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In vivo antiplasmodial evaluation of methanol mesocarp extract of *Citrillus lanatus* in *Plasmodium berghei berghei* infected mice.

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ABSTRACT

Aim: The aim of this study was to evaluate the *in vivo* antiplasmodial activities of the methanol mesocarp extract of *Citrillus lanatus* in mice infected with *Plasmodium berghei berghei.* **Materials and Methods**: The extract (125, 250, and 500 mg/kg) was administered orally to mice and were assessed in suppressive, repository and curative tests using Chloroquine (5 mg/kg) and Pyrimethamine (1.2 mg/kg) as positive controls. **Results:** A dose-dependent, significant ($p < 0.001$) antiplasmodial effect was recorded in the suppressive test relative to control. The extract also demonstrated a dose-dependent, significant ($p < 0.01 – 0.001$) prophylactic and curative effects when compared to the controls. These antiplasmodial effects of the extract compared favourably with those of the standard drugs. The extract in addition, increased the mean survival times of the infected mice. **Conclusions**: The methanol mesocarp extract of *C. lanatus* possesses antiplasmodial activities, thereby corroborating its use in natural medicine in the treatment of malaria.

Keywords: *Citrillus lanatus*, antiplasmodial, *Plasmodium berghei berghei*, extract, mice.

INTRODUCTION

There are six species of Plasmodium that cause malaria in human beings and these are *Plasmodium ovale wallikeri, Plasmodium malariae, Plasmodium ovale curtisi, Plasmodium falciparum, Plasmodium vivax and Plasmodium knowlesi*. *P. knowlesi* is zoonotic but is capable of causing malarial infections in many people in South Eastern parts of Asia [1, 2]. The World Malaria Report of December, 2019, recorded 228 million cases of malaria in 2018 compared to 231 million cases observed in 2017 along with 251 million recorded cases in 2010. Africa recorded the highest number of malaria cases in 2018 (93%), followed by South-East Asia (3.4%) and Eastern Mediterranean (2.1%). Deaths resulting from malaria globally was put at 405,000 in 2018 as compared to 416 000 estimated deaths in 2017, and 585, 000 in 2010 respectively. Children who aged under 5 years were those most affected by malaria which accounted for 272,000 deaths (about 67%) caused by malaria globally in 2018 [3].

Citrillus lanatus (Watermelon) is one of the most popular fruits in the world and contains good quantity of nutrients. Watermelon seeds are great sources of essential and non-essential amino acids and oil. They are known to be rich in nutrients such as calcium, phosphorous, zinc, magnesium, potassium as well as iron [4, 5]. The plant is an annual plant, herbaceous with long stems of up to 10 m in length whether it is lies or creeps on the ground and usually has curly tendrils. The leaves are often 5-20 by 3-19 cm and hairy. The lobes are usually palmate with 3 – 5 lobes. Its fruits vary greatly in morphology of color, odour and taste [6]. The fruit and seeds have laxative, antimicrobial, anti-giardial, ant-infalmmatory, antioxidant, anti-ulcer, hepatoprotective and anti-hyperlipedimic properties [7]. The fruit is reported to have diuretic effect and is reported to be efficacious when used to treat renal stones and oedema [8]. The fruit peel is used to treat diabetes and alcoholic poisoning [9]. The root of the plant has purgative effect and when given in large doses, causes vomiting [10]. Its seeds are useful for their anthelminthic and antihypertensive effects. The seeds have fatty oil and extracts obtained using aqueous or solvents are reported to cause paralysis in tapeworms and roundworms. The plant is also used in Northern Sudan to treat burns, rheumatism, inflammation, gout and also used for its laxative effect [11]. *Citrullus lanatus* (Thunb.) is a medicinal plant that has been used traditionally to treat malaria Southern Nigeria, especially among the Ibibios but there is poverty of scientific literature to ascertain this claim. The aim of this study was to evaluate the antiplasmodial activity of *Citrullus lanatus* mesocarp extract in order to provide necessary scientific information that will confirm and rationalize its medicinal uses in the treatment of malaria.

MATERIALS AND METHODS

Collection and Identification of Plants

The plant material was collected from Uyo in Akwa Ibom State, Nigeria, identified and authenticated by a Taxonomist (Prof. Mrs. Margaret Bassey) in the Department of Botany and Ecological Studies, University of Uyo. A voucher specimen is in the Herbarium at the Faculty of Pharmacy, University of Uyo, Nigeria.

