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Research article

Evaluation of antimalarial effect of methanol root extract of *Costus Lucanusianus* in the treatment of malaria-infested albino mice

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Abstract

This study is one of its kinds because there is little or no published work on the plant in question. **Aim**: This study was conducted to evaluate the antimalarial activity of methanol root extract of *Costus lucanusianus* on chloroquine-sensitive *Plasmodium berghei berghei* infection in mice. **Method**: The plant extract was screened for blood schizontocidal activity against chloroquine-sensitive *Plasmodium berghei* infection in the mice. The schizontocidal activity was monitored at stages of early and established infection. The methanol extract of the roots at 100, 200 and 300 mg kg^{-1} body weight/day dose levels were used to treat the test groups immediately after infection for the suppressive test and 72 hours post infection for the curative test while a standard antimalarial drug, Chloroquine, at a dose of 5 mg kg^{-1} body weight was administered as the reference drug. The control group was left untreated. The levels of parasitemia in the different groups were monitored throughout the period of study. **Result**: The methanol extract at 100, 200 and 300 mg kg^{-1} body weight/day suppressed parasitemia by 112/µl, 128/µl, 192/µl after treating for four days in the suppressive test as against 144/µl for the standard drug with significance of $p<0.0001$, while the level of parasitemia was reduced by 528/µl, 320/µl and 240/µl, respectively after treating for three days in the curative test as against 160±/µl for the standard drug. **Conclusion**: These results show that the methanol root extract of *Costus lucanusianus* has suppressive and also potent curative effect against *P. berghei* in infected mice. Thus it may therefore offer the potential for a safe, effective and affordable antimalarial drug. It therefore justifies its use by those in rural areas to treat malaria.The mechanism behind the antiplasmodial activity displayed by *C. lucanusianus* is yet to be demonstrated. However, some plants have been shown to elicit antiplasmodial effects either by inducing an elevation of erythrocyte oxidation or by inhibiting the synthesis of proteins.

Keywords: *Costus lucanusianus*, schizontocidal activity, parasitemia, suppressive activity, *Plasmodium berghei*, Chloroquine.

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1. Introduction

Malaria, which predominantly occurs in tropical areas, is a potentially life-threatening disease caused by infection with *Plasmodium* protozoa transmitted by an infective female Anopheles mosquito vector. Individuals with malaria may present with fever and a wide range of symptoms which may include; headache (noted in virtually all patients with malaria), cough, fatigue, malaise, shaking chills, arthralgia, myalgia, paroxysm of fever and sweats (every 48 or 72 hours, depending on species) etc. [1]

In this study, the methanol root extract of *Costus lucanusianus* was used to treat malaria in mice and was compared with a reference drug- Chloroquine. *Costus lucanusianus*, commonly known as monkey sugar cane in Nigeria [2], is a small to large non-aromatic perennial rhizomatous herbs, terrestrial or less commonly epiphytic which grow in seasonally or permanently humid localities in forest, up to 1200m altitude. In cultivation it prefers a humus-rich soil and partial shade [3].*Costus lucanusianus* is

commonly used as a medicinal plant in tropical Africa. An infusion of the inflorescence is used to treat tachycardia and stomach complaints. A stem decoction, warmed stem sap or the pounded fruit are taken to treat cough, bronchitis and a sore throat; the stem is also mashed or chewed to treat cough [4].Leaf sap is acidic and is used as eye drops to treat eye troubles and headache with vertigo, and in frictions to treat edema and fever. Leaf sap is used as nose drops and leaf pulp is rubbed on the head to calm insanity [5]*Costus lucanusianus* is commonly used for ceremonial and religious purposes. It is sold in Western countries as an ornamental container plant [3].

