

# Evaluation of antimalarial potential of aqueous stem bark extract of *Bombax buonopozense* P. Beauv. (Bombacaceae)

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**Abstract.** The treatment of malaria in Africa is becoming increasingly difficult due to rising prevalence of *plasmodium falciparum* resistant to antimalarial drugs. This study was undertaken to investigate the *in vivo* antiplasmodial effect of the aqueous stem bark extract of *Bombax buonopozense* in mice infected with chloroquine-sensitive *plasmodium berghei* NK65. The *in vivo* antiplasmodial effect against early infection and curative effect against established infection were studied. The extract at all doses (100, 200 and 400 mg/kg, *p.o.*) used, exhibited significant ( $P < 0.05$ ), dose-dependent activity against the parasite in suppressive and curative tests. These results show that the stem bark extract of *Bombax buonopozense* plant has significant antiplasmodial effect. The reduction in parasitemia levels in the two employed models suggest that a part of its antimalarial activity is mediated by direct plasmocidal effect on the parasite.

Key words: Antimalaria, bombax buonopozense, medicinal plant, mice, plasmodium berghei

## 1. Introduction

There are 300 million acute cases of malaria each year globally, resulting in more than a million deaths. Majority of these disease cases and deaths occur in sub-Saharan Africa where the disease is endemic (1, 2). Malaria is Africa's leading causes of under-five mortality and constitutes 10% of the continents overall disease burden. The alarming rate at which the parasite particularly *plasmodium falciparum*, has developed resistance to currently used antimalarial drugs makes it imperative to search for newer, more effective therapeutic agents.

One of the areas for the search for new antimalarial drugs is from the claimed antimalarial plants from the African flora (3). Only few of these claims have been authenticated by scientific investigation (4). Plants form the major part of treatments used by traditional healers in many societies, thus, many plants have acquired reputation for being useful against malaria (5).

*Bombax buonopozense* which belong to the family *Bombaceae* is a large tropical tree that grows to 40 m in height with large buttress roots that can spread to 6 m. The bark is covered with large conical spines, especially when young, shedding them with age to some degree. The branches are arranged in whorls; the leaves are compound and have 5-9 leaflets and 5-25 secondary veins. The individual leaflets have entire margins and are also large. The under side of the leaflets may be glabrous or puberulous (6). It is widely distributed in Africa, from Ghana to Sierra Leone, Uganda and Gabon. In Northern

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Nigeria, the Hausas call it 'kurya' while the Dagbanis of Ghana call it 'vabga'. Many parts of the plant are used for medicinal purposes. The leaves are taken as a decoction for the treatment of malaria, feverish (7) and pains (8). The roots are used as antimicrobial and stomach aches. The fruits are eaten by animals such as the water chevrotain (9).

The aim of this study therefore is to authenticate the potential of the aqueous stem bark extract of *Bombax buonopozense* by evaluating its antiplasmodial activity.

## 2. Materials and methods

### 2. 1. Collection and preparation of plant material

The plant material was collected in March, 2009, from Chaza, Niger State, Nigeria. It was identified and authenticated by Mrs Jemilat Ibrahim, a taxonomist in the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, where a voucher specimen (no. NIPRD/H/6402) was deposited at the herbarium for reference. The bark was cleaned, cut into pieces and air-dried at room temperature for 7 days and ground to powder using mortar and pestle.

### 2. 2. Extraction of plant material

Four hundred grams of the bark powder was extracted in distilled water by maceration for 24 h with constant shaking. The filtrate was dried on a water bath and the yield calculated to be 75 g (18.75%) of the aqueous extract. The bark extract was subsequently reconstituted in distilled water at appropriate concentrations for the experiment.

### 2. 3. Phytochemical tests

The aqueous stem bark extract of *Bombax buonopozense* was subjected to phytochemical analysis for identification of constituents using conventional procedures (10, 11).

### 2. 4. Acute toxicity test

The acute toxicity of the extract was tested to determine the safety of the agent using Lorke's (12) method. The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three mice per cage and were administered orally with graded concentration (10, 100 and 1000 mg/kg<sup>-1</sup> of the extract. The mice were observed for signs of toxicity which include paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first

four hours and subsequently for 4 days. In the second phase, another set of nine mice were also randomized into three groups of three mice per cage and were administered orally with graded concentrations (1600, 2900 and 5000 mg/kg<sup>-1</sup> of the bark extract also, based on the result of the first phase. The animals were also observed for signs of toxicity and mortality for the first four hours and thereafter for 4 days. The oral LD<sub>50</sub> was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

### 2. 5. Animals

A total of seventy-eight Swiss albino mice (18-25 g) of both sexes were used in the study. Eighteen for acute toxicity study while thirty were used for each of the *in vivo* antiplasmodial test. The animals were obtained from animal facility centre of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The animals were housed in cages at room temperature and moisture under naturally illuminated environment of 12:12 h dark/ light cycle. They were fed on standard diet and had water *ad libitum*. A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulation [ENV/MC/CHEM (98)] (13). The principle of laboratory animal care [NIH publication No. 85-23(14)] was also followed in this study.