Preparation of Extract

The fruit was washed, drained and cut and separated into its different parts. The mesocarp was chopped into small cubic sizes but not ground because of its texture and then macerated in 99.5% methanol (Sigma, USA) for 72 h and filtered using Whatman filtered paper No. 7. The filtrate was evaporated to dryness using water bath regulated at 45 ºC to get the dry extract.

Phytochemical Screening of the extract

Phytochemical screening of the crude extract of *C. lanatus* mesocarp was carried out using the standard procedures and tests [12].

Experimental Animals

Swiss albino mice (20 – 26 g) selected from both female and male sexes were used for the experiments. These animals were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Uyo, Nigeria. They were housed in cross-ventilated wooden cages with wire netting with wood shaving as their beddings. The animals were allowed to acclimatize to the environment for 7 – 14 days and fed with pelleted feed obtained from Livestock Feed Nigeria Ltd and water given *ad libitum*.

Malaria Parasite

Plasmodium berghei berghei (chloroquine-sensitive) was obtained from the Department of Microbiology and Parasitology, National Institute of Medical Research (NIMER), Lagos, Nigeria and kept in mice using sub-passage.

Parasite Inoculation

Blood from mice with high parasitaemia was obtained after anaesthesia with chloroform by cardiac puncture and put in sterile heparinized bottle. Parasitaemia (percentage) was thereafter calculated through counting the number of red blood cells (RBCs) which are parasitized against the total number of RBCs. The required volume of blood collected from the parasitized mouse was then accordingly diluted using normal saline such that the final inoculum of 0.2 ml for each mouse would contain the expected number of parasitized RBCs (1.0×10^7), which is the standard inoculum for the infection of a single mouse [13].

Administration of drug and extract

The standard drugs chloroquine and pyrimethamine and the mesocarp extract of *C. lanatus* were administered to the experimental animals via oral route.

Determination of acute toxicity

The median lethal dose (LD_{50}) of *C. lanatus* mesocarp extract was determined through administering different doses of the extracts (10 – 5000 mg/kg) to groups of 3 mice per group at different phases (phase 1 and phase II). In phase I different doses of extract 10, 100 and 1000 mg/kg were administered while in phase II, the doses of extract doses administered were1600, 2900 and also 5000 mg/kg body weight. The animals were observed for cardinal signs of toxicity (writhing, grooming, sedation, paw licking, hyperactivity, drowsiness etc.) and mortality within 24 h. They were further observed for 13 days for any delayed manifestation of toxicity and death [14].

The LD_{50} was determined as a geometrical mean of maximum dose that recorded 0 % mortality (a) and minimum dose that recorded 100 % mortality (b).

$$LD_{50} = \sqrt{ab}$$

Determiniation of Antiplasmodial Activities

Evaluation of Suppressive Activity of the Extract

This screening evaluated the schizontocidal effects of the methanol extract of *C. lanatus* and chloroquine against *Plasmodium berghei berghei* infected mice. Twenty five mice were divided randomly into 5 groups of five mice per group. On the first day (D0), the mice were inoculated with 0.2 mL (i.p) of infected blood and thereafter divided into their different groups. After 10 min, Group 1 mice received 10 mL/kg of distilled water orally, which constituted the negative control group. Group II mice were administered with 125 mg/kg of the extract, Group III got 250 mg/kg of the extract; Group IV, 500 mg/kg and Group V, 5 mg/kg/day of chloroquine.

Administration of the extract and drug alongside the distilled water was thereafter given every day for 4 days (D0-D3) in the morning between 8 am and 9 am. Thin blood films were collected from the tail of each mouse on the fifth day (D4) and thereafter stained with Leishman's stain to reveal parasitized erythrocytes. Parasitaemia (%) was determined by counting the number of RBCs parasitized out of 500 RBCs from random fields under the microscope.

$$\%\ Parasitaemia = \frac{\text{No. of RBCs parasitized}}{\text{Total RBCs counted}}\ X\ 100$$

The mean percentag suppression of parasitaemia was determined in comparison with the controls:

$$\%\ \text{Suppression of Parasitaemia} = \left(\frac{A-B}{A}\right) X\ 100$$

A is the mean percentage parasitaemia in negative control group. B is the mean percentage parasitaemia in the test group [15, 16].

