



## **Antimalarial Activity of Ethanolic Extract of Root Bark of *Salacia nitida* L. Benth in Mice Infected with *Plasmodium berghei***

**Barine I. Nwiloh<sup>1\*</sup>, Joyce O. Akaninwor<sup>1</sup> and Augustine A. Uwakwe<sup>1</sup>**

<sup>1</sup>Department of Biochemistry, Faculty of Science, University of Port Harcourt, P.M.B. 5323 Choba,  
Port Harcourt, Rivers State, Nigeria.

### **Authors' contributions**

All the authors collaborated to carry out this scientific work. Author AAU designed the work and some literature searches. Author BIN managed the laboratory animals, performed the laboratory investigation, managed the analyses of the study and wrote the manuscript. Author JOA performed the statistical analyses and some literature searches. All authors read and approved the final version of the manuscript.

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### **ABSTRACT**

**Aim:** This study was designed to evaluate the *in vivo* antimalarial activity of ethanolic extract of root bark of *Salacia nitida* in mice infected with *Plasmodium berghei*.

**Methods:** Prophylactic and curative antimalarial activity of ethanolic extract of root bark of *S. nitida* was studied using sixty-six healthy mice infected with chloroquine sensitive *Plasmodium berghei*. 430 mg/kg body weight/day of the extract was given orally infected mice in the prophylactic test for 11 days, while 280 – 580 mg/kg body weight/day of extract was orally administered to the malaria infected mice in the curative test for 5 days, against 4 mg/kg body weight/day of artesunate drug. The antimalarial effect of the ethanolic extract of root bark of *S. nitida* on the parasites was determined.

**Results:** The ethanolic extract of root bark of *S. nitida* at a dose of 430 mg/kg body weight/day caused 89.77% inhibition in the levels of parasitaemia in the prophylactic test and 33.78% - 88.45% inhibitions of parasitaemia levels in the curative tests.

\*Corresponding author: E-mail: bin4him@yahoo.com;

**Conclusion:** The results showed that the ethanolic extract of root bark of *S. nitida* possessed significant ( $p < 0.05$ ) antimalarial activity, thus supporting its traditional use in the treatment of malaria.

**Keywords:** Antimalarial activity; mice; parasitaemia levels; *Plasmodium berghei*; prophylactic test; *Salacia nitida*.

## 1. INTRODUCTION

Every year about 212 million cases of malaria are reported, with about 429,000 deaths, majority of who are African children [1,2]. Malaria which is caused by *Plasmodium* species, that is transmitted by mosquitoes, is a life threatening disease ranked third among the major infectious diseases causing deaths in the world [3]. It poses a very important public health concern in the world, and will continue to remain an epidemic of great concern in the sub-Sahara Africa, causing death every year. In Africa, this disease has posed difficult health problems as a result of resistance to most synthetic antimalaria drugs, high cost of effective drugs and their non-availability, especially in rural areas [4]. This has made the use and dependence on traditional medicines, particularly the plant based antimalaria products an important source for treatment [5]. The use of indigenous plants in Africa, especially among the Ogonis, a tribe in Rivers state, in the Niger delta region of Nigeria, still play an important role in the treatment of malaria and could possibly be interesting sources for the development of antimalaria agents in the near future.

The plant *Salacia nitida* L. Benth (“Akorkorn” in Ogoni) belonged to the family Celastraceae. It is use for the treatment of malaria and typhoid fever by the tribal people of Ogoni. The alcoholic decoction of the root bark, root, leaves or whole plant of *Salacia nitida* is taken orally.



**Fig. 1. *Salacia nitida***

The phytochemical constituents of the root bark of *S. nitida* have been reported [6]. Therefore, this study is designed to “evaluate the antimalaria activity of ethanolic extract of root bark of *Salacia nitida* in mice infected with *Plasmodium berghei* so as to ascertain the acclaimed therapeutic values of the plant part in the treatment of malaria”.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Preparation of Plant Materials

Collection of *Salacia nitida* (“Akorkon”) plant was done in the month of April, 2016, from Wiyor farm land in Nyogor-Beerri, Khana Local Government Area of Rivers state, Nigeria. It was identified and authenticated by Dr. N. L. Edwin-Wosu of the department of plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria, with herbarium voucher number UPHV-1033, and plant sample deposited at the University herbarium. Fresh plant roots were uprooted with a spade properly washed in clean water to remove soil particles and air dried. The barks were removed from the roots by hitting the root with a hammer and the root bark peeled with hand onto a clean leather material. Root barks were then cut into smaller pieces with a knife and air dried under shade for one week.

