# Antiplasmodial Activity of Methanolic Stem Bark Extract of *Anthocleista grandiflora* in Mice

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## Abstract

*Malaria is an infectious disease affecting a large number of the world's population. The search for new antimalarial chemotherapies has become increasingly urgent due to the parasites' resistance to current drugs. The invivo antiplasmodial effect of Anthocleista grandiflora in mice was investigated in this study. Anthocleista grandiflora is a medicinal plant used for the management of malaria and other ailments. Four models were investigated such as the oral acute toxicity of the methanolic extract of Anthocleista grandiflora stem bark, suppressive effect against early infection, curative effect against established infection and prophylactic effect against residual infection in chloroquine-sensitive Plasmodium berghei berghei infected mice. Acute toxicity ($LD_{50}$) test of the crude extract on malaria parasites P. berghei gave a value of 1000mg/kg body weight. The in vivo antimalaria activity of the Anthocleista grandiflora extract doses (300, 500 and 700mg/kg body weight) against P. berghei berghei showed significant ($p<0.05$), dose-dependent activity for suppressive, curative and prophylactic tests. These results showed the importance of Anthocleista grandiflora plant with a pharmacological activity for the development of new drug for treatment or prophylaxis against malarial and other ailments.*

**Keywords:** Antimalaria, malaria, *Plasmodium berghei*, *Anthocleista grandiflora*

## *1.0 Introduction*

Malaria is a mosquito-borne infectious disease of humans and other animals caused by eukaryotic protists of the genus *Plasmodium*. The disease results from the multiplication of Plasmodium parasites within red blood cells, causing symptoms that typically include fever and headache, in severe cases progressing to coma or death. It is widespread in tropical and subtropical regions, including much of Sub-Saharan Africa, Asia, and the Americas.
There were an estimated 225 million cases of malaria worldwide in 2009 (WHO, 2010). An estimated 655,000 people died from malaria in 2010 (WHO, 2011) a 5% decrease from the 781,000 who died in 2009 according to the World Health Organization's 2011 World Malaria Report, accounting for 2.23% of deaths worldwide (WHO, 2010). Ninety percent of malaria-related deaths occur in sub-Saharan Africa, with the majority of deaths being young children. In Nigeria, the disease is a major health problem with stable transmission throughout the country. It accounts for about 50% of out-patient consultation, 15% of hospital admission and is the prime amongst the top three causes of death in the country. More importantly, it is a social and economic problem which consumes about US $5 million in various control attempts (WHO, 2004).

Five species of *Plasmodium* can infect and be transmitted by humans. Severe disease is largely caused by *Plasmodium falciparum* while the disease caused by *Plasmodium vivax*, *Plasmodium ovale* (Sutherland et al, 2010) and *Plasmodium malariae* is generally a milder disease that is rarely fatal. *Plasmodium knowlesi* is a zoonosis that causes malaria in macaques but can also infect humans (Fong et al, 1971 and Singh et al, 2004).
Malaria transmission can be reduced by preventing mosquito bites by distribution of mosquito nets and insect repellents, or by mosquito-control measures such as spraying insecticides and draining standing water (where mosquitoes breed). The challenge of producing a widely available vaccine that provides a high level of protection for a sustained period is still to be met, although several are under development (Kilama, W and Ntoumi, F. 2009).

A number of medications are also available to prevent malaria in travelers to malaria-endemic countries (prophylaxis). The acquisition of multidrug resistance resulting to reduction in the effectiveness of a drug in curing a disease or improving a patient's symptom has led to a serious impediment to improved health care issues. The diversity of resistance types will require that public health measures to control malaria be region specific (Bennett, P. M. 2008). Plants have always been considered to be a possible alternative and rich source of new drugs. The search for malaria remedies in plants and improved interest in plant drugs by many communities staying in endemic area led to the use of *Anthocleista grandiflora* plant in establishing the scientific basis for the treatment of malaria.

*Anthocleista grandiflora*, commonly known as the forest fever tree is a large tree of moist forests in the eastern and south-eastern African tropics and the comores. It is a member of the family *Gentianaceae* and a small genus of only 14 species. It is a tall, slender tree up to 30m with a preference for forests in high rainfall areas.

The tree is sometimes epiphytic with auxiliary spines or tendrils, leaves opposite, occasionally alternate, rare venticillate, fascicute or in a whorl, often reduced to lines connecting petiole bases. The flowers are in cymes these are often grouped into thyrses; sometimes umbel-like, scorpioid or reduced to single flower bracts usually small. The flowers are usually bisexual and cream coloured. It is not edible as food but possesses root, stems, bark, leaves and flowers which are claimed to have medicinal properties (Palmer and Pitman; 1972). .

