Antiplasmodial activity of methanol leaf extract of *Salacia senegalensis* Lam (Dc) in Albino Mice infected with chloroquine-sensitive *Plasmodium berghei berghei* (NK65)

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The antiplasmodial effect of methanol leaf extract of *Salacia senegalensis* were evaluated in albino mice infected with chloroquine-sensitive *Plasmodium berghei berghei* (NK65) in order to justify or otherwise its use as antimalarial remedy in Nigeria folk medicine. Activities investigated were suppressive effect against early infection, curative effect against established infection and prophylactic effect against residual infection. Results showed a dose dependent blood schizontocidal activity at all the phases of malarial infection studied. The *in vivo* antiplasmodial effect of the extract (1000, 1200 and 1400 mg/kg body weight) against *P. berghei* showed significant (p < 0.05) dose-dependent activity for suppressive, curative and prophylactic test. When the extract dose increased from 1000 to 1400 mg/kg/day, chemosuppressive activity of the extract increased from 66.47 % to 80.33 %. There was also an increase from 66.57 % to 75.41 % and from 64.90 % to 82.72 % for the repository and curative activities respectively. The schizontocidal activities were comparable to that of chloroquine -which had percentage suppression of parasitaemia as 87.03 %, 85.12 %, and 91.68 % for suppressive, prophylactic and curative activities respectively. It was thus concluded that the herbal extract possesses significant antimalarial potency which could be exploited in the formulation of antimalarial drugs.

**Keywords:** Antiplasmodial, *Salacia senegalensis*, *Plasmodium berghei*, chemosuppression, schizontocidal.

**INTRODUCTION**

Malaria is a disease caused by the parasite called *Plasmodium* characterized by fever, headache, and anaemia and in severe cases causes coma or death. The World Health Organization estimated the mortality rate of malaria in Africa at 781,000 people per year (WHO, 2010). Despite various declarations by African governments and complementary efforts promised in the main content of the Roll back Malaria declaration, malaria remains a major health challenge in Africa and Nigeria. The vulnerable groups are mostly pregnant women and children under 5 years.
The causative parasites, *Plasmodium* species have acquired resistance to most common drugs (Boland, 2001). New drugs thus have to be sourced to replace the ones already compromised by this phenomenon. Therefore, there is need to evaluate scientifically important medicinal plant species commonly used in the herbal treatment of the disease (Okunji et al., 2000). Medicinal properties of plant lie in some bioactive substances that produce a definite physiological action on the human or animal body (Edeoga et al., 2005). Decoctions of some plants are of great value in treatment of malaria, typhoid fever, diarrhea or gastrointestinal disorder, urinary tract infection and abscesses (Meyer et al., 1996).

*Salacia senegalensis* Lam (DC) is a shrub erect or climbing with white or pale greenish cream petals and orange or yellow flowers. It is found in forest. It belongs to the family Celestraceae. Traditionally, the extract of its leaf is used in malaria treatment, lotion for sick children and in the treatment of skin problem like eczema by the people of South-East zone of Nigeria (NNMDA, 2011). A decoction or alcohol extract of *Salacia senegalensis* leaf is used by the Orij people of Owerri North Local Government Area (L.G.A), Imo State, South-East Nigeria as a remedy for malaria; although no scientific data on its antimalarial activity has been reported. Therefore, the study, the blood schizontocidal activity of the leaf extract of *Salacia senegalensis* against *Plasmodium berghei* infection in Swiss albino mice was evaluated as to support or discourage its use in the treatment of malaria in Nigeria folk medicine.

**MATERIALS AND METHODS**

**Plant Materials collection and authentication**

The plant *Salacia senegalensis* (figure 1) was obtained from the forest at Orji, Owerri North L.G.A, Imo State, Nigeria, identified and authenticated by taxonomists Prof. Okeke, SE and Dr. Mbagwu, FN of the Department of Plant Science and Biotechnology Imo State University, Owerri, Nigeria.