### 2.2 Preparation of Ethanolic Extract of Root Bark

The dried root barks were ground with grinding machine (corona-16D) and further pulverized into powder form using wooden mortar and pestle. Extraction was done with soxhlet extractor, using 350 g of the powdered material of the root bark and 170 ml of ethanol. The set-up was heated at a temperature of 80°C with a water bath for about 20 hours and then concentrated to dryness for two weeks in a water bath at same temperature. The dried ethanolic extract of the root bark was stored in a refrigerator at 4°C in an air-tight container until required.

### 2.3 Acute Oral Toxicity (AOT)

The acute oral toxicity of ethanolic extract of root bark of *S. nitida* was determined according to the OECD TG 423 method [7], using a total of twelve mice of mixed sexes and LD<sub>50</sub> determined by taking the cube root of the product of all the doses that did not caused dead of the mice and the up – and – down procedure used for the selection of doses used for treatment [8-10].

### 2.4 Experimental Animals

Healthy Swiss albino mice of mixed sex, 6-8 weeks old, weighing between 19 g and 35 g, procured from the Department of Pharmacology, Faculty of Medical Sciences, College of Medicine, University of Port Harcourt, and acclimatized for a period of two weeks, were used for this study. They were maintained in plastic cages at ordinary room temperature, and under standard conditions of humidity and moisture, with 12 hours light and 12 hours darkness cycle. The animals were given free access to food (grower's marsh) and clean water. The research was carried out according to the United States National Institute of Health "Principle of Laboratory animals care" [11] and that of the University of Port Harcourt ethic committee guidelines on use of laboratory animals.

### 2.5 Inoculation of Mice with the Parasites

*Plasmodium berghei* (NK-65 strain) parasites obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria were used. They were contained in five (5) *P. berghei* inoculated mice, which were conveyed to the World Bank-Assisted Malaria and Phytomedicine Research Laboratory, University of Port Harcourt, where they were maintained by passage into ten (10) other healthy albino mice after one week to protect against any lost of the parasites. The parasitized mice carrying *P. berghei* at varying parasitaemia levels were used as the donor mice. Before inoculation, percentage levels of parasitaemia in the donor mice were determined. Blood from all the donor mice were collected by cardiac puncture using sterile disposable syringe and needle into one small beaker. Then 1 ml of the parasitized blood from the donor mice was added to 9ml of normal saline solution. Each healthy mouse was administered 0.2 ml of the diluted parasitized blood containing  $1 \times 10^7$  *P. berghei* infected red blood cells intraperitoneally.

### 2.6 Administration of Extract and Standard Drug

Ethanolic extract of root bark of *S. nitida* and the standard drug (artesunate) were administered to the mice by oral route, using stomach canula. The dosage volumes of the drug and the extract were calculated from their stocks according to the method reported by Erhirhie et al. [12].

### 2.7 Evaluation of Prophylactic Potential

In this study thirty (30) Swiss albino mice were used. The mice were divided into five groups (1 – 5) consisting of six (6) mice each, and the study was conducted for eleven days (D<sub>0</sub> – D<sub>11</sub>). The mice were inoculated with 0.2 ml of infected blood containing for  $1 \times 10^7$  *P. berghei* parasites as described above, on day eight (D<sub>8</sub>), and treated as follows:

- Group 1** - were given only physiological saline (4 ml/kg body weight/day) for 11 days infected on day eight (negative control group – NC).
- Group 2** - were orally administered 430 mg/kg body weight/day of ethanolic extract of root bark of *S. nitida* for 7 days, inoculated on day eight.
- Group 3** - were administered 430 mg/kg body weight/day of ethanolic extract of root bark of *S. nitida* for 11 days and inoculated on day eight.
- Group 4** - were administered 4 mg/kg body weight/day of artesunate (ART) for 7 days and inoculated on day eight.
- Group 5** - were administered 4 mg/kg body weight/day of artesunate (ART) for 11 days and inoculated on day eight.

