# ANTIPLASMODIAL ACTIVITY OF STEM-BARK EXTRACT OF *MILICIA EXCELSA* (WELW.) C.C.BERG AGAINST RODENT MALARIA PARASITES (*PLASMODIUM BERGHEI*) IN MICE.

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## ABSTRACT

Plants have played significant role as a reservoir of active phytochemicals in the treatment of malaria infection worldwide. In the present study, we investigated the antiplasmodial activity of ethanolic extract of *Milicia excelsa*. *Plasmodium berghei* (NK) infection in mice was used as a model for malaria infection. Ethanolic extract of *M. excelsa* at doses of 250, 500 and 1000 mg/kg/day were evaluated in the suppressive (early infection), prophylactic (residual infection) and curative (established infection) phases. Results showed that the ethanolic extract of *M. excelsa* demonstrated a dose-dependent chemo-suppression in early and residual infections. At 250, 500 and 1000 mg/kg ,the suppression were 76.7%, 80% and 96.7% respectively while in the prophylactic; at the lowest dose of 250mg/kg, the extract suppressed parasite growth by 42.43%, 45.28%, 59.82% and 66.61% after treatment for 2, 3, 4 and 5 days respectively. These results are significantly ($p<0.5$) different from the control. In the curative assay, the extract demonstrated a significant non-dose-dependent antiplasmodial activity in established infection known as curative; even at the lowest dose of 250mg/kg, the extract reduced parasite growth by 42.43%, 45.28%, 59.82% and 66.61% after treatment with the extract for 2, 3, 4 and 5 days respectively. These results showed that the extract possesses significant antiplasmodial activities, therefore the extract could be a potential candidate for antimalarial drug.

## INTRODUCTION

Malaria is undoubtedly the most destructive and dangerous infectious disease in the developing countries of the world (Greenwood *et al*., 2005; Winter *et al*., 2006). This vector-borne infectious disease is a classic example of one that affects the productivity of individuals, families and the whole society, since it causes more energy loss and more debilitation than any other human parasitic diseases (Sachs and Malaney, 2002). Malaria infection used to be regarded as a poor man's disease associated with poverty but recent reports has shown that malaria no longer discriminates between the rich and the poor. There were an estimated 247 million malaria cases among 3.3 billion people at risk of infection in 2006, causing nearly a million deaths, mostly of children under 5 years of age. It is widespread in the tropical and subtropical regions, including parts of America, Asia and Africa. A total of 109 countries were endemic for malaria in 2008, 45 of these countries are found within the African region (WHO, 2008). The most widespread and severe malaria is caused by *P. falciparum*, which transiently infects the liver before invading red blood cells of the mammalian host; resulting in chronic anemia. Clinical manifestations occur at the erythrocytic stage and can include fever, chills, anemia, as well as delirium, cerebral malaria and multi-organ system failure, which may be followed by coma and death (Jones and Good, 2006).

Considering increasing resistance of malaria parasites to available drugs, there is broad consensus that there is a need to develop new anti-malarial drugs. Drug development can follow several strategies, ranging from minor modifications of existing agents to the design or isolation of novel agents that can act against new targets in the parasite. Plants no doubt have been and are still a veritable source of drugs to mankind and it is certain that plants will continue to provide leads and template for new drugs (Faraz, 2012). Plant sources as anti-malarial agents has gained a lot of interests since the discovery of *Artemisinin* from a plant known as *Atemisia annua,* the compound was found to be very active against drug-resistant malaria parasites. The plant has been used to treat malaria infection for thousands of years in Africa and China. This was the starting point for the isolation of *Artemisinin* and the

subsequent synthesis of its derivatives; which has now become the frontline therapy for malaria infection, in combination with currently available anti-malaria drugs such as piperaquine, mefloquine, lumefantrine and naphthoquine (Nsagha *et al*., 2012).