**Extraction procedures**

The fresh leaves of *Salacia senegalensis* were subsequently cleaned, cut into pieces and air dried at room temperature. Dry leaves were ground into a coarse powder using a mortar, and milled to fine powder using electric blender (Q-link). Five hundred grams (500 g) of the powder was macerated in 1600 ml of 95 % methanol for 72 hours. The methanol extract was concentrated using rotary evaporator at temperature of 45-50 °C.

**Mouse strain**

Healthy Swiss albino mice of both sexes weighing between 18-22 g were obtained from the Animal House of Department of Biochemistry University of Port Harcourt. The mice were appropriately grouped so that their cumulative weights and sex were taken care off. The mice were kept in plastic cages and allowed to acclimatize for a period of one week before the commencement of the study. They were allowed unrestricted access to standard feed (Vital feed growers) obtained from Brand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria and water ad libitum throughout the experimental period. The mice were handled in accordance with the guidelines for the care and use of laboratory animals (US- NRC, 2003).

**Malaria Parasite**

Mice already parasitized with chloroquine-sensitive *Plasmodium berghei berghei* (NK65) were obtained from National Institute for Medical Research (NIRM), Lagos, Nigeria and maintained in the laboratory by serial passage in mice.

**Inoculation of *P. berghei* Parasite**

Albino mice previously infected with *Plasmodium berghei* having different levels of parasitaemia were used as donors. The parasitaemia levels of donor mice were first determined by cutting the tails of the mice with a sterile pair of scissors, and the blood collected into 0.5 ml normal saline. A drop of the diluted blood was placed on a microscope slide and observed under the light microscope using 40 x magnifications. The stained blood showed the presence of the parasite. The donor mice were then sacrificed and blood collected by cardiac puncture. The parasitized blood was subsequently diluted with normal saline. 0.05 ml donor blood was added to 9.95 % normal physiological saline. Then, 0.2 ml of the diluted blood which contained about 1 x 10^7 *Plasmodium berghei* infected erythrocytes was administered intra-peritoneally to each mouse.
Safe dose and acute toxicity (LD_{50})

The modified method of Lorke (1983) was used to determine the LD_{50} of *Salacia senegalensis*. The concentrated extract was dissolved in dimethylsulphoxide (DMSO -Sigma chemicals, St. Louis, M.O. USA) and used to determine the LD_{50} which showed that dose of less than or equal to 5000 mg per kg body weight (b.w) is safe, i.e. no death was recorded at this maximum concentrations used, but extremely high doses may not be advisable (Adumanya et al., 2014). i.e. LD_{50} > 5000 mg/kg b.w.

\[
LD_{50} = \sqrt{xy} \quad \text{Where: } x = \text{least tolerable dose}, \quad y = \text{maximum tolerable dose (Lorke, 1983)}
\]

**In vivo test**

Both the extract and the drug chloroquine phosphate tablets (obtained from Dana Pharmaceuticals Nig. Plc.) used were administered orally using sterile orogastric tubes.

**Chemosuppressive Test:** Evaluation of schizontocidal activity on early infection

The chemosuppressive test was done using 4-day suppressive test against *P. berghei* infection in mice (Knight and Peters, 1980). About one hour after parasite inoculation, the animals were divided into five groups of five mice each and were administered 1000, 1200 and 1400 mg/kg/day doses of the plant extract, chloroquine phosphate 5 mg/kg/day and equivalent volume of normal saline (control group) for four consecutive days. On the fifth day, the mice were inoculated with the *P. berghei* parasite. Seventy-two (72) hours later, the levels of parasitaemia were assessed by thick blood smears as described in chemosuppressive test and average percentage suppression of parasitaemia calculated using formula (2).

**Evaluation of schizontocidal activity in Test group**

**Prophylactic (Repository) Activity:** Evaluation schizontocidal activity on residual infection

The repository activity was assessed using the method described by Peters (1967). The mice were divided into five groups of five mice each, and administered 1000, 1200 and 1400 mg/kg/day extract, 5 mg/kg/day of chloroquine phosphate and 5 ml/kg/day of normal saline (control group) for four consecutive days. On the fifth day, the mice were inoculated with the *P. berghei* parasite. Seventy-two (72) hours later, the levels of parasitaemia were assessed by thick blood smears as described in chemosuppressive test and average percentage suppression of parasitaemia calculated using formula (2).

