

Pharmacological screening and evaluation of antiplasmodial activity of *Croton zambesicus* against *Plasmodium berghei berghei* infection in mice

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ABSTRACT

Objective: To evaluate the antiplasmodial activity of leaf extract of *Croton zambesicus* on chloroquine-sensitive *Plasmodium berghei berghei* infection in mice and to confirm its traditional use as a malarial remedy in Africa.

Materials and Methods: The ethanolic leaf extract of *Croton zambesicus* (50-200 mg/kg) was screened for blood schizontocidal activity against chloroquine-sensitive *Plasmodium berghei berghei* infection in mice. The schizontocidal activity during early and established infections as well as the repository activity were investigated.

Results: The extract demonstrated a dose-dependent chemosuppression or schizontocidal effect during early and in established infections, and also had repository activity. The activity was lower than that of the standard drugs (chloroquine 5 mg/kg, pyrimethamine 1.2 mg/kg/day).

Conclusion: The leaf extract possesses considerable antiplasmodial activity, which can be exploited in malaria therapy.

KEY WORDS: Malaria, schizontocide.

Introduction

Croton zambesicus Muell Arg. (Euphorbiaceae) syn *C. amabilis* Muell. Arg.; syn. *C. gratissimus* Burch is an ornamental tree grown in villages and towns of Nigeria. It is a Guineo-Congolese species widely spread in tropical Africa. The leaf decoction is used in Benin as antihypertensive and antimicrobial (urinary infections).^[1] The Ibibios in Uruan area of Akwa Ibom State of Nigeria use the leaf traditionally as a remedy for malaria. Block *et al* reported that ent-trachyloban-3 β -ol, a trachylobane diterpene, isolated from dichloromethane extract of the leaves has cytotoxic activity on HeLa cells.^[2] The alkaloidal fractions of the leaf have been reported to possess weak activity against *Kelebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Aspergillus niger*, *Microsporium species* and *Penicillium species*.^[3] The alkaloidal fractions of the stem have also been reported to be active against all micro-organisms mentioned above.^[3] The essential oil found in the leaves contains P-cymene, linalool and beta-caryophyllene.^[4] The constituents

of the essential oil also found in the flowering tops are pinene, limonene linalool, menthol, carvone, thymol, alpha-humulene and ceisnerolidol.^[5]

In spite of the large number of phytochemical and pharmacological studies carried out worldwide on *Croton zambesicus*, no scientific report has been quoted in the index medicus and NAPRALERT database on the antiplasmodial activity of this plant grown in Nigeria. We, therefore, evaluated the blood schizontocidal activity of the leaf extract of *C. zambesicus* against *Plasmodium berghei* infection in mice.

Materials and Methods

Plant

The leaves of *Croton zambesicus* Linn (Euphorbiaceae) were collected in March 2003, at Uyo area of Akwa Ibom State, Nigeria and authenticated by Dr. Uduak Eshiet, a taxonomist in the department of Botany, University of Uyo, Uyo, Nigeria. A specimen was deposited at the Faculty of Pharmacy herbarium, University of Uyo, Uyo (Voucher no. FPUU 209.)

Preparation of extract

Material: The fresh leaves (2 kg) of the plant were shade

dried for 8 days and then powdered. 100 g of the powder was macerated in ethanol (300 ml) for 72 h. The liquid extract obtained was concentrated in vacuum at 40 °C. The yield was 3.81%.

Phytochemical screening: A preliminary phytochemical screening of the powdered material was carried out employing the standard procedures to reveal the presence of saponin, flavonoids, tannins, alkaloids and glycosides.^[6,7]

Animals

Swiss albino mice (25-32 g) of both sexes were obtained from the University of Uyo animal house, Uyo, Nigeria. The animals were housed in standard cages and acclimatized for a period of 10 days. The mice were maintained on standard pelleted diet and water *ad libitum*. Approval for the study was obtained from the Animal Ethics Committee, University of Uyo.

Parasite inoculation

The chloroquine-sensitive *Plasmodium berghei berghei* strain was obtained from the National Institute of Pharmaceutical Research and Development, Abuja, Nigeria and was maintained in mice. The inoculum consisted of 5×10^7 *P. berghei berghei* parasitized red blood cells per ml. This was done by determining both the percentage parasitaemia and the red blood cell count of the donor mouse using the hemocytometer and diluting the blood with isotonic saline in proportions indicated by both determinations. Each mouse was inoculated on day 0, intraperitoneally, with 0.2 ml of infected blood containing about 1×10^7 *P. berghei berghei* parasitized red blood cells obtained from a donor mouse having about 64.0% parasitaemia.^[8]

Drug administration

The drugs and the extract used in this study were orally administered with the aid of a stainless metallic feeding cannula.

