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## ANTIMALARIAL ACTIVITY OF MAMMEA AFRICANA

\*<sup>1</sup>Jude E. Okokon; <sup>2</sup>Aniekan E. Udokpoh; <sup>3</sup>Grace A. Essiet

<sup>1</sup>Dept. of Pharmacology and Toxicology, faculty of Pharmacy, university of Uyo, Uyo, Nigeria, <sup>2</sup>Dept. of Pharmaceutics and Pharmaceutical Technology, faculty of Pharmacy, University of Uyo, Uyo, Nigeria, <sup>3</sup>Dept. of Pharmacology, College of Medical Sciences, University of Calabar, Calabar, Nigeria.

**E-mail:** [judeefiom@yahoo.com](mailto:judeefiom@yahoo.com), **Tel:** +234-80234-3678

### Abstract

The antimalarial activity of ethanolic stem bark extract of *Mammea africana* was studied in vivo in mice infected with *Plasmodium berghei berghei* during early and established infections. *Mammea africana* extract (30 – 90mg/kg/day) exhibited a significant ( $P < 0.05$ ) blood schizontocidal activity both in 4-day early infection and in established infection with a significant mean survival time comparable to that of the standard drug, chloroquine, 5mg/kg/day. The stem bark extract possesses a promising antiplasmodial activity which can be exploited in malaria therapy.

### Introduction

*Mammea africana* Sabine (Guttiferae) (syn. *Ochrocarpus africana* Oliv.) is a large forest tree of 50 to 100 feet high with bark often yellow with pale scales and resinous yellow sap (Daziel, 1956). The plant is widely distributed in tropical Africa. The stem bark of the plant is used traditionally by the Ibibios of Niger Delta region of Nigeria in the treatment of malaria related fever, internal heat, and microbial infections. The chloroformic and ether stem bark extract are reported to possess cytotoxic activity on cell culture (Chapuis *et al.*, 1988). Ouahou *et al.*, (2004) reported cytotoxic coumarins with antimicrobial activity against *Staphylococcus aureus* from the plant stem bark. Methanolic fractions of the stem bark have been reported to contain compounds that are potent urease inhibitors (Rahman and Choudhary, 2001). The stem bark has been reported to contain 5,7-dihydroxy-8-(12-methyl-butyl) -4-N-Pentyl coumarins (Carpenter *et al.*, 1971; Crichton and Waterman, 1978; Carpenter *et al.*, 1970), Mesuxanthone B (Carpenter *et al.*, 1971).

Alkaloids have been reported to be absent in the entire plant parts (Gartlands et al., 1980). Although reports of scientific studies on *Mammea africana* have been widely published, there is no information regarding the antiplasmodial activity of the stembark extract in mice. Therefore this work was aimed at evaluating the antiplasmodial activity of ethanolic extract of the stembark of *M. africana* on *Plasmodium berghei berghei* infection in mice.

## **Materials and Methods**

### **Plant material**

Fresh stembark of *M. africana* was collected in November, 2004 from Anwa forest in Uruan area of Akwa Ibom State and authenticated by Dr. (Mrs) Margaret Bassey, a taxonomist in Botany Dept, University of Uyo, Uyo – Nigeria. Herbarium specimen was deposited at Faculty of Pharmacy herbarium, University of Uyo with voucher no. FPHU 381. The fresh stembark were cut into pieces and dried on a laboratory table for 2 weeks and reduced to powder. The powder (300g) was macerated in ethanol (500ml) for 72 hours. The liquid extract obtained was concentrated in vacuo at 40°C. The yield was 3.51%. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

### **Animals**

The animals used in the study were adult male and female Swiss albino mice (22-30g) obtained from University of Uyo animal house, Uyo, Nigeria. The animals were used after acclimatization period of 10 days to room temperature and relative humidity of 28 ± 5°C and 50% respectively. They were housed in standard cages and maintained on standard animal pellets and water ad libitum. The study was approved by University of Uyo College of Health Sciences Animal Ethics Committee.

### **Acute toxicity**

Mice were treated intraperitoneally with doses ranging from 50 to 1000mg/kg of the crude extract. The animals were inspected for appearance of signs of toxicity for 24 hours and number of deaths was also recorded. LD<sub>50</sub> value was calculated using the method of Lorke (1983).

### **Phytochemical screening**

Phytochemical screening of the ethanolic stembark extract of *Mammea africana* was carried out employing standard procedures (Trease and Evans, 1989) to reveal the presence of saponin, terpenes, flavonoids, cardiac glycosides, tannins and alkaloids.

### **Parasite inoculation**

The chloroquine sensitive *Plasmodium berghei berghei* was obtained from National Institute of Medical Research, Lagos, Nigeria and maintained in mice. The

inoculum consisted of  $5 \times 10^7$  *P. berghei berghei* parasitized red blood cells per ml. This was prepared by determining both the percentage parasitaemia and the red blood cell count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations. Each mouse was inoculated on day 0, intraperitoneally, with 0.2ml of infected blood containing about  $1 \times 10^7$  *P. berghei berghei* parasitized red blood cell.