In Southern Africa, bark decoctions are used traditionally to treat malaria (Palmer and Pitman; 1972). Regionally, preparations of the bark has also found use as an anthelmintic specifically for roundworms (Githers 1949), antidiarrhoeal (Watt and Bieyer-Brandwijk 1962; Mabogo 1990) and to treat diabetes, high blood pressure and venereal diseases (Mabogo 1990). Furthermore, in the north continent, epilepsy is remedied with the aid of the stem bark decoction (Neuwinger 2000).

## *2.0 Materials and Methods*

### 2.1 Plant Collection and Authentication

Fresh stem bark of *Anthocleista grandiflora* was collected in March, 2011, at Choba area, Port Harcourt, Rivers State. The plant specimen was identified and anuthicated by Dr. l.K. Agbagwa, a taxonomist in the dependent of plant science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria.

### 2.2 Preparation of plant materials

The plant stem barks were sorted to eliminate any dead matter and other unwanted particles. The voucher specimen was thinly on the flat clean tray (to prevent spoilage by moisture condensation) and allowed to dry at room temperature for seven days (Sofowora 1982). The dried plant materials were grounded into powder using an electric mill.

The crude extract was prepared by cold maceration technique (O'Neill at el 1985). The plant material was extracted by refluxing 45g of the specimen in 2.5L of methanol (80%) for three consecutive days at room temperature. The extracts were then filtered using cotton and then filtrate was passed through whatman filter paper (No.3, 15cm size with retention down to 0.1um in liquids). The methanol (80%) extract was concentrated in a rotary evaporator (Buchi type TRE121, Switzerland) to a yield of 5.08%. the extracts were kept in a tightly closed bottle on a refrigerator until used for anti-malaria testing.

### 2.3 Animal and inoculation

A Swiss albino mouse (18-20g) of 72 mice of both sexes of two months old were used for the experiments and was obtained from the University of Nigeria animal house, Nsukka Enugu, Nigeria. The animals were housed in a standard six group each, and acclimatized for a period of twelve days. The animals were housed in wooden cages under standard conditions (ambient temperature, 28.0 $\pm$ 2.0$^{o}$C, and humidity 46%, with a 12 hour light/dark cycle), were fed with growers mash. All the mice were allowed free access to food and water ad libitum, throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of feces and spilled feed from cages daily.

A strain of Plasmodium berghei that was Chloroquine sensitive was gotten from the University of Nigeria, Nsukka, Enugu, Nigeria. The *P. berghei* was subsequently maintained in the laboratory by serial blood passage from mouse to mouse every 5-7 days.

Three animals at a time were used as infected donors and as parasite reservoir. The donor mice were monitored for signs of infection which include lethargy, anorexia, ruffled appearance, shivering and heat-seeking behavior. Blood was taken from the second day, to confirm level of parasitaemia in the donor mice, using the WBC count method (Iqbal, 2003). Blood collected from the tail of the infected donor mouse was diluted with normal saline to produce a standard inoculums of 0.2ml containing $1x10^7$ *P. berghei* infected erythrocytes (NIH publication, 2001). Test mice were then inoculated with 0.2ml of infected erythrocytes IP using a 1ml syringe and ½ inch 23-gauge needle.

Each mouse (Group 1-5) except Group 6 used in the experiment was infected intraperitonally with 0.2ml of infected blood containing about $1x10^7$ of *P. berghei* – parasitized erythrocyte per mL. This was prepared by determining both the percentage parasitemia and the erythrocyte count of the donor mouse and diluting the blood with isotonic 0.88% saline in proportions indicated by both determinations (Odetola and Basir, 1980).

### 2.4 Acute Toxicity Study ($Ld_{50}$)

The crude extract of Anthocleista grandiflora stem bark was evaluated for their toxicity in P. berghei non infected swiss albino mice aged 2 months weighing 18-20g using, modified Locke's (1983) method of determining toxicity level of extract in mice. The test was carried out in two phases. In the phase one of the study, twelve mice randomized into three groups of four mice each and were given 50, 100 and 200 mg/kg body weight respectively of the extract orally. The mice were observed for changes in physical appearance, gross behavioral change and death in the first four hours and subsequently daily for ten days. In view of the result obtained from phase 1 treatment, phase 11 treatment was carried out using another fresh set of twelve mice randomized into three groups of four mice each and were given 500, 1000 and 2000 mg/kg b.wt. of the extract orally. These were observed for signs of toxicity and mortality for the first four hours and thereafter daily for ten days. The LD50 was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose, i.e., he geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase. The oral median lethal dose was calculated using the formula:

LD50 =√maximum dose for all survival × minimum dose for all death. (Lorke 1983)

### 2.5 Antiplasmodial Activity of the Drug

#### 2.5.1 Evaluation of Blood Schizontocidal Activity on Early Infection (Suppessive Test)

Schizontocidal activity of the extract fraction and chloroquine against early *P. berghei* infection was evaluated using the method of Knight and Peters (1980). Each mouse was inoculated on the first day (Day 0), intraperitoneally, with 0.2ml of infected blood containing about 1 X $10^7$ Plasmodium berghei berghei parasitized erythrocytes. The animals were divided into five groups of six mice each and orally administered shortly after inoculation with 300, 500 and 700mg/kg/day doses of the *Anthocleista grandiflora* stem bark extract, chloroquine 5mg/kg/day and an equivalent volume of distilled water (Negative control) for four consecutive days, (day 0 to day 3). On the $5^{th}$ day (day 4), thin films were made from the tail blood of each mouse and smear on to a microscope slide to make a film (Saidu *et al*., 2000). The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 min and parasitaemia examined microscopically and the parasitaemia level was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage chemosuppression was calculated as: $100[(A-B)/A]$, where $A$ is the average percentage parasitaemia in the negative control group and $B$ is the average parasitaemia in the test/standard group.

#### 2.5.2 Evaluation of Schizontocidal Activity in Established Infection (Curative or Rane Test)

The curative potential evaluation of the extract was done using a method of Ryley and Peter (1970) Standard inoculums of $1x10^7$ *P. berghei* infected erythrocytes were injected intraperitoneally on the mice, on the first day (Day 0). Seventy two hours later (day 3) the mice were divided into six groups of 6 mice each. The groups were orally administered with *Anthocleista grandiflora* stem bark extract (300, 500, 700mg/kg/day), chloroquine (5mg/kg) was given to the positive control group and an equal volume of normal saline and distilled water to the negative control group.

The drug extract was given orally once daily for 5days. Thin films stained with 10% Giemsa at pH 7.2 for 10 min and parasitaemia examined microscopically on each day of treatment to monitor parasiteamia level.

### 2.5.3 Evaluation of prophylactic activity (Repository test)

The method of Peters (1967) was adopted in the evaluation of the prophylactic potential of *A. grandiflora*. The mice were randomly divided into five groups with 6 mice in each group. Negative control group (Normal Saline) was given 10 mL distilled water $kg^{-1}$ b.wt orally. While the positive group was given 5 mg chloroquine per kg body weight intraperitoneally, the experimental groups were administered with extract of 300, 500 and 700 mg/kg/day body weight. Treatments were initiated on day 0 and continued until day 4; the mice were all infected with the parasite. Blood smears were then made from each mouse 72 hours after treatment (Abatan and Makinde, 1986).Increase or decrease in parasitaemia was then determined.

### 2.6 Statistical Analysis

Results of the study were presented as a mean plus or minus standard error of mean (M ± SEM). Statistical significance was determined by Students paired t-test and one way analysis of variance with multiple comparison tests (Tukey's test) to compare parameters within groups using computer software spss version 17. Data from the test groups were compared with their respective controls and differences at $P<0.05$ were considered significant.

## *3.0 Results*

### 3.1 Acute Toxicity Test

The acute toxicity study indicated that none of the different doses of extract in all the groups and phases caused mortality of mice for over ten days up to 2000mg/kg. The median lethal dose $LD_{50}$ was estimated to be 1000mg/kg b.wt, which is about 1.5 times the maximum effective dose tested (667mg/kg). Gross physical and behavioral observation of the experimental mice also revealed no visible signs of acute toxicity like hair erection, weakness and reduction in their motor and feeding activities. They were physically active.

### 3.2 Effect of methanolic extract of *Anthocleista grandiflora* stems bark in early malaria infection (4- Day Suppressive Test)

The results of the 4-day suppressive study of the extract stem bark of *A. grandiflora* showed dose dependent chemosuppressive effect at various doses in mice infected with *Plasmodium berghei* malaria parasite. The highest extract suppression of parasitaemia was observed at the dose of 700mg/kg body weight of mice with a mild suppression of parasite in 500mg/kg and 300mg/kg body weight of mice when compared to the negative control group (Normal Saline) with the highest % parasitaemia (Table 1). Percentage suppression was observed to increase as extract concentration increased. From Table 1, the extract caused a statistically significant ($P<0.05$) chemosuppression of 14.40%, 32.78% and 68.20% for the 300, 500 and 700 mg/kg/day respectively when compared to the control. The standard drug, chloroquine (Positive control) caused chemosuppression of 81.11%, which was higher than those of the extract treated groups (Table 1).