Evaluation of prophylactic activity of extract

The prophylactic activity of the extract and pyrimethamine as standard drug was also assessed. The method described by Ryley and Peters with slight modification [15, 16] was adopted. The animals were divided randomly into five groups of five per group. Group I animals were administered with 10ml/kg of distilled water and served as negative control while Groups 2, 3 and 4 were administered with 125, 250 and 500 mg/kg/day of the extract respectively. Group 5 mice were administered with the standard drug of pyrimethamine (2 mg/kg/day)

orally and served as positive control. The extract, drug and distilled water were thereafter administered daily for three days (D0-D2). These mice were inoculated on the fourth day (Day 3) with 0.2 mL of blood infected with *Plasmodium berghei berghei* as earlier described. The level of parasitaemia was assessed by blood smear from the tail after 72 h. Percentage parasitaemia and the mean chemosuppression were thereafter determined.

Evaluation of Curative activity of extract

In order to screen the curative effect of the extract, thirty mice were inoculated with 0.2 mL (i.p) of infected blood as described earlier on the first day (DO). Seventy two hours later, these mice were thereafter divided into 5 groups of 5 animals each. Group 1 animals were administered with distilled water (10 ml/kg). Group 2 – 4 animals were administered orally with extract 125 – 500 mg/kg/day while Group 5 animals were administered with chloroquine (5 mg/kg/day). All the drugs and extract were repeated daily for next four days and thereafter, tail blood samples from each mouse were collected daily for four days. Thin films were then prepared and stained with Leishman's stain. The percentage parasitaemia and the average chemosuppression were calculated. Subsequently, the Mean Survival Time (MST) of each group of mice was calculated over a thirty-day period (D0 – D29) [17].

$$MST = \frac{\text{No. of days mice survived}}{\text{Total No. of days (30)}} X\ 100$$

Statistical Analysis of Data

Results obtained were expressed as multiple comparison of Mean ± SEM. The level of significance was assessed using ANOVA (One-way Analysis of Variance) and followed by Turkey-Kramer multiple comparisons post-test and a probability level of $p < 0.05$ was considered significant.

RESULTS

Phytochemical Screening

Phytochemcial screening of the extract revealed that the extract tested positive to the following phytochemical constituents: saponin, tannin, flavonoids, alkaloids, cardiac glycosides and anthraquinones.

Acute toxicity studies

No deaths were recorded in all the animals in 24 hours following administration of the extract. There were no visible signs of toxicity such as excitation, paw-licking, depressed motor activity, increased respiration,gasping, coma or death for up to thirteen days following extract administration. The LD_{50} of methanol mesocarp extract of *C. lanatus* was > 5000 mg/kg

Antiplasmodial Activities

Suppressive Activity

There was a dose-dependent increase in the percentage chemosuppression relative to negative control. This increase was significant ($p < 0.001$). The level of percentage chemosuppression was less than that of standard drug chloroquine for the low and middle doses of the extract. However, the highest dose of the extract (500 mg/kg) attained a percentage chemosuppresion (70.15 %) comparable to the standard drug chloroquine (70.66%) as shown in Table 1.

Table 1: The suppressive test of methanol mesocarp extract of *Citrillus lanatus*

Treatment (Drug/Extract)	Dose (mg/kg)	Average Parasitaemia	Percentage Chemosuppression
Distilled water	10ml	7.84 ± 0.46	
Extract	125	3.40 ± 0.85*	56.63*
	250	3.32 ± 0.89*	57.65*
	500	2.34 ± 0.10*	70.15*
Chloroquine	5.0	2.30 ± 0.10*	70.66*

Data expressed as mean ± SEM; *p < 0.001 relative to control, n=5

Prophylactic test

In prophylactic test, there was a dose-dependent increase in the percentage chemosuppression relative to the negative control and this increase was significant ($p < 0.001$). However, the level of percentage chemosuppression (62.84 %) was less than that of standard drug Pyrimethamine (67.69 %) as shown in Table 2.