Previous studies have shown that more than 1200 medicinal plants from 160 families are used worldwide to treat malaria or fever [6] and still many anti-malarial plant species remain to be discovered.Some of the plants include;

- *Vernonia amygdalina* (Bitter leaf): It has broad greenish leaves that contain natural quinine which has bitter taste. The medicinal constituent (quinine) cures malaria, cleans the liver and lymphatic system and lungs for smokers [7]. The major part of the plant used is the leaf and young stem [8].
- *Mangifera indica* (Mango): The major part used for treating malaria is the bark and leaves, which is boiled in water for 50 minutes (decoction) [9].
- *Azadirachta indica* (Neem plant): The leaf is found to be of high medicinal value and is used to treat all forms of malaria caused by *Plasmodium* parasite living in the blood stream. The main part of the plant used is the bark and leaves. [9].
- *Carica papaya (Pawpaw)*: A combination of its leaf extract with the plants stated above is an effective medicine used in the treatment of malaria. The part used is the leaves and seed [8]. The method of extraction is via infusion [9].
- *Ocimum gratissimum* (Scent leaf): The part of the plant used for medicinal purpose is its leaf. The leaves are washed and pounded or squeezed and the juice filtered and taken until malaria symptoms disappear [8].
- *Cymbopogon citratus* (Lemon grass): The part used for treating malaria is its leaves. Fresh leaves are macerated in water and the extract is administered [8].
- *Psidium guajava* (Guava): The leaves and bark of this plant is used for treatment of malaria and the method of extraction is infusion [9].
- *Citrus aurantifolia* (Lime): The major part used include root, bark, stem-twigs, leaves and fruit. In southwest Nigeria, the roots, bark, stem twigs, leaves and fruits are used in the treatment of malaria [9].

In this study we report the antimalarial activity of methanol root extract of *Costus lucanusianus* using mice as our model organism.

2. Materials and methods

Preparation of Plant Extract

After collection of the plant, the roots were shade-dried at room temperature (32 – 35^{o}C) to constant weight over a period of seven (7) days. The cold maceration extraction method of Cowan (1999) was used. Fifty grams of *Costus lucanusianus* was weighed and grinded to fine powder and dissolved in 1000ml of seventy percent methanol inside a 2-liter conical flask. The flask was shaken vigorously at 30 minute intervals and left to stand for 72 hours at room temperature for effective extraction. The resultant mixture then was filtered with Watman's No. 1 filter paper and cotton wool to remove particles of plant sample. The clear solution obtained was concentrated with rotary evaporator at 45^{o}C under low pressure and later transferred to evaporating dish over a steam bath. The solid dried powder obtained was stored in sterile pre-weighed screw capped bottles and labeled accordingly. The extract was now stored at room temperature [10].

Experimental animals

The animals used for this study were 6 week-old-albino mice weighing 20-35g obtained from the Animal house, Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port-Harcourt. They were housed in plastic cages with saw dust as beddings and were given food and water ad libitum.

Rodent parasites (*Plasmodium berghei berghei*)

Plasmodium berghei was obtained from Malaria Research Laboratory, Centre for Malaria Research and Phytomedicine, University of Port-Harcourt. The parasites were kept alive by continuous intraperitoneal injection in mice every seven (7) days. Prior to the beginning of the study, one of the infested mice was kept and observed to show disease symptoms similar to human infection such as shaking chills, anemia, fatigue.

Parasite inoculation

The method of Peter (1967) was used for the inoculation of parasites into the experimental animals. The inoculums consisted of *Plasmodium berghei*-parasitized erythrocytes. Each mouse was inoculated on day 1, intraperitoneally with 0.2mL of infected blood containing approximately 1×10^{7}*Plasmodium berghei*-parasitized red blood cells. In addition, the newly inoculated animals were monitored daily to determine expression of parasites in circulation [11]. The inoculation of the parasites into the animals was done at the Malaria Research Laboratory, Centre for Malaria Research and Phytomedicine, University of Port-Harcourt.