Evaluation of schizontocidal activity on early infection

A method described by Knight and Peters was used.^[9] The animals were divided into six groups of five mice each, and were administered 50, 100, 150 and 200 mg/kg/day doses of the extract, chloroquine 5 mg/kg/day and an equivalent volume of distilled water (control group) for four consecutive days (D0 to D3). On the fifth day (D4), thin blood films were made from the tail blood and fixed with methanol. The films were then stained with Giemsa stain and the percentage parasitemia was determined by counting the number of parasitized blood cells out of 500 red blood cells in random fields of the microscope. The average % suppression of parasitemia was calculated in comparison to controls as shown below.

$$\text{Average \% suppression}^{[10]} = \frac{\text{Average \% parasitemia in control groups} - \text{Average \% parasitemia in treated group}}{\text{Average \% parasitemia in control}} \times 100$$

Evaluation of the repository activity

The repository activity was assessed by using the method described by Peters.^[11] The mice were divided into six groups of five animals each and administered 50, 100, 150 and 200 mg/kg/day doses of the extract, 1.2 mg/kg/day pyrimethamine (standard group) and distilled water (control) for four consecutive days (D0 to D3). On day five (D4), the mice were inoculated with *P. berghei berghei*. Seventy-two hours later,

the parasitemia level was assessed by blood smears.

Evaluation of schizontocidal activity in established infection (Rane test)

A modified method similar to Ryley and Peters was used.^[12] On D0, standard inocula of 1×10^7 infected erythrocytes was injected in mice, intraperitoneally. Seventy-two hours later, the mice were divided into six groups (n = 5). Different doses of *C. zambesicus* extract (50, 100, 150 and 200 mg/kg/day) were orally administered to these groups. Chloroquine (5 mg/kg/day) was given to the standard group, and an equal volume of distilled water to the control group. The drug/extract was given once daily for 5 days. Thin films stained with Giemsa stain were prepared from tail blood for each mouse daily for 5 days to monitor the parasitemia level. The mean survival time for each group was determined arithmetically by finding the average of the survival time (days) of the mice (post inoculation) in each group over a period of 28 days (D0 to D28).

Statistical analysis

The data are expressed as mean \pm SEM. The data were statistically analysed using one-way ANOVA followed by Tukey-Kramer post test and values of $P < 0.05$ were considered significant.

Results

Phytochemical screening

Phytochemical screening of the ethanolic leaf extract of *Croton zambesicus* revealed the presence of compounds like saponins, alkaloids, terpenes, flavonoids and cardiac glycosides.

4-Day test

The ethanolic leaf extract of *C. zambesicus* produced a dose-dependent chemosuppressive effect at the different doses employed. (200, 150, 100 and 50 mg/kg doses with a chemosuppression of 80.7%, 75.0%, 70.7% and 38.4%, respectively - Table 1). The standard drug, chloroquine 5 mg/kg/day, caused 90 % suppression [Table 1], which was significantly ($P < 0.05$) more than that of the extract-treated groups.

Repository test

The ethanolic extract of *C. zambesicus* exerted a dose-dependent repository activity at the various doses employed (200, 150, 100 and 50 mg/kg/day doses causing 78.5%, 71.3%, 63.0% and 50.0% chemosuppression, respectively - Table 2). However, the standard agent pyrimethamine (1.2 mg/kg/day) caused a considerably higher (89.93%) chemosuppression than the extract-treated groups [Table 2] in which the highest dose produced 78.5% chemosuppression.

Rane test

There was a dose-dependent reduction in parasitaemia with the extract, similar to the chloroquine-treated group, while the control group showed a daily increase in parasitemia. However, there was a daily reduction in the parasitemia level of the chloroquine-treated group [Figure 1]. Data in Table 3 show that chloroquine (5 mg/kg/day) gave a mean survival time (m.s.t) of 27.1 ± 0.7 days as compared to 11.5 ± 3.5 , 20.5 ± 0.6 , 21.3 ± 0.8 and 25.5 ± 0.5 days observed with 50, 100, 150 and 200 mg/kg/day doses of plant extract, respectively. It is noteworthy that some of the mice in the 200 mg/kg/day

Table 1

Blood schizontocidal activity of *C. zambesicus* extract during early *P. berghei berghei* infection (4–day test) in mice

Drug/extract	Dose (mg/kg/day)	Average % parasitemia	Average % suppression
<i>C. zambesicus</i> extract	200	8.27 ± 0.09*	80.7
	150	10.68 ± 0.21*	75.0
	100	12.53 ± 0.07*	70.7
	50	26.34 ± 1.73*	38.4
Chloroquine (standard)	5	4.27 ± 0.09	90.0
Distilled water (control)	0.2 ml	42.77 ± 0.05	-
One-way ANOVA	F	133.70	
	P	<0.05	

Data are expressed as mean±SEM for five animals per group; df=4,24; *P<0.05 when compared to control.