Table 2: Repository effect of methanol mesocarp extract of *Citrillus. lanatus*

Treatment (Drug/Extract)	Dose (mg/kg)	Parasitaemia	Percentage Chemosuppression
Distilled water	10ml	12.38 ± 0.40	
Extract	125	5.64 ± 0.75*	54.44*
	250	4.96 ± 0.72*	59.94*
	500	4.60 ± 0.52*	62.84*
Pyrimethamine	1.2	4.00 ± 0.11*	67.69*

Data are expressed as mean ± SEM; *p < 0.001 relative to control, n=5

Curative test

The extract demonstrated schizonticidal activity which was dose-dependent and significant ($p < 0.001$) relative to the negative control. But when compared with the standard drug Chloroquine, its effect was much less (Table 3). As well, the average survival time of animals administered with the extract was longer than that of the negative control but lesser than that of chloroquine as shown in Table 4.

Table 3: Curative effect of methanol mesocarp extract of *Citrillus. lanatus*

Drug/Extract	Dose (mg/kg)	% Parasitaemia				
		3rd Day	4th Day	5th Day	6th Day	7th Day
Distilled water	10ml	16.16 ± 0.28	27.50 ± 0.80	28.82 ± 0.78	27.20 ± 0.23	26.14 ± 0.66
Extract	125	10.56 ± 0.51*	18.14 ± 0.05*	18.82 ± 0.50*	15.27 ± 0.40*	14.76 ± 1.70*
	250	8.58 ± 0.40c	15.14 ± 0.60*	15.08 ± 0.90*	10.74 ± 0.54*	5..58 ± 1.32*
	500	8.10 ± 0.70*	12.42 ± 0.98*	12.22 ± 0.42*	5.50 ± 0.37*	3.08 ± 0.59*
Chloroquine	5.0	8.02 ± 0.96*	11.52 ± 0.65*	6.84 ± 0.13*	5.04 ± 0.69*	0.80 ± 0.33*

Data are expressed as mean ± SEM; *p < 0.001 relative to control, n=5

Table 4: Mean survival time (MST) of mice treated with the extract of *Citrillus lanatus*

Treatment (Drug/Extract)	Dose (mg/kg)	MST (days)
Distilled water	10ml	11.60 ± 0.55
Extract	125	16.20 ± 0.86*
	250	18.20 ± 0.49**
\	500	18.20 ± 0.85**
Chloroquine	5	20.40 ± 0.57**

Data are expressed as mean ± SEM; *p < 0.05; **p < 0.001 relative to control, n=5

DISCUSSION

From this evaluation of the antiplasmodial activities of ***Citrillus. lanatus***, the results indicate that the mesocarp extract possesses antiplasmodial potentials in mice inoculated and infected with *Plasmodium berghei berghei*. When screening for antimalrial activity of plants *in vivo*, rodent malaria parasites are usually used especially for antimalarial activity is usually done with rodent malaria parasites, especially *Plasmodium berghei berghei* which has been extensively used in antimalarial drug discovery and development. The suppressive, prophylactic (repository) and curative tests have been used in predicting treatment outcome of infection in humans [18]. *C. lanatus* extract dose-dependently and significantly suppressed parasitaemia in both the 4-day and prophylactic tests which suggests that this natural plant product pcould be useful as a potent phylactic antimalarial drug.Similarly, the extract also demonstrated a dose-depent and significant schizonticidal activity in established infection, which could make it useful in the treatment of malariain the tropics and especially developing countries where the malaria burden is great. Furthermore,the extract reduced parasitaemia and increased mean survival times in mice which depicts its curative capacity. The phytochemical screening showed that the methanol mesocarp extract of *C. lanatus* contains saponin, tannin, flavonoids, alkaloids, cardiac glycosides and anthraquinones. Alkaloids, tannins, flavonoids and triterpenoids have been reported to possess antiplasmodial activity [19, 20, 21] and being present in this extract might have conferred, at least in part, on the plant the observed antiplasmodial activities. The acute toxicity results of LD50 > 5000 mg/kg indicates that this extract is practically nontoxic and therefore safe [22].

CONCLUSION

These results demonstrate that the methanol mesocarp extract of *Citrillus lanatus* has antiplasmodial properties as can be seen from its significant, dose-dependent suppressive and curative activities and prolongation mean survival times in mice. The observed antiplasmodial properties may be attributed to the presence of some secondary metabolites in the extract. Taken together, this study justifies the ethnomedicinal use of this plant in the treatment of malaria by the Ibibios of South-South Nigeria.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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