In Africa, up to 80 per cent of the population still relies on herbal medicine to treat malaria and other diseases because of their affordability and accessibility (Agbedahunsi, 2000). The report by Adebayo and Krettli (2010) on the potential anti-malaria plants from Nigeria revealed that, about 125 plants have so far been scientifically screened for anti-malaria activities out of the numerous plants used by different tribes in Nigeria to treat malaria infection. It is therefore necessary to intensify research in this area to discover new potent anti-malaria drugs from medicinal plants used locally that have not been scientifically investigated (Oladele and Adewumi, 2008).

The plant *M. excelsa* (commonly known as Iroko or African Teak) is a famous tree found in tropical Africa. It is one of the two tree species yielding timber known as *Iroko*, the other being *M. regia. M. excelsa* is a large deciduous tree growing to 50 metres (160 ft) high. The trunk is bare lower down with the first branch usually at least 20 meters (66 ft) above the ground. It often has several short buttress roots at the base, the bark is pale or dark grey, thick but little fissured and if it gets damaged it oozes milky latex (Jones *et al*, 2011). In ethnomedicine, various parts of the plant are used for different purposes among diverse tribes in Africa. Young leaves are used as vegetables; decoction of the leaf is used for steam bath and in the treatment of filariasis in Cameroon (Adjanohoun *et al*., 1991) and in the treatment of viral infection in southern Cameroon (Noumi and Eloumou, 2011). The decoction of the stem bark is used to treat malaria infection and rheumatism in Southwest Nigeria (Odugbemi *et al.,* 2007).

*M. excelsa* is one of the revered medicinal plants in Africa that have been used for the treatment of malaria infection among the Yoruba speaking people of South-Western Nigeria for many centuries. As a result of this, the toxicity of ethanol extract of this plant was investigated in our laboratory in 2015; the $LD_{50}$ was found to be greater than 5g/kg body weight of rats (Areola *et al*. 2015). This study was designed, to investigate the *in vivo* anti-malaria potential of *M. excelsa* extract against rodent malaria parasites.

## MATERIALS AND METHODS

### Plant Materials

Fresh stem-bark of *M. excelsa* was collected from a matured *Milicia excelsa* tree at the main Campus of Obafemi Awolowo University, Ile-Ife, Nigeria. The plant was identified and authenticated by Dr. F. A. Oloyede, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The plant specimen was deposited at IFE Herbarium with specimen voucher number UHI 16469.

### Experimental Animals

Healthy albino mice, (*Mus musculus*), average weight of 20 ± 2.05 g were obtained from the Animal House, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were acclimatized for two weeks before the commencement of the experiments.

### Rodent Malaria Parasites

Chloroquine sensitive *Plasmodium berghei* (NK65) from a donor mouse was obtained from the Institute for Advanced Research and Training (IMRAT), College of Medicine, University of Ibadan, Nigeria.

### Methods

### Preparation of Ethanol Extract

The fresh stem-bark of *M. excelsa* were washed, cut into tiny bits, shade-dried for four weeks and ground into powder with electrical milling machine. The powdered stem bark (1kg) was macerated in 70% (v/v) ethanol (3L) for 72 hr at room temperature with regular shaking. The suspension was filtered; the residue was re-suspended and re-extracted until the filtrate became colourless. The filtrates were combined, concentrated under reduced pressure in rotary evaporator (Edman High Vacuum Pump) at 40°C and lyophilized.

### Evaluation of Anti-plasmodial Activity of EE

Three biological assay methods were employed to evaluate the *in vivo* antiplasmodial activity of the extract, namely: suppressive (test on early

infection), according to Knight and Peters (1980), prophylactic (test on residual infection), and curative according to the methods reported by Peters, (1982)

**Parasite inoculation**

The rodent malaria parasite (Chloroquine sensitive *Plasmodium berghei* (NK65)) was maintained in albino mice by weekly inoculation of $1 \times 10^7$ infected red blood cells in naïve mice.