Table 2

Repository activity of *C. zambesicus* extract against *P. berghei berghei* infection in mice

Drug/extract	Dose (mg/kg/day)	Average % parasitemia	Average % suppression
<i>C. zambesicus</i> extract	200	10.12±1.50*	78.5
	150	13.49±7.38*	71.3
	100	17.38±1.73*	63.0
	50	23.54±1.91*	50.0
Pyrimethamine (standard)	1.2	4.74±1.02	89.9
Distilled water (control)	0.2 ml	47.08±1.3	-
One-way ANOVA	F	3.176	
	P	<0.05	

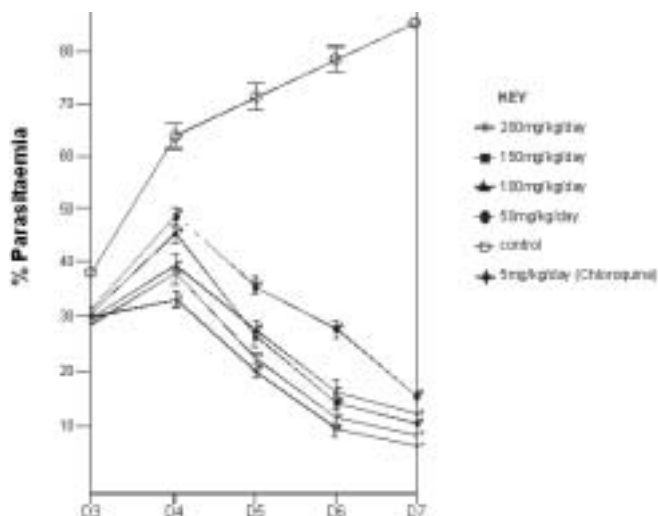
Data are expressed as mean±SEM for five animals per group; df=4, 24; *P<0.05 when compared to control.

Table 3

Mean survival time of the mice receiving the various doses of ethanolic extract of *C. zambesicus* during an established *P. berghei berghei* infection in mice

Drug/extract	Dose (day)	Mean survival time (mg/kg/day)
<i>C. zambesicus</i> extract	50	11.5 ± 3.51*
	100	20.5 ± 0.63*
	150	21.3 ± 0.81*
	200	25.5 ± 0.54*
Chloroquine (standard)	5	27.5 ± 0.73
Distilled water (control)	0.2 ml	7.5 ± 0.76
One-way ANOVA	F	3.176
	P	<0.05

Data are expressed as mean±SEM for five animals per group; df=4,24; *P<0.05 when compared to control.

Figure 1. Ethanolic extract of *C. zambesicus* on Rane test. Each point represents mean of 5 observations. Error bars represent SEM.

dose group survived the total duration of the study, as was the case with the chloroquine group. Although the values of m.s.t show that the plant extract significantly ($P < 0.05$) suppressed established infection at the doses studied relative to the control group, they were lower than that induced by the group treated with the standard drug (chloroquine).

Discussion

In this study, preliminary phytochemical screening and evaluation of antiplasmodial activity of ethanolic leaf extract of *Croton zambesicus* were carried out. Alkaloids, terpenes and flavonoids, which have been variously implicated in antiplasmodial activities of many plants,^[13] are also found in the extract studied. The antiplasmodial activity observed in this study could have resulted from a single or combined action of these compounds. However, the active principle responsible is yet to be identified.

The results indicate that the leaf extract possessed blood schizontocidal activity as evident from the chemosuppression obtained during the 4-day early infection test. The plant extract also exhibited repository activity, though the doses used could not produce suppression comparable to that of the standard drug (pyrimethamine 1.2 mg/kg/day). However, on established infection, the plant extract exhibited significant ($P < 0.05$) blood schizontocidal activity. It is noteworthy that the antiplasmodial activity of the extract at all doses during early and established infections was not comparable to that of the standard drugs. There was a dose-dependent chemosuppression of parasitemia seen with the extract. This was confirmed by the significant ($P < 0.05$) high m.s.t values particularly in the group administered 200 mg/kg/day of the extract. The fact that some of the mice in this group survived throughout the 28 days of observation similar to the chloroquine-treated group shows a high level of efficacy of the extract, which was comparable to that of chloroquine, the referenced drug.

The plant has exhibited promising antimalarial activity, which can be exploited in malaria therapy. However, in the course of the study it was discovered that the 5 mg/kg/ day of chloroquine used as the standard in the 4-day test and Rane test did not cure the mice of malaria. This could be due to the development of resistance as the parasite is passaged from one mouse to another in the course of maintenance of the parasite strain. The extract and chloroquine doses tested in this study do not seem to be effective in chloroquine-resistant malaria. However, the possibility of increased doses of both the extract and chloroquine producing 100% chemosuppression cannot be ruled out as this observation is based on the tested doses and also preliminary. The low doses of the extract in this preliminary study may have affected the results obtained with the tested extract in which curative activity could not be ascertained particularly in the two tests mentioned above. Although the mechanism of action of this extract has not been elucidated, some plants are known to exert antiplasmodial action either by causing elevation of red blood cell oxidation^[14] or by inhibiting protein synthesis^[15] 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.

To conclude, the results of the present study have shown that the ethanolic leaf extract of *Croton zambesicus* possesses antimalarial activity as seen in its ability to suppress *Plasmodium berghei berghei* infection in mice in all the three evaluation tests. These observations confirm the traditional use of the plant to treat malaria. Therefore, it would be interesting if the active principle could be isolated, identified and characterized from this promising medicinal plant.

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