All the experimental animals were orally administered equal volume of 0.3 ml of the extract, drug and physiological saline once daily by about 8.00 am for the period of the study (D<sub>0</sub> - D<sub>11</sub>), using stomach canula. The animals were also given free access to food (grower's marsh) and clean drinking water. At the end of the study period, that is, on day 12 (D<sub>12</sub>), thin blood films were prepared by cutting the tip of the tails of the mice with a pair of sterile scissors and the blood smeared on microscopic slides. The films were fixed in 98% methanol and air-dried for about 2-5 minutes. Giemsa stain was poured carefully on the slides using a dropping pipette and allowed to stay for about ten (10) minutes before flushing it with tap water to removed excess stain. The back of the slide was wiped with clean tissue

paper and placed in a draining rack to dry the films. The prepared blood films were examined under a light microscope using the special 7x eye piece and 100x oil immersion objective, with the condenser sufficiently adjusted to give a good contrast [13]. Parasites were searched for on the thin blood film without moving the slide and the number of infected red blood cells counted against the total number of red blood cells in that field. The number of parasites was determined by counting the total number of parasitized red blood cells in 200 RBCs counted, and the percentage (%) parasitaemia was calculated for each dose level using the formula:

$$\% \text{ parasitaemia} = \frac{\text{No of parasitize d Rbc}}{\text{Total no. of Rbc}} \times \frac{100}{1}$$

Chemosuppression (% inhibition) was determined using the formula:

$$\% \text{ chemosuppression} = \frac{X - Y}{X} \times \frac{100}{1}$$

Where; X = Average % parasitaemia in negative control group (NC), and Y = Average % parasitaemia in treated group.

## 2.8 Evaluation of Curative Activity of *S. nitida*

A modified method of Ryley and Peters [14] was used to evaluate the curative potential of the extract in mice. In this study thirty six (36) swiss albino mice were used. The mice were inoculated with 0.2 ml of infected blood containing  $1 \times 10^7$  *P. berghei* parasites as described above; on day zero ( $D_0$ ). Seventy two (72) hours later the inoculated mice, were randomly divided into six (6) groups labeled A - F; consisting of six (6) mice per group which were treated as follows:

- Group A:** Were given only saline solution (4 ml/kg body weight/day) and which served as the negative control group (NC).
- Group B:** Were given 280 mg/kg body weight/day of ethanolic extract of root bark of *S. nitida*.
- Group C:** Were given 430 mg/kg body weight /day of ethanolic extract of root bark of *S. nitida*.
- Group D:** Were given 580 mg/kg body weight/day of ethanolic extract of root bark of *S. nitida*.

**Group E:** Were given 4 mg/kg body weight/day of artesunate (ART) (positive control, PC).

**Group F:** Were not infected but given only saline solution (4 ml/kg body weight/day) (reference control, RC).

All the experimental animals were orally administered equal volume of 0.3 ml of the extract, drug and physiological saline once daily by 8.00 am for five consecutive days post inoculation using stomach canula. The animals were also given free access to food (grower's marsh) and clean drinking water. Bleeding of the mice were done on day 4 ( $D_4$ ), 6 ( $D_6$ ) and 9 ( $D_9$ ), for assessment of parasitaemia levels, by cutting the tip of the tail with a pair of sterile scissors. Blood were collected onto microscopic slides and thin blood films prepared as described above. The % parasitaemia and % inhibition were also determined for each dose level using the formulae stated above.

## 2.9 Statistical Analyses of Data

Data were analyzed using the SPSS version 22. The one way ANOVA was used to compare results and multiple comparisons were done using the Schiffe's post hoc test to compare results at 95% confidence level ( $P = 0.05$ ). The results were expressed as mean values  $\pm$  SEM. Bar graph were also used to illustrate results.

## 3. RESULTS

All the mice tested did not show any signs of toxicity after the 72 hours of the oral toxicity test with the extract. The  $LD_{50}$  was determined to be 865mg, which was three times greater than the minimum effective dose (MED) of 280 mg.

The result of this study is shown in Fig. 2. It showed that the levels of parasitaemia in the negative control (group 1) ( $31.00 \pm 2.51\%$ ) was significantly ( $p = 0.05$ ) higher than those of infected treated mice with  $9.33 \pm 3.22$  for group 2,  $7.83 \pm 1.09$  for group 3,  $3.17 \pm 0.11$  for group 4, and  $1.33 \pm 0.90\%$  for group 5 mice. This showed that parasitaemia levels in the extract and artesunate pretreated mice (groups 2 to 5) were significantly ( $p = 0.05$ ) decreased compared to the infected untreated mice of group 1.

