pm 0.19$  to  $1.46 \pm 0.19$ ) in the untreated control and chloroquine standard control groups respectively (Figures 2 and 3).

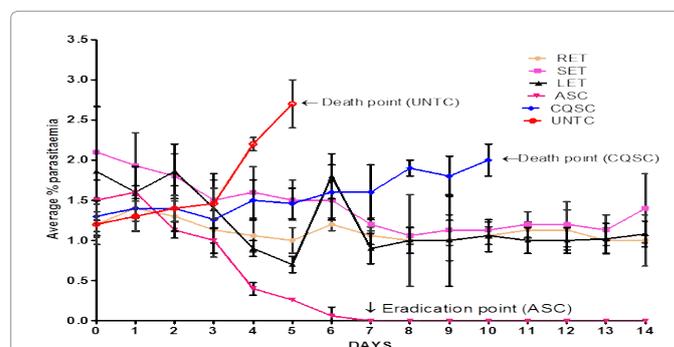
The comparisons were made between days 0, day 3, day 7 and day 14. It showed that the parasitemia level of the infected animals treated

with the ethanol extracts of the root and leaf of *A. cordifolia* showed a very significant difference ( $p < 0.05$ ) when compared with the other treatment groups, untreated infected animals and the chloroquine standard control group (Figure 3). However, there was no significant difference ( $p > 0.05$ ) observed when the infected mice treated with chloroquine were compared with untreated infected mice (Figures 2 & 3). It should be noted though that the other extracts of *A. cordifolia* showed anti-malarial activity, but was not as significant as the activity exhibited by the ethanol extracts of leaf and root, with the leaf extract having the greater activity (Figures 2 and 3).

The parasitemia was considerably reduced but not completely cleared in the groups treated with ethanol and aqueous extracts, as noted in Figures 2 and 3. However, this did not lead to the survival of the experimental animals as some of the mice started dying as the parasite load increased after treatment was withdrawn as opposed to the group treated with artesunate which survived.

## Discussion

The use of ethanol and water as solvents for the extraction of active metabolites in plant is in consonance with folkloric procedure of the use of decoctions and alcohol extracts. From Table 1, it shows that ethanol as solvent generally but non-significantly ( $\chi^2 = 1.201$ ;  $p = 0.5485$ ) yielded more extracts from the leaves and stem bark than water. For both ethanol and water, the trend of extract yield was leaves > stem bark > root. The percentage (%) yield of the extracts varied, probably due to the solvent medium. The methods employed required no heat, thus preserving most of the thermo-labile metabolites in their active forms.



**Figure 3:** *In vivo* effect of crude ethanol extract of different parts of *Alchornea cordifolia* on chloroquine resistant *Plasmodium berghei* NK 65. RET, Ethanol root extract; SET, ethanol stem extract; LET, ethanol leave extract; ASC, Artesunate Standard Control; CQSC, Chloroquine standard control; UNTC, Untreated control.

Results on Table 2 show that tannins, flavonoids, anthraquinones, alkaloids and glycosides were widely distributed in both ethanol and aqueous extracts of the leaves, stem bark and roots of *A. cordifolia*. On the other hand, saponins and terpenes were found present in the aqueous extracts, but absent in the ethanol extracts, while phlobatannins were detected only in the aqueous root extract. The high preponderance of these phytochemicals may be responsible for the antimalarial activity exhibited by the plant [16,19].

Administration of both the ethanol and aqueous extracts of *A. cordifolia* elicited percentage increases in the animals' body weights with only the ethanol leaves extract causing significantly ( $p < 0.05$ ) increased gain in total body weight (Figure 1). On the other hand, there were observed significant ( $p < 0.05$ ) decreases in percentage body weights of the animals administered chloroquine standard drugs and the untreated control animals. The observed increases in body weights of the animals administered the extracts may not be attributed to the presence of phytochemicals because such secondary metabolites are known to be non-nutritional. Furthermore, there were observed increases in body weights of animals administered Artesunate standard drug which is a purified chemical substance. This indicates that the body weight increases in these groups of animals can directly be associated with their anti-malaria activities, helping the animals overcome the infection and hence giving them leverage to continue to metabolize and grow without serious hindrances.

In this study, the ethanol extract demonstrated higher anti-malarial activity than the crude aqueous extract. Ethanol is less dense than water and might possess greater diffusibility in the same medium than water. This might account for the greater efficacy exhibited by ethanol extract over the aqueous extract. It might also be possible that the active metabolites were more soluble in ethanol than water conferring this advantage on the ethanol extract. It can be clearly seen from the results that percentage parasitemia was reduced by the plant's crude extracts in *Plasmodium berghei* infected mice, pointing to the fact that the plant is endowed with antimalarial activity. Evidence comes for this assertion from studies that reported antimalarial activity of other species of the same genus such as *Phyllanthus amarus*, as can be seen in the study 'In vivo antimalarial effects of ethanol and crude aqueous extracts of *Phyllanthus amarus*'.

However, complete eradication of parasitemia was not achieved with any of the extracts when compared with the use of artesunate, but the observation of overall high percentage antimalarial activity of the plant, especially with ethanol leaves and stem bark extracts, indicates the need for further studies towards identification, isolation and purification of the active components apparently present in the ethanol extract.

## Conclusion

The results of this study have shown that the ethanol leaves and stem bark extracts of *Alchornea cordifolia* exhibited high anti-malarial activities than the aqueous extracts. It is noteworthy that the other extracts of *A. cordifolia* showed mild anti-malarial activity. The study therefore justifies the traditional use of the plant leaves in the treatment of malaria. Nevertheless, further work is encouraged.

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