### 2. 6. Inoculums

Parasitized erythrocytes were obtained from a donor-infected mouse maintained at animal facility centre, NIPRD. Parasites are maintained by continuous re infection in mice. The inoculums consisted of *plasmodium berghei* parasitized erythrocytes. This was prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and diluting them with normal saline in proportion indicated by both determinations. Each mouse was inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 1x10<sup>7</sup> *plasmodium berghei* parasitized red blood cells on day zero. Infected mice with parasitemia of 5-7% were allocated to five groups of six mice each (15).

### 2. 7. In vivo antiplasmodial test

#### Suppressive test

A total of thirty mice were used for the study using the methods of Akuodor *et al.*, 2010 (16) and Ryle and Peter, 1970 (17). Each mouse

Table 1. Suppressive activity of *B. buonopozense* Stem bark in *plasmodium berghei*-infected mice

Treatment	Dose (mg/kg)	Mean parasitemia density	% suppression
		D <sub>5</sub>	
Control	0.2 ml/kg	15.32± 0.91	-----
<i>B. buonopozense</i>	100	2.45± 0.15	84**
	200	1.67±0.08	89**
	400	0.5± 0.10	96.74**
CQ	10	0.47± 0.06	96.93**

D<sub>5</sub>= Day five, CQ=Chloroquine \* significantly different from control at P< 0.05.

\*\* High significantly different from control at P<0.01

was inoculated intraperitoneally with standard inoculums of  $1 \times 10^7$  *plasmodium berghei* infected erythrocytes. The mice were randomly divided into five groups of six per cage and treated with 100, 200 and 400 mg/kg/day of the extract. Chloroquine diphosphate 10 mg/kg/day was given as positive control and 0.2 mL of normal saline to the negative control group. All administered orally for four consecutive days (D<sub>0</sub>-D<sub>3</sub>). On the fifth day (D<sub>4</sub>), blood was collected from each mouse and thin films made on a slide. The films were fixed with methanol, stained with Giemsa and parasitemia density examined (Nikon YS2-H, Japan) by counting the parasitized red blood cells on at least 1000 red blood cells in 10 different fields.

#### Curative test

Evaluation of curative potential of *Bombax buonopozense* stem bark extract was done by adopting the method of Chandel and Bagai, 2010 (18) and Akuodor *et al.*, 2010 (19) with slight modification. A total of thirty mice were inoculated intraperitoneally with standard inoculums of  $1 \times 10^7$  *P.berghei* infected erythrocytes on the first day. Seventy-two hours later, the mice were divided into five groups of six per cage and treated with 100, 200 and 400 mg/kg/day of the extract. Chloroquine diphosphate 10 mg/kg/day was given to positive control and 0.2 mL of normal saline to the negative control group, all administered orally. Treatment continued daily until the eighth day when thin films were prepared with the blood collected from the tail of each mouse. The films were fixed with methanol, stained with Giemsa and parasitemia density examined by counting the parasitized red blood cells on at least 1000 red blood cells in 10 different fields. The mean survival time for each group was determined by finding the average survival time (days) of the

mice (post inoculation) in each group over a period of 30 days (D<sub>0</sub>-D<sub>29</sub>) (20).

#### Statistical analysis

Results obtained were expressed as mean ± S.E.M. The data was analyzed using One-way ANOVA followed by Dunnett's post hoc test. P<0.05 was considered as significant.

### 3. Results

#### 3. 1. Phytochemical test

Phytochemical analysis of the stem bark extract of *Bombax buonopozense* revealed the presence of alkaloids, terpenes, sterols, flavonoids, tannins, saponins and carbohydrates.

#### 3. 2. Acute toxicity test

There was no mortality observed in mice after oral administration of the aqueous extract, even at doses as high as 5000 mg/kg signifying that the oral LD<sub>50</sub> was greater than 5000 mg/kg. Hence, the experimental doses used (100,200 and 400 mg/kg *p.o.*) were within safe margin.