The results of the effect of the extract on the levels of parasitaemia in the established infection are represented in Fig. 3. From the figure it was

seen that there was a significant ( $p = 0.05$ ) increase in the mean parasitaemia levels of infected untreated mice in the negative control (group A) which had  $12.58 \pm 3.21\%$  compared to the normal mice in the reference control (group F) with  $0.00\%$  and significant ( $p = 0.05$ ) decrease in mean parasitaemia (%) levels of mice in the positive control (group E) which had  $6.83 \pm 0.11\%$  on day 4 ( $D_4$ ). Also, reductions in mean percentage parasitaemia levels of infected treated mice in groups B through D compared to the group A mice were observed. On day 6 ( $D_6$ ), the mean percentage parasitaemia levels, in the treated groups B ( $10.57 \pm 1.57\%$ ), C ( $9.50 \pm 0.48\%$ ), D ( $7.58 \pm 3.33\%$ ) and E ( $4.50 \pm 0.98\%$ ), treated with 280, 430, 580 mg/kg body weight/day of extract, and 4 mg/kg body weight/day of artesunate were significantly ( $p = 0.05$ ) reduced compared to their group A counterparts with  $31.17 \pm 0.74\%$ . Also, there was no significant ( $p > 0.05$ ) difference between group D (580 mg/kg of extract) and group E (4 mg/kg of artesunate drug) mice. On day 8 ( $D_8$ ), a significant ( $p = 0.05$ ) decrease of  $2.58 \pm 3.09\%$  parasitaemia level was observed in the positive control mice (group E) treated with 4 mg/kg body weight/day, compared to the negative control (group A) mice that were infected and not treated, with  $40.42 \pm 2.40\%$  parasitaemia level, and  $0.00\%$  inhibition. There were also significant ( $p = 0.05$ ) decreases in mean percentage parasitaemia of mice in groups B ( $8.75 \pm 0.15\%$ ), C ( $7.67 \pm 0.99\%$ ), D ( $4.67 \pm 3.01\%$ ) that were treated with 280, 430, 580 mg/kg body weight/day of the extract compared to the negative control (group A) mice. It was seen that the reduction in mean percentage parasitaemia was dose-dependent.

#### 4. DISCUSSION

The  $LD_{50}$  which was three times greater than the minimum effective dose (MED) is an indication that the extract is good and non-toxic [15].

The slight difference between the infected treated mice of groups D and the positive control (group E) observed in this study, showed that the extract of the root bark of *S. nitida* possessed very strong antimalarial potential against *P. berghei* when compared to the standard drug (artesunate), showing that both the drug and the extract (at high doses) exhibited comparable antimalarial strength. This was seen in both the prophylactic and curative studies with the extract on *P. berghei* malaria infections (Figs. 2 and 3).

It was shown from this study that the extract of the plant part under investigation has proven promising anti-malarial activity that can be exploited in malaria therapy. This is because the extract has shown more than 30% inhibitory effect in the reduction of percentage parasitaemia, which agreed with the findings of Carvalho and colleagues, that a compound is active in the reduction of parasitaemia if its inhibitory effect is  $\geq 30\%$  [16]. In this study the percentage of inhibitions of parasitaemia exhibited by the extract is within 78.35% to 88.45%, which is greater than 30%; thus supporting the findings in this work that the extract contained antimalarial principles. The findings in this work are in line with previous reports on *Croton zambesicus* with 80.70%, *Anona senegalensis* with 76.30%, *Stachytarpheta cayennensis* with 78.20% and *Pteridium aquilinum* with 79.45% inhibitions [17–20].

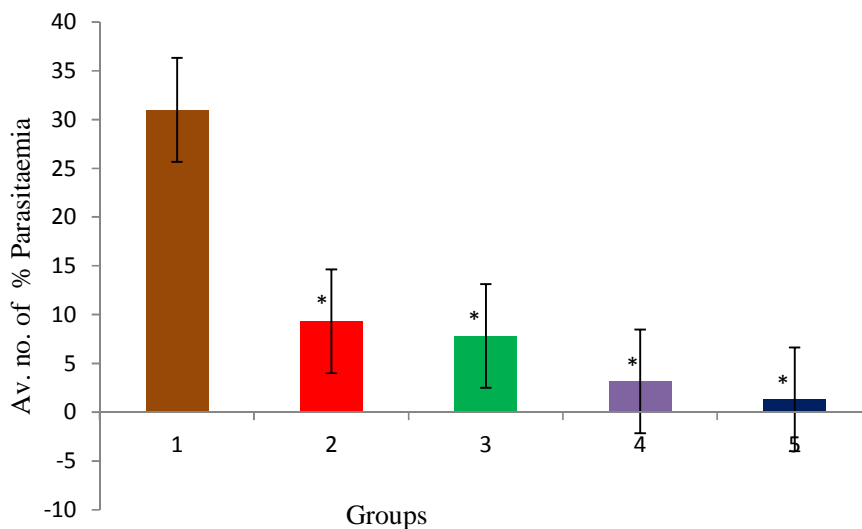
The antimalarial activity of the ethanolic extract of root bark of *S. nitida* might be attributed to the presence of pharmacologically active phytoconstituents with diverse biological activities. The phytochemical constituents of root bark of *S. nitida* have been reported to contain spartein, epicatechin, phytate, anthocyanin, tannin, phenol, lunamarine, sapogenin, ribalinidine, catechin, rutin, and Kaempferol [6].