**Evaluation of the Suppressive Anti-plasmodial Activity of Ethanol Extract (Early Infection)**

The suppressive test against *Plasmodium berghei* infection in mice was determined according to the method of Knight and Peters (1980). The mice (25) were inoculated with 0.2ml of infected blood containing about $1.0 \times 10^7$ parasites of chloroquine sensitive *Plasmodium berghei* (NK65) and randomly divided into 5 groups of 5 mice per group. They were marked, weighed and kept in five separate cages. Mice in groups A, B and C were treated with 250, 500 and 1000 mg/kg body weight of ethanol extract for 4 consecutive days, while chloroquine (5mg/kg/day) was administered to group D (positive control), and group E (negative control group) received 0.2ml of distilled water for four consecutive days; starting from the day of inoculation (D1 – D4). All test materials were administered orally. On the fifth day (D5), thin blood films were prepared from the tail of the mice. Smears were fixed in methanol and stained with Giemsa stain. The average percentage of parasitaemia suppression of 5 fields was calculated relative to controls as follows:

Average % Suppression = $(\{X - Y\} / X) \times 100$

X = Mean % parasitaemia of negative control group.

Y= Mean % parasitaemia in treated groups.

Mean = Mean $\pm$ SEM

**Evaluation of the Prophylactic Antiplasmodial Activity of Ethanol Extract (Residual infection)**

The prophylactic activity of ethanol extract against chloroquine sensitive *plasmodium berghei* (NK65) was determined as described by Peters (1982). The mice (25) were randomly divided into 5 groups of 5 mice each. They will be marked, weighed and caged separately. Different doses of EE (250, 500 and 1000 mg/kg body weight) were orally administered to mice in groups A, B and C respectively for 4 consecutive days. Groups 4 and 5 were also administered with 5 mg/kg of pyrimethamine and 0.2 ml of distilled water respectively. On the fourth day, the mice in all the groups were inoculated with 0.2 ml of standard inoculums. Percentage parasitaemia in the mice were determined 72 hours after inoculation as follow:

Percentage parasitaemia = $(P / T) \times 100$

P = Total parasitized red blood cells in a field

T = Total red blood cells in a field

**Evaluation of the Curative Antiplasmodial Activity in Established infection (Rane test)**

Twenty five (25) mice were inoculated on the first day as described above and randomly divided into five groups of 5 mice per group. Seventy two hours later, different doses of ethanol extract (250, 500 and 1000 mg/kg) were administered orally to groups A, B and C respectively while chloroquine (5mg/kg/day) was administered to group D (positive control) and group E (negative control group) received 0.2ml of distilled water for five consecutive days. Thin blood smears were made from the tail of each mouse everyday to monitor changes in parasitaemia for 5 consecutive days. Mean % parasitaemia for each dose per day were determined for day 1 to day 5 of the experiment.

## RESULTS

Table 1. Antiplasmodial activity of *M.excelsa* extract on early infection (suppressive)

| Doses (mg/kg/day) | Chemo-suppression (%) | Mean survival time (days) |
|---|---|---|
| Extract 250 | 76.7 | 8 |
| 500 | 80 | 10 |
| 1000 | 96.7 | 13 |
| Chloroquine 5 | 94.4 | 22 |