**Curative Test:** Evaluation of schizontocidal activity in established infection

A modified method similar to that of Ryley and Peters (1970) was used. On the first day, standard inoculum of 1 x 10^7 infected red blood cells were injected in the mice, intra-peritoneally. Seventy-two (72) hours later, the mice were distributed into five groups of six mice each and the parasitaemia levels were determined. Subsequently different doses of the extract (1000, 1200 and 1400 mg/kg b.w. /day), chloroquine phosphate (5 mg/kg/day) and normal saline (5 ml/kg/day) were given to the respective groups. These treatments were administered once daily for five consecutive days. For each mouse, thick blood films were made with 10% Giemsa stain was prepared daily for five consecutive days to monitor the levels of parasitaemia. Average percentage suppression of parasitaemia calculated using formula (3).

\[
A = \left( \frac{D - E}{D} \right) \times 100 \quad \text{…….. “3”}
\]

Where, \( A = \text{average percentage suppression of parasitaemia} \), \( D = \text{average percentage parasitaemia before treatment} \) and \( E = \text{average percentage parasitaemia after treatment} \)

**Statistical Analysis**

The results of the study were presented as mean ± standard deviation. The statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) version 17.0. One way analysis of variance (ANOVA) was used to compare the means at 95 % level of confidence.

**RESULTS AND DISCUSSION**

The effects of the chemosuppressive, prophylactic and curative tests showed a dose-dependent manner as shown in Tables 1, 2 and 3 respectively. The in vivo...
Table 1. Chemosuppressive Effect of methanol extract of Salacia senegalensis leaves against P. berghei infection in albino mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chemosuppressive activity (% suppression of parasitaemia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline (5 ml/kg b.w)</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Extract (1000 mg/kg b.w)</td>
<td>66.47 ± 3.41</td>
</tr>
<tr>
<td>Extract (1200 mg/kg b.w)</td>
<td>72.74 ± 2.99</td>
</tr>
<tr>
<td>Extract (1400 mg/kg b.w)</td>
<td>80.33 ± 3.23</td>
</tr>
<tr>
<td>Chloroquine (5 mg/kg b.w)</td>
<td>87.03 ± 2.87</td>
</tr>
</tbody>
</table>

Means with different superscripts in the same column are significantly different from each other (P < 0.05). Values are means ± standard deviation of five (5) replicates.

Table 2. Repository Activity (prophylactic effect) of methanol extract of Salacia senegalensis leaves against P. berghei infection in albino mice

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Prophylactic (Repository) activity (% suppression of parasitaemia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline (5 ml/kg b.w)</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Extract (1000 mg/kg b.w)</td>
<td>66.57 ± 4.72</td>
</tr>
<tr>
<td>Extract (1200 mg/kg b.w)</td>
<td>71.17 ± 5.96</td>
</tr>
<tr>
<td>Extract (1400 mg/kg b.w)</td>
<td>75.41 ± 6.99</td>
</tr>
<tr>
<td>Chloroquine (5 mg/kg b.w)</td>
<td>85.12 ± 5.49</td>
</tr>
</tbody>
</table>

Means with different superscripts in the same column are significantly different from each other (P < 0.05). Values are means ± standard deviation of five (5) replicates.

Table 3. Curative Effect of methanol extract of Salacia senegalensis leaves against P. berghei infection in albino mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Curative activity (% suppression of parasitaemia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline (5 ml/kg b.w)</td>
<td>-</td>
</tr>
<tr>
<td>Extract (1000 mg/kg b.w)</td>
<td>64.90 ± 7.06</td>
</tr>
<tr>
<td>Extract (1200 mg/kg b.w)</td>
<td>78.01 ± 5.21</td>
</tr>
<tr>
<td>Extract (1400 mg/kg b.w)</td>
<td>82.72 ± 3.63</td>
</tr>
<tr>
<td>Chloroquine (5 mg/kg b.w)</td>
<td>91.68 ± 2.16</td>
</tr>
</tbody>
</table>

Means with different superscripts in the same column are significantly different from each other (P < 0.05). Values are means ± standard deviation of six (6) replicates.