The antimalarial mechanism of actions of the extract of *S. nitida* has not been proved, but it could be said to be related to the mechanism of actions of the phytochemical compounds present in the extract. The extract contained the quinoline alkaloids such as linamarine, ribalinidine and spartein. Compounds containing quinoline nucleus are known for their high antimalarial potency in inhibiting the formation of hemozoin [21–31], which might cause the death of plasmodium parasites [32–34]. The extract is also rich in flavonoids, which have been severely implicated in antimalarial activities [35,36]. Flavonoids such as rutin which is present in the extract has been reported to inhibit the synthesis of nucleic acid and DNA gyrase by formation of hydrogen bonding with nucleic acid bases via hydroxylation of the B-ring of the flavonoids [37–40]. Catechin, another phytoconstituent present in this extract is known to cause alteration of cell membrane fluidity [41].

The use of extract in therapy might produce more beneficial, efficient and effective pharmacological and therapeutic actions than the isolated

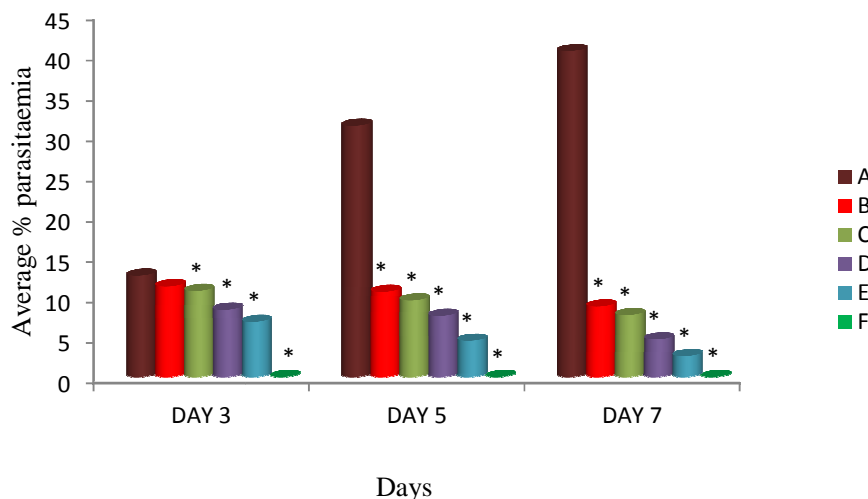
compounds. This might be due to the synergistic effects of bioactive phytochemicals in the plant extract [42–44]. So, the combined effects

different bioactive compounds present in the extract of *S. nitida*, might be responsible for its antimalarial activity.



■ = 4ml/kg saline for 11 days; ■ = 430mg/kg of Extract for 7 days; ■ = 430 mg/kg of extract for 11 days; ■ = 4 mg/kg of ART for 7 days; ■ = 4mg/kg of ART for 11 days.

**Fig. 2. Prophylactic activity of ethanolic extract of root bark of *S. nitida* on parasitaemia levels (%) in mice infected with *Plasmodium berghei***  
*n* =6; \* = values are significant from the negative control (*p* = 0.05)



A = 4 ml/kg (NC); B = 280 mg/kg of extract; C = 430 mg/kg of extract; D = 580 mg/kg of extract; E = 4 mg/kg of ART (PC); F = RC.

**Fig. 3. Effect of treatments with ethanolic extract of root bark of *S. nitida* on levels of parasitaemia (%) in established infection in mice infected with *P. berghei***  
*n* =6, \* = values are significant from the negative control (*p*=0.05)

## 5. CONCLUSION

The results of this study showed that the ethanolic extract of root bark of *S. nitida* is effective against the treatment and prevention of malaria, as seen in its ability to inhibit *P. berghei* infection in mice. Also, this study scientifically supported the acclaimed traditional use of the plant part for the treatment of malaria. Notwithstanding, the bioactive compounds that confer on the plant part being investigated, the antimalarial effect, need to be elucidated.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the ethics committee of the University of Port Harcourt, Rivers state, Nigeria.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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