Data are expressed as Mean ± SEM, n=5

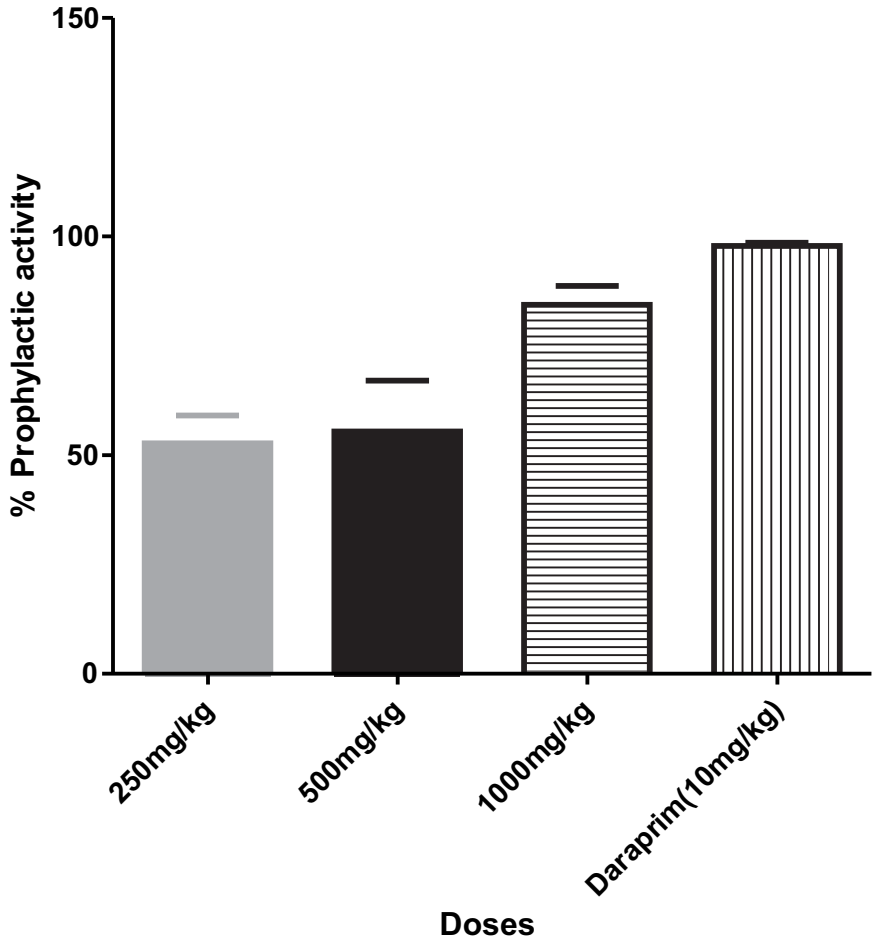


Fig. 1 Antiplasmodial activity of *M. excelsa* extract on residual infection (Prophylactic)

Table 1 shows the chemo-suppressive effects of the ethanolic stem-bark extract of *M. excelsa*. The suppressive effect of the extract was dose-dependent, even at the lowest dose of 250 mg/kg the extract suppressed parasite growth by 76.7% while a dose of 1000mg/kg caused a suppression of 96.7%. Survival time of the extract after the stoppage of extract administration was also dose-dependent. The mean survival time was 8, 10 and 13days for 250, 500 and 1000 mg/kg of the extract respectively.

In Figure 1, the prophylactic activity was also dose-dependent. At the lowest dose of 250 mg/kg, a significant antiplasmodial activity was observed (52.70%) and at 1000 mg/kg, the activity was 84.41%.

The curative activity of the extract is presented in Figure 2, the figure showed that even in established infection, the extract demonstrated significant ($p < 0.05$) activity at all the doses tested. At a dose of 500 mg/kg, the percentage chemo-suppression for day 1 to 5 are 87.33, 90.98, 94.25, 86 and 76% respectively.