#### **Evaluation of schizontocidal activity on early infection (4-day test)**

A method described by Knight and Peters (1980) was used. The animals were divided into five groups of five mice each and were orally administered with 30, 60 and 90mg/kg/day of *M. africana* stem bark extract, chloroquine 5mg/kg/day (positive control) and an equivalent volume of distilled water (negative control group) for four consecutive days (day 0 to day 3) between 8.00am and 9.00 am. On the fifth day (D4), 24 hours after the administration of the last dose, thin blood films were made from the tail blood and stained with Giemsa stain and the percentage parasitaemia 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, average parasitaemia in the test group.

#### **Evaluation of schizontocidal activity in established infection (Rane test)**

A modified method similar to that described by Ryley and Peters (1970) was used. On the first day (day 0), standard inoculum of  $1 \times 10^7$  *P. berghei berghei* infected erythrocytes was injected intraperitoneally into mice. Seventy-two hours later, the mice were divided into five groups of five mice each. Different doses of *M. africana* extract (30, 60 and 90mg/kg/day) were administered orally to these groups. Chloroquine (5mg/kg/day) was given to the positive control group and an equal volume of distilled water to the negative control group. The drug/extract was given once daily for 5 days. Thin films stained with Giemsa stain were prepared from tail blood of each mouse daily for 5 days to monitor the parasitaemia level. The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post inoculation) in each group over a period of 30 days (D0 to D29). The parasitaemia level of the animals that survived after 30 days of monitoring were determined using thin blood film made from tail blood of each surviving animal.

#### **Statistical analysis**

Data obtained from the study were analyzed statistically using Student's test and values of  $P < 0.05$  were considered significant.

## Results

### Acute toxicity

Ethanollic stembark extract of *M. africana* (30 -90mg/kg) produced various signs of toxicity on mice treated with it depending on the dose administered ranging from writhing, gasping, decreased respiratory rate, decreased limb tone and death. Animals treated with 250mg/kg and above of the extract died. The LD<sub>50</sub> was calculated to be 223.6mg/kg.

### Phytochemical screening

The result of phytochemical screening of the ethanolic stembark extract of *Mammea africana* showed that the stembark extract contains flavonoids ,terpenes, saponins ,anthraquinones ,cardiac glycosides, tannins ,and deoxy-sugar; while alkaloids were found to be absent.

**Table 1:** Blood schizontocidal activity of *Mammea africana* stembark extract during early infection

| Drug/Extract              | Dose (mg/kg/day) | Average % Parasitaemia | Average % Suppression |
|---------------------------|------------------|------------------------|-----------------------|
| <i>Africana</i> extract   | 30               | 9.0 ± 2.94*            | 82.92                 |
|                           | 60               | 7.3 ± 3.39*            | 86.59                 |
|                           | 90               | 4.0 ± 1.63*            | 91.66                 |
| Chloroquine               | 5                | 6.3 ± 0.23*            | 86.87                 |
| Control (distilled water) | 0.2ml            | 48.0 ± 7.4             | -                     |

Data are expressed as mean ± SD for five animals per group (\*P<0.05) when compared with control

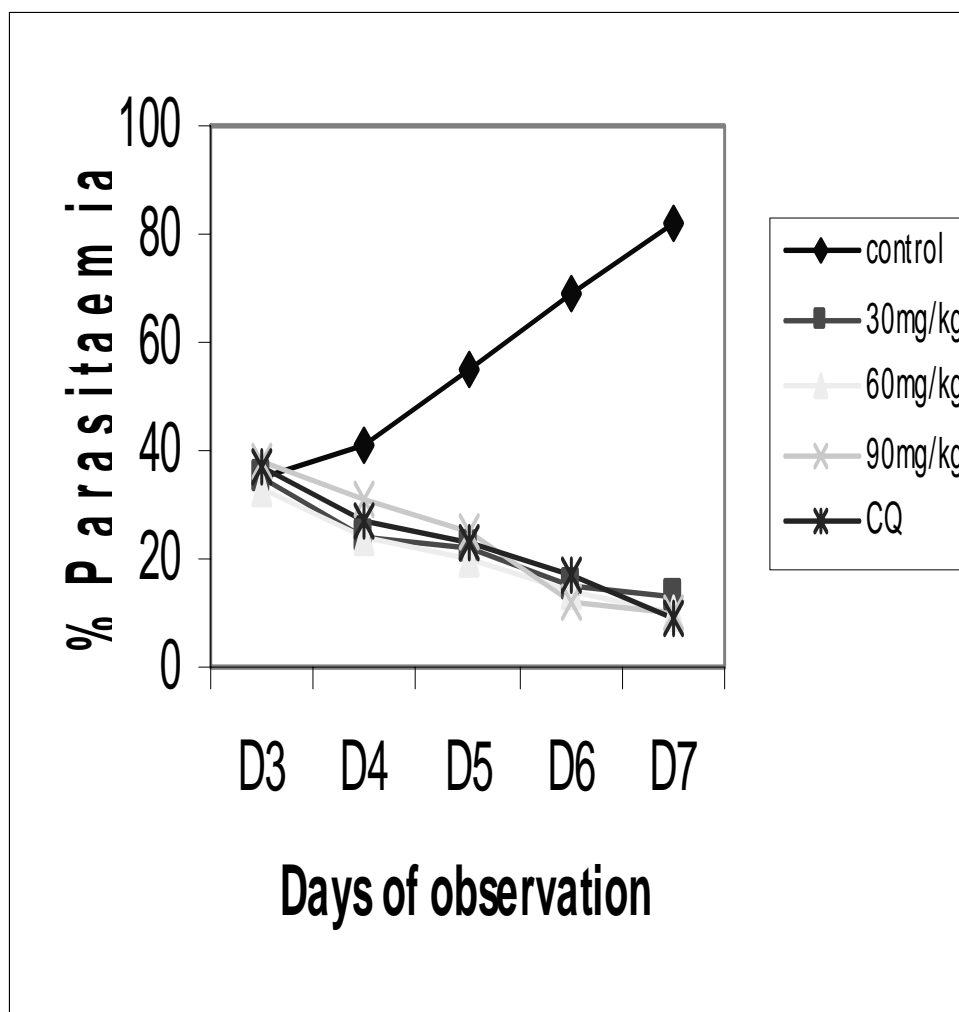
**Table 2:** Mean Survival Time of mice receiving ethanolic stem bark extract of *Mammea africana*