#### 3. 3. Suppressive effect

The aqueous stem bark extract of *Bombax buonopozense* exhibited a dose dependent chemosuppressive effect at the different doses employed. Doses of 100, 200 and 400 mg/kg caused chemosuppression of 84%, 89% and 96.74% respectively. The effect of the extract was significant (P<0.05) when compared with the control. The standard drug, chloroquine (10 mg/kg/day), caused 96.93% suppression (Table 1).

#### 3. 4. Curative effect

The aqueous stem bark extract of *Bombax buonopozense* caused a dose-dependent decrease in parasitemia in the extract treated group similar to chloroquine treated group unlike the saline treated group in which there was a consistent increase in the blood parasite density. The

Table 2. Curative effect of *B. buonopozense* Stem bark in *P. berghei*-infected mice

Treatment	Dose (mg/kg)	Mean parasitemia density		Survival time
		(Days)	Pre (D <sub>3</sub> )	post (D <sub>7</sub> )-treatment
Control	0.2ml/kg	30.73±1.77	39.62±3.2	10.67±0.49
<i>B. buonopozense</i>	100	29.42±2.22	5.72±0.49**	24.83±0.95
	200	30.34±3.10	4.45±0.37**	27.17±0.75
	400	30.98±4.30	2.90±0.11**	29.67±0.21
CQ	10	29.68±3.11	2.32±0.07**	30.0±0.0

D3=Day three, D7=Day seven, CQ=Chloroquine \* significantly different from control at P< 0.05.

\*\* High significant different from control at P<0.01

survival values showed that the plant extract significantly (P<0.05) suppressed established infection at the doses employed. Death was observed in the control group on day 9, and by day 12, all mice in the group died. On the other hand, mice in extract treated groups survived beyond 24 days. However, some of the mice in the 400 mg/kg/day group survived the 30 days observation period, while chloroquine treated group recorded no death at all (Table 2).

#### 4. Discussion

Although primate models provide a better prediction of efficacy in human than the rodent models, the later have also been validated through the identification of several conventional antimalarial, such as chloroquine, mefloquine, halofantrine and more recently artemisinin derivatives (21). *Plasmodium berghei* has been used in studying the activity of potential antimalarials in mice (22) and in rats (23). It produces diseases similar to those of human plasmodium infection (24, 25). As this parasite is sensitive to chloroquine, this drug was used as a standard drug in the study. Chloroquine has been used for suppressive and curative antiplasmodial activities. In early and established infection, chloroquine interrupts with the hem polymerization by forming a PF-chloroquine complex. This complex is responsible for the disruption of the parasite's cell membrane function and ultimately leads to auto digestion.

The 4-day suppressive test is a standard test commonly used for antimalarial screening and the determination of percent inhibition of parasitemia is the most reliable parameter. A mean group parasitemia level of less than or equal to 90% of the mock-treated control animals usually indicate that the test agent is active in standard screening studies (26). The results obtained showed that in *Plasmodium berghei* infected mice treated with aqueous stem bark extract of *Bombax buonopozense*, there was significant decrease in

parasitemia. The extract exhibited a dose dependent activity. In addition, the result of the chemosuppressive activity suggests that the stem bark extract of this plant can suppress parasite growth to non-detectable levels in erythrocytes. It is important to know that scientific evaluation of traditional medicine preparations for claimed antimalaria efficacy be carried out even up to the level of finding out the degree of suppression of parasite growth in erythrocytes (27).

The stem bark extract of this plant also exerted significant curative effect during established infection. Curative activity of potential antimalarial agents of ethnomedicinal materials should be discernable during testing for antimalarial. The observed antimalarial activity of the plant extract is consistent with the traditional use of the plant as herbal medication against the disease and indicative of its potential as a chemotherapeutic antimalarial agent. This was confirmed by the mean survival time values which at doses employed were twice or more than that of control group. In untreated mice, the parasite count increased daily until the death of the animal, which was also observed in our previous studies (28).

However, the traditional use of *Bombax buonopozense* could be attributed to the presence of certain phytochemicals that constitute the bioactive principles in the plant. Numerous plants containing a wide variety of phytochemicals as their bioactive principle have shown antiplasmodial activities (29-31). The antiplasmodial activity of this plant extract might be attributed to the presence of alkaloids, flavonoids and terpenes which have been variously implicated in antiplasmodial activities of many plants (32). These compounds have also been shown to exert antiplasmodial activity by elevating the red blood oxidation and inhibiting the parasite's protein synthesis. This counteracts the oxidative damage induced by the malaria parasite (33).

In conclusion, this study has however established the rationale for the traditional use of this plant in Nigeria and like many others, proved that medicinal plants which have folkloric reputations for antimalarial properties can be investigated in order to establish their efficacy and to determine their potentials as sources of new antimalarial drugs.

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