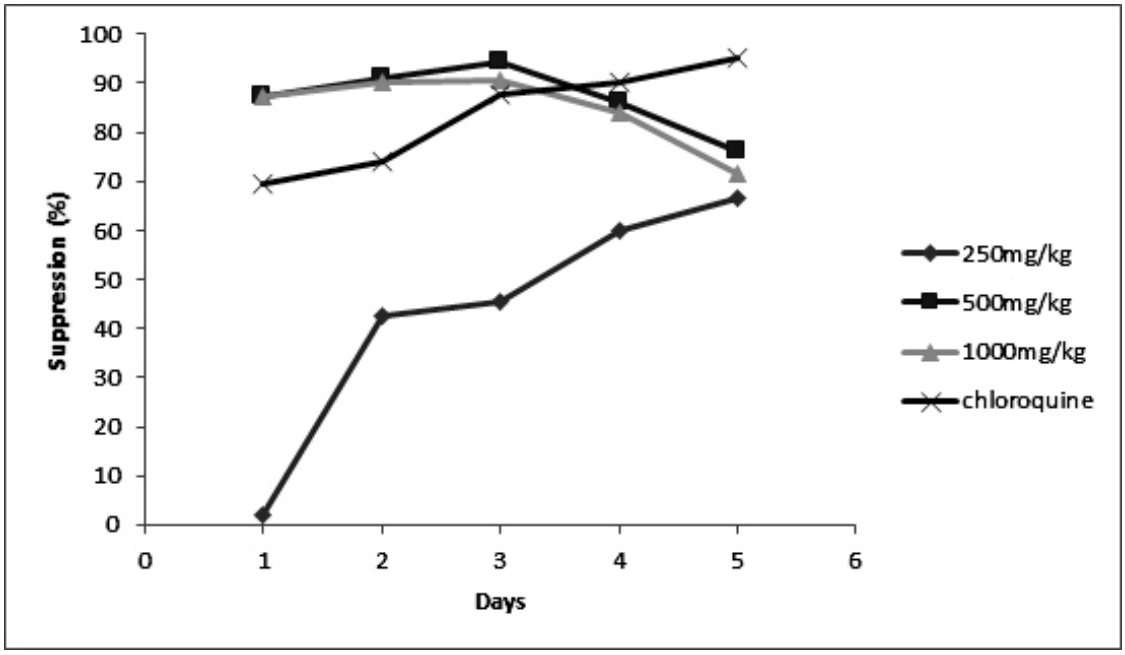


Fig. 2 Antiplasmodial activity of *M.excelsa* extract in established infection (Curative)

## DISCUSSION

The rodent malaria model in mice is the most extensively used and most ideal for the primary *in vivo* tests of new anti-malaria drugs. Reports from various *in vitro* antiplasmodial assays have revealed that many natural or synthetic compounds with promising *in vitro* results gave disappointing *in vivo* results.

During early infection (suppressive) antiplasmodial activity, the extract elicited significant suppressive activity at the three doses investigated; at 250 and 500mg/kg/day, the chemo-supression were 76.7 and 80 percent respectively. At the highest concentration of 1000 mg/kg/day the percentage suppressive activity (96.7%) was comparable to chloroquine activity (94.4%) at 5 mg/kg/day (Table 1). The dose-dependent activity was also observed in suppressive activity (Figure 2). Compounds that are capable of reducing parasitemia by 30% or more at a dose of 1000 mg/kg are considered active and recommended for further study (Carvalho *et al.*, 1991).

The prophylactic antiplasmodial activity of the extract on chloroquine sensitive *Plasmodium berghei* in albino mice revealed a dose-dependent activity (Fig. 1). At the lowest concentration of 250 mg/kg of ethanolic extract, appreciable antiplasmodial activity was observed (52.70 %). The prophylactic activity at 1000 mg/kg (84.41%) was comparable to the standard drug, Daraprim (97.88%). These activities are statistically significant ($p< 0.05$) compared with the negative control.

The ethanolic extract of *M. excelsa* equally demonstrated a significant non-dose dependent antiplasmodial activity in the established infection known as curative; even at the lowest dose of 250mg/kg, the extract suppressed parasite growth by 42.43%, 45.28%, 59.82% and 66.61% for day 2, 3, 4 and 5 respectively, these results are significantly ($p<0.5$) different from control. At a dose of 250mg/kg, the extract demonstrated a progressive increase in antiplasmodia activities. These results showed that the extract contains phytochemicals such as tannins and terpenoids that are toxic to the malaria parasites (Areola *et al.*, 2015). Secondary metabolites have been found to exert their antiplasmodial effect by one or more of the following mechanisms: inducing oxidative stress, elevating red blood cell oxidation or through inhibition of protein biosynthesis (Chandel and Bagai, 2010).

Based on the results obtained in this study, the extract could be regarded as a potential candidate for development of antimalarial drug. The results also justified the reason why the stem-bark is used as antimalaria therapy in traditional medicine

(Odugbemi *et al*., 2007). The other advantage this extract has is that it may not be toxic to experimental animals (Areola *et al*., 2015).

Further studies are recommended to isolate and elucidate the structure of the compound(s) responsible for its antiplasmodial activities. It is also important to investigate its biochemical mechanism of action.

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