Evaluation of antimalarial activity

Evaluation of suppressive activity on early infection (4-day test)

Suppressive activity of the extract was evaluated using the method of Ryley and Peters (1970). Each mouse was inoculated on the first day (day 1), intraperitoneally, with 0.2ml of infected blood containing about 1×10^7 *Plasmodium berghei*-parasitized erythrocytes. The animals were randomly divided into five groups of five animals each. Shortly after infection (20 minutes), mice in the first group were orally administered 10ml/kg distilled water, and they served as control; the second to fourth groups received 100, 200, and 300mg/kg. Chloroquine, 5mg/kg/day, was given to the fifth group, which served as the reference group [12].
Administration of the extract and reference drug continued daily for four (4) days between 9:00am and 10:00am. On the fifth day, thin blood films were made from tail blood of each mouse. The films were stained with Giemsa stain to reveal the parasitized erythrocytes. The parasitemia level was determined by counting the number of parasitized red blood cells out of one hundred (100) white blood cells in twenty-five (25) fields of the microscope.

Evaluation of curative activity

The curative activity of the extract was evaluated when the infection was established. The method used was the one proposed by Ryley and Peters (1970). Twenty mice were infected intraperitoneally with standard inoculum of 1×10^7 *P. berghei berghei* parasitized erythrocytes on the first day (Day1). Seventy-two (72) hours later, the mice were randomly assigned to five groups (A-E) of four mice each. Group A served as the control and received distilled water 10ml/kg. Group B, Group C and Group D were administered with 100, 200 and 300mg/kg of the extract orally respectively. Group E served as the reference group and received Chloroquine (5.0mg/kg/day).
The reference drug/extract was given to the animals once daily for 3 days. Thin films, stained with Geimsa stain, were prepared from the tail blood of each mouse daily for three (3) days to monitor the parasitemia level. In the process of administering the extract and reference drug, for both curative and suppressive activity, to the animals, six (6) animals died.

Determination of parasitemia

The parasite density or parasitemia was achieved by counting one hundred WBCs with the aid of a Neubauer Chamber and reporting the number of parasites per 100 WBCs on the smear. The value gotten was converted to the number of parasites per µl of blood by dividing the number of parasites per 100 WBCs by 100, and multiplying that value by the standard number of WBCs/µl (8000/µl).

$$\text{Parasites/µL blood} = \frac{\text{Number of parasites counted} \times 8000 \text{ white blood cells/µL}}{\text{No. of white blood cells counted}}$$

Data analysis

Data from this study was analyzed using the Graph pad prism 7.02 statistical software. Numeric data was presented as Mean±SEM. The significance of the mean difference among the groups was determined using one-way analysis of variance (ANOVA) followed by Post-hoc test (Dunnet method) multiple comparison while the confidence intervals were 95%, 99%, 99.9% and 99.99% significance.

3. Results

Suppressive activity of methanol root extract of *Costus lucanusianus*

The methanol root extract of *Costus lucanusianus* exerted dose dependent chemosuppressive effect against *Plasmodium berghei berghei* malaria parasite. The extract caused a significant ($p<0.0001$) chemosuppression when compared to the control. The reference drug, Chloroquine, caused suppression which was only higher than the 300mg/kg dose.

Table No 1: Group Classification

SN	Groups	Specification
1	Group a	10ml/kg h_2o
2	Group b	100mg/kg of plant extract
3	Group c	200mg/kg of plant extract
4	Group d	300mg/kg of plant extract
5	Group e	5mg/kg chloroquine

Table No 2: Suppressive activity of methanol root extract of *Costus lucanusianus* on *Plasmodium berghei*-infested mice.

SN	Groups	Parasite Density
1	A(10ml/kg H_2O)	1472±114.8
2	B(100mg/kg of plant extract)	112±40.79****
3	C(200mg/kg of plant extract)	128±40.79****
4	D(300mg/kg of plant extract)	192±32****
5	E(5mg/kg chloroquine)	144±29.93****

****= $p<0.0001$

Curative activity of methanol root extract of *Costus lucanusianus*

The methanol root extract of *Costus lucanusianus* produced significant ($p<0.0001$) dose dependent reduction in parasitemia levels after day one in the extract treated groups of C & D, with a similar reduction in the Chloroquine treated groups (positive control). (Table 2)
It also produced significant ($p<0.01$) dose dependent reduction in parasitemia levels after day two in the extract treated groups of B & D, with a similar reduction with significant ($p<0.001$) dose in the Chloroquine treated group and group C. On the third day, It produced significant ($p<0.0001$) dose dependent reduction in parasitemia levels in the extract treated groups of B, C & D, with a similar

reduction with significant ($p<0.0001$) dose in the Chloroquine treated group.