**Table 1: Suppressive Effect of *Anthocleista grandiflora m*ethanolic stem bark extract and chloroquine against *P. berghei berghei* infection in mice**

| TREATMENT | DOSE | % PARASITAEMIA + SEM | % CHEMOSUPPRESSION (% INHIBITION) |
|---|---|---|---|
| Normal Saline (Control) | 5ml/kg/day | 42.28± 2.41 | 0.00 |
| Extract | 300mg/kg/day | 38.63± 1.72[a] | 14.40[a] |
| Extract | 500mg/kg/day | 32.4± 3.6[a,b] | 32.78[a,b] |
| Extract | 700mg/kg/day | 23.34±4.09[a,b] | 68.20 [a,b] |
| Chloroquine | 5mg/kg/day | 13.6±2.42[a] | 81.11[a] |

The results are expressed as mean ± SEM (n=5),Values with Superscript a are significantly different from the negative control and those with superscript b are not significantly different from the positive control at $P\leq0.05$

### 3.3 Effect of methanolic extract Stem Bark of *Anthocleista grandiflora* in established malaria infection (Curative or Rane Test)

On established infection, it was observed from Table 2 that there was a daily significant ($p\leq0.05$) increase in parasitaemia level of the control group (Normal Saline) from day 0 to day 7 (day 1 test to day 5 test).

However, a daily reduction in the parasitaemia levels of the extract treated groups as well as that of positive control (Chloroquine) was also observed. However on Day 7, the average percentage chemosuppression of parasitaemia of the extract groups were 36.48%, 54.23%, 74.20% for 300, 500, 700mg/kg/day methanolic stem bark extract of A. grandiflora and 90.54% for 5mg/kg/day b.wt of chloroquine respectively.

**Table 2: Curative Effect of *Anthocleista grandiflora m*ethanolic stem bark extract and chloroquine against *P. berghei berghei* infection in mice**

The data are represented in Mean+SEM when n=5 and the values with superscript a are significantly different

| TREATMENT | DOSE | % PARASITAEMIA ± SEM | % CHEMOSUPPRESSION (% INHIBITION) |
|---|---|---|---|
| Normal Saline (Control) | 5ml/kg/day | 48.80± 2.46 | 0.00 |
| Extract | 300mg/kg/day | 41.40± 1.32[a] | 36.48[a] |
| Extract | 500mg/kg/day | 33.22± 2.8[a] | 54.23[a] |
| Extract | 700mg/kg/day | 30.49±5.2[a] | 74.20 [a] |
| Chloroquine | 5mg/kg/day | 8.75±1.32[a] | 90.54[a] |

from the control at P≤0.05.

### 3.4 Prophylactic Effect of *Anthocleista grandiflora* methanolic stem bark extract against *P. berghei berghei* infection in mice

The methanolic extract of *Anthocleista grandiflora stem bark* produced a significant ($P \leq 0.05$) dose-dependent prophylactic activity at the different doses with a reduction in the level of parasitaemia of 11.23, 28.54 and 59.20% for 300, 500 and 700mg/kg/day extract treated groups while 5mg/kg/day chloroquine caused a chemosuppression of 76.37% when compared to control (Table 3)

**Table 3: Prophylactic Effect of *Anthocleista grandiflora* methanolic stem bark extract against *P. berghei berghei* infection in mice**

The data are represented in Mean+SEM when n=5 and the values with superscript a are significantly different

| TREATMENT | DOSE | % PARASITAEMIA ± SEM | % CHEMOSUPPRESSION (% INHIBITION) |
|---|---|---|---|
| Normal Saline (Control) | 5ml/kg/day | 45.09± 4.53 | 0.00 |
| Extract | 300mg/kg/day | 40.34± 6.11[a] | 11.23[a] |
| Extract | 500mg/kg/day | 32.4± 4.5[a] | 28.54[a] |
| Extract | 700mg/kg/day | 23.34±1.4[a] | 59.20 [a] |
| Chloroquine | 5mg/kg/day | 10.6±1.67[a] | 76.37[a] |

from the control at P≤0.05.