The antiplasmodial effect of the extract (1000, 1200 and 1400 mg/kg body weight) against P. berghei showed significant (p < 0.05) dose-dependent activity for suppressive, curative and prophylactic test. At the extract doses of 1000, 1200 and 1400 mg/kg/day, chemosuppressive activity was 66.47 %, 72.74 % and 80.33 % respectively. There were 66.57 %, 71.17 % and 75.41 % and 64.90 %, 78.01 % and 82.72 % suppression for the repository and curative activities respectively.

The results obtained in the study showed that the methanol extract of Salacia senegalensis exhibited blood schizontocidal activity at all phases of malarial infection. The result of the chemosuppressive activity of the extract as shown in Table 1 showed a dose dependent manner.

The highest suppressive effect (87.03 %) was observed with the standard drug chloroquine. The value was however comparable to that observed when the extract dose was increased to 1400 mg/kg/day which gave 80.33 % suppression.

The repository effect of the extract also occurred in a dose dependent manner as shown in Table 2, for instance when the extract dose increased from 1000 mg/kg/day, to 1400 mg/kg/day (the highest dose used), repository activity increased from 66.57 % to 75.41 % which is also comparable to the value (85.12 %) obtained with the standard drug (chloroquine), although significantly different (p < 0.05).

The result of the curative effect of the plant extract equally showed a dose-dependent activity as shown in Table 3.
The highest curative effect was observed for the plant extract when the highest dose (1400 mg/kg/day) was administered. This gave 82.72 % curative effect compared with 91.68 % obtained for chloroquine phosphate.

The better performance observed for chloroquine compared with the extract in this study agreed with report by Kamei et al., (2000), that when a standard antimalarial drug is used in the management of *Plasmodium berghei* in mice, it suppressed parasitaemia. This highest percentage chemosuppression activity of chloroquine recorded in the study showed that it could still serve as antimalarial drug (Fidock et al., 2004). It also supports previous work on antimalarial effect of chloroquine by Oyewole et al., (2008) and Odeghe et al., (2012). The antiplasmoidal properties of this plant could be as result of its phytochemical composition. Study in our laboratory showed that the methanol extract of *Salacia senegalensis* leaf contains alkaloids, flavonoids, and saponins (unpublished yet). These constituents have been found in other natural products which exhibited antimalarial activity (Ayoola et al., 2008). Saganuwan et al., (2011) reported that plants which contain many phytochemicals with biological activities like alkaloids and flavonoids could serve as sources of antimalarial drugs. These phytochemicals are antioxidants. Phytochemicals have been implicated in creation of an intracellular environment that is unfavourable to *Plasmodia* growth (Kirby et al., 1989; Lavender and Ager, 1993; Alli et al., 2011). This suggests that the antiplasmoidal activity of *Salacia senegalensis* extract could be based on the antioxidant and antiparasitic effects of these phytochemicals (Ayoola et al., 2008). This observation is validated by Etkin (1997) who reported that artemisinin (a modern antimalarial drug of plant origin) depends on its oxidant action for its potency against *Plasmodium* species. This does not however rule out plants that lack oxidant property from having antiplasmoidal activity since they may be active through other biochemical mechanisms (Ali et al., 2011). Therefore, the antiplasmoidal activity of the *Salacia senegalensis* extract observed could be attributed to the presence of these phytochemicals. Flavonoids are known to elevate red blood cell oxidation and inhibit the parasites protein synthesis (Chandel and Bagai, 2010). Also antimalarial properties of flavonoids have been reported by Amponsah et al., (2012) and Addai (2010).

**CONCLUSION AND RECOMMENDATION**

From the results of the study, the methanol leaf extract of *Salacia senegalensis* has antiplasmoidal/antimalarial activity as it has been shown to suppress the development of *Plasmodium berghei* parasite infection in mice at the different phases of malaria infection. The results justify the use of the plant extract in the treatment of malaria in Nigerian folk medicine. Therefore, further studies on isolation, characterization and identification of active chemical component(s) present in methanol leaf extract of *Salacia senegalensis* are recommended for development of antimalarial drug.

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**Conflict of interest:** None

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