Table No 3: Curative activity of methanol root extract of *Costus lucanusianus* on *Plasmodium berghei*-infested mice. (Day 1-3)

Groups	Parasite Density		
	Day One	Day Two	Day Three
A (10ml/kg H_2O)	960±43.2	1408±256.2	1904±207.7
B (100mg/kg of plant extract)	752±40.79^{ns}	528±106.1**	528±40.9****
C(200mg/kg of plant extract)	624±68.82**	480±97.98***	320±56.57****
D(300mg/kg of plant extract)	416±68.82****	672±99.92**	240±35.78****
E(5mg/kg clq)	352±89.8****	352±82.37***	160±43.82****

ns= Not Significant; **= $p<0.01$; ***= $p<0.001$; ****= $p<0.0001$

Discussion

Plasmodium berghei berghei parasite is used in predicting treatment outcomes of any suspected antimalarial agent due to its high sensitivity to chloroquine making it the appropriate parasite for this study [13].

In this study, the antimalarial activity of methanol root extract of *Costus lucanusianus* in mice was evaluated. The result obtained in this work has shown that the methanol root extract of *Costus lucanusianus* demonstrated a significant ($p<0.0001$) dose-dependent anti-plasmodial activity for both suppressive and curative tests. The highest suppressive effect was observed with 100mg/kg having parasite density of 112/µl. This is followed by the 200mg/kg dose with parasite density of 128/µl. The third dose of 300mg/kg gave the least suppression of 192/µl, while chloroquine, the reference drug, gave a minimal suppression when compared to 100 and 200mg/kg doses. The observed lower efficacy of chloroquine may be due to the fact that chloroquine is a cidal antimalarial drug and hence will not effectively exhibit suppressive action. Studies have shown that chloroquine, at lower concentration; exhibit an inhibitory effect rather than its normal cidal effect it is supposed to cause [14].

The result of the curative effect of the plant extract equally showed a concentration-dependent activity. It was observed that the methanol root extract of *C. lucanusianus* produced a dose dependent reduction in parasitaemia levels in the extract treated groups, with a similar reduction in the chloroquine treated group, while, there was a daily increase in parasitaemia in the control group. The dose of 100mg/kg gave a minimal reduction in parasitaemia level (752/µl) after the first day of administration. On the second and third day, the parasitaemia levels reduced but slowly, making this dose a less likely dose that can be used for cidal action. It can be inferred that 100mg/kg of the plant extract will serve as an excellent inhibitory (suppressive) dose, but will not be very effective as a cidal dose like the dose of chloroquine. As the dose increased, the efficacy of the extract also increased in its cidal action, thus the 300mg/kg dose had higher cidal action than 100 and 200mg/kg dose. The reference drug (chloroquine) produced the highest efficacy with respect to cidal action. This observed higher efficacy of chloroquine may in part be due to non-selectivity of the extract or slow absorption and poor bioavailability of the extract. Similar observation was reported with the use of *Vernonia amygdalina*by Adzu and Haruna (2007).

The mechanism behind the antiplasmodial activity displayed by *C. lucanusianus* is yet to be demonstrated. However, some plants have been shown to elicit antiplasmodial effects either by inducing an elevation of erythrocyte oxidation or by inhibiting the synthesis of proteins [12] [16].

Conclusion

From the results of this study, it could be concluded that the methanol root extract of *C. lucanusianus* has antiplasmodial/antimalarial activity as it has been shown to suppress the development of *Plasmodium berghei* parasite infection in mice at the different stage of malaria infection.

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