## *4.0 Discussion and Conclusions*

The anti-malarial activities exhibited by the extract were perhaps due to the possible presence of active compounds. The acute toxicity study indicated that extract did not cause mortality of mice within 24 hours up to 1000mg/kg. In general, if the lethal dose (LD50) of the test substance is three times more than the minimum effective dose (MED), the substance is considered a good candidate for further studies (Carol et al 1995). It was also suggested that oral administration is about 100 times less toxic than (Carol et al., 1995). However, the *A. grandiflora* extract showed no lethality to mice at 1,000 mg/kg, which is more than three times the MED and the gross physical and behavioral observation of the experimental mice also revealed no visible signs of acute toxicity like lacrimation, hair erection, and reduction in their motor and feeding activities. They were physically active. Therefore, observations that no death with upto an oral dose of 1000mg/kg could indicate that the test extracts are very safe. The absence of death in the oral administration of *Anthocleista grandiflora* stem bark methanolic extract at 1000 mg extract $kg^{-1}$ b.wt. observed in the rats suggests that the extract is practically non-toxic acutely (Salawu *et al*., 2009).This could also explain the safe use of the plant by the local people, who have been using it in traditional treatment of malaria, in choba area in southern Nigeria.

The extract of *A. grandiflora* stem bark showed a moderate antiplasmodial activity with a dose dependent inhibition against *P. berghei* infection in mice. The result of the suppressive activity of the plant extract is shown in Table 3.5. The extract at 300mg/kg, 500mg/kg and 700mg/kg and chloroquine at 5mg/kg body weight of mice yielded 14.4%, 32.78%, 68.20% and 81.11% chemosuppression respectively when compared to the control. The observed highest chemosuppression effect in the standard drug, chloroquine may be due to the inability of the parasite to develop resistance against the drug as compared to that of the extract. Also, this parasitaemia suppression effect is similar to the effect of the extract reported by previous studies such on Zizyphus spina-christi (Adzu and Haruna 2007), Alstonia boonei (Iyiola et al., 2011), Result for the 4 days of treatment showed a significant ($p<0.05$) difference both in the extract and chloroquine with a mean parasitaemia of 38.63$\pm$ 1.72%, 32.43$\pm$ 2.82%, 23.34$\pm$5.2 %, 13.69$\pm$1.32% and 49.28$\pm$ 2.41% for 300mg/kg, 500mg/kg, 700mg/kg extract, 5mg/kg chloroquine and 5ml/kg normal saline respectively (Table 3.7). But there was no significant ($p<0.05$) difference in the 500mg/kg and 700mg/kg of the extract when compared to chloroquine. Percentage chemosuppression was also observed to increase as extract concentration increased but with a decrease in parasitaemia as the dosage increases in the curative test. In the rane test (curative study), it was shown that *A. grandiflora* produced a dose dependent reduction in parasitaemia levels in the extract treated groups, with a similar reduction in the cloroquine treated group (positive control). But, with a daily increase in parasitaemia in the negative control group, the average percentage suppression of parasitaemia of the extract treated groups on day seven were 82.79, 83.20 and 84.84% for 300mg/kg, 500mg/kg and 700mg/kg/day of the extract, respectively and 5mg/kg/day chloroquine exerted 88.11% decrease in parasite count. This study is similar to the reports of Idowu et al., (2010), Titanji et al. (2008), Tantchou et al., (1986) , Odeku et al., (2008) and Iyiola et al., (2011).

The prophylactic study of the methanolic stem bark extract of *A. grandiflora* produced a significant ($p<0.05$) dose dependent reduction in the level of parasitaemia and possesses blood schizontocidal activity which is in agreement with the suppressive test of this study with a chemosuppression of 11.23, 28.54 and 59.20% whereas 5mg/kg b.wt. of chloroquine exerted 76.37% reduction in prasitaemia level when compared to the control (Normal saline). When a standard antimalarial drug is used in mice infected with *P. berghei*, it suppresses parasitemia to non-detectable levels (Kiseko et al, 2000), which is in agreement with the effects of chloroquine in this study.

However, the mechanism of action of the extract has not been elucidated, some plants are known to exert antiplasmodial activity either by causing red blood cell oxidation (Etkin, N.L.,1997) or by inhibiting protein synthesis (Kirby et al 1989) depending on their phytochemical constituents. The extract could have exerted its action through either of the two mechanisms mentioned above or by some other unknown mechanism.

In conclusion, the results of this study shows that the extract of *Anthocleista grandiflora* is safe and possess potent antimalarial activity as found in its ability to suppress *Plasmodium berghei* infection in the three models investigated. This justifies its continuous use as an antimalarial remedy.

Also further researches are on-going in our laboratories to isolate, identify and characterize the active component of the plant and also to fractionate and test for their activity against *P. falciparum and P. vivax* in order to consider them as potential sources for antimalarial drug development for human malaria.

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