| Drug/Extract               | Dose (mg/kg/day) | Mean survival time (day) |
|----------------------------|------------------|--------------------------|
| <i>M. africana</i> extract | 30               | 14.0 ± 0.47*             |
|                            | 60               | 19.6 ± 2.05*             |
|                            | 90               | 28.3 ± 2.35*             |
| Chloroquine                | 5                | 30.0 ± 0.00*             |
| Control (distilled water)  | 0.2ml            | 11.5 ± 0.94              |

Data are expressed as mean ± SD for five animals per group (\*P<0.05) when compared with control

#### 4-day test

The stem bark extract of *M. africana* produced a dose dependent chemosuppression effect at the different doses of the extract employed. Doses of 30, 60 and 90mg/kg of the extract administered orally caused chemosuppression of 82.97, 86.59 and 91.66% (table 1) respectively, which was significant ( $P < 0.05$ ) when compared to control. The standard drug, chloroquine, caused 86.87% chemosuppression, which was higher than that of the extract treated group (table 1).



**Figure 1:** Effect of *Mammea africana* extract on established infection

### **Schizontocidal activity during established infection (curative test)**

There was a dose dependent reduction in parasitaemia of the extract treated grouped, while the control group showed a daily increase in parasitaemia chloroquine (5mg/kg/day) also produced a daily reduction in parasitaemia. The percentage suppression of parasitaemia of the extract treated groups on day 7 were 13.0, 10.0 and 9.0% for 30, 60 and 90mg/kg/day of the extract (Figure 1), respectively, while that of control and chloroquine treated groups respectively were 82.5 and 8.0%. The mean survival time (MST) of the mice in various groups were  $14.0 \pm 0.47$ ,  $19.6 \pm 2.05$ ,  $28.3 \pm 2.35$ ,  $30.00 \pm 0.00$  and  $11.3 \pm 0.94$  days for 30, 60 and 90 mg/kg/day of extract, chloroquine and control groups respectively (Table 2). The animals that survived in the extract treated group as well as chloroquine group were found to be parasite free.

### **Discussion**

In this study ,the acute toxicity evaluation of the extract revealed that doses of 250 mg/kg and above were lethal to the animals and the determined LD50 of the extract, 223.6 mg/kg shows that the extract is moderately toxic(Homburger,1989). Antiplasmodial screening of plants have implicated alkaloids, terpenes and flavonoids in this activity (Philipson and Wright, 1990; Christensen and Kharazmi, 2001). These compounds ,except alkaloids, were found to be present in the extract studied and may be responsible for the observed antiplasmodial activity of the extract, though the active principle is yet to be identified. The results indicate that the stembark extract possess blood schizontocidal activity as evident from the chemosuppression obtained during the 4-day early infection test. A significant ( $P < 0.05$ ) activity was also recorded during established infection, which was comparable to the standard drug (Chloroquine, 5mg/kg/day). The highest dose of the extract (90mg/kg/day) was observed to sustain some mice throughout the 30 days period of study similar to that of the standard drug, Chloroquine. Thus demonstrating a considerable antiplasmodial activity.

### **Conclusion**

The results of this study shows that the stembark extract of *M. africana* possesses antimalarial activity and justifies its folkloric use as an antimalarial remedy. Further work is suggested to isolate, identify and characterize the active principle(s) from this plant.

### **Acknowledgement**

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