# Antimalarial Activity of Leaf Extract of *Solanum anomalum* (Solanaceae)

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## ABSTRACT

The ethanol leaf extract of *Solanum anomalum* was evaluated for antiplasmodial activity in rodents to ascertain the folkloric claim of its antimalarial activity. The crude leaf extract (70 – 210 mg/kg) of *Solanum anomalum* was investigated for antiplasmodial activity against chloroquine sensitive *Plasmodium berghei* infections in mice. Antimalarial activities during early and established infections as well as prophylactic potentials were investigated. Artesunate 5 mg/kg and pyrimethamine 1.2mg/kg were used as positive controls. The extract dose-dependently reduced parasitaemia induced by chloroquine sensitive *Plasmodium berghei* infection in prophylactic, suppressive and curative models in mice. These reductions were statistically significant ($p<0.001$). it also improved the mean survival time (MST) from 12.66 to 15.66 days relative to control ($p<0.001$). The activity of extract was incomparable to that of the standard drugs used (artesunate and pyrimethamine). *Solanum anomalum* leaf extract has antiplasmodial activity which may in part be mediated through the chemical constituents of the plant.

**KEYWORDS:** *Solanum anomalum*, antimalarial, *Plasmodium berghei*

## INTRODUCTION

*Solanum anomalum* Thonn. ex Schumach.( family *Solanaceae*) is a shrub growing up to 2 metres tall. The stem, branches and midribs of the leaves are usually armed with prickles up to 5 mm long.

The edible fruits are gathered from the wild and consumed locally. Both the fruits and the leaves are used medicinally. The plant is sometimes cultivated or semi-cultivated for its fruits. It is found in West tropical Africa - Sierra Leone to southern Nigeria, Cameroon and DR Congo. It is Known as 'childrens' tomatoes', they are more commonly used as a condiment in soups and sauces and the fruits are eaten raw or cooked (Burkill,2000). The sap from the leaves and fruits is drunk, or taken by enema 1 - 2 times daily, as a treatment for leprosy and gonorrhoea (Burkill, 2000). The fruits are used as a laxative and digestive (Burkill, 2000). They are also served ground up in soups and sauces as an appetizer for sick persons, sometimes mixed with fruits of Parkia (Burkill, 2000). The crushed fruits are applied to maturate inflammations on fingers or toes (Burkill, 2000). The fruit juice is applied to sores on the ears to alleviate pain (Bukenya and Hall, 1988). The fruits and leaves decoctions of the plant are used in Ibibio traditional medicine to treat various ailments including malaria. Offor and Ubengama (2015) reported the antidiabetic activity of the fruit of this plant. Although there is little information on the fruit of this plant, there is no report of phytochemical constituents and biological activity of the leaf of *S. anomalum*. In this study, we report in this study the phytochemical composition and antimalarial activity of the leaf extract of the plant.

## MATERIALS AND METHODS

### Plants collection

The plant material *Solanum anomalum* (leaves) were collected in compounds in Uruan area, Akwa Ibom State, Nigeria in August, 2015

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The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

**Extraction**

The plant parts (leaves) were washed and shade-dried for two weeks. The dried plants' materials were reduced to powder using mortar and pestle. The powdered material was soaked in 50% ethanol. The liquid filtrate was concentrated and evaporated to dryness in vacuo at 40°C using rotary evaporator and stored in a refrigerator at - 4°C.

**Phytochemical Screening**

Phytochemical screening of the crude extract was carried out employing standard procedures and tests (Trease and Evans, 1989; Sofowora, 1993), to detect the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides among others.

**Animals**

Albino Swiss mice (19 – 28g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*.

**Determination of median lethal dose ($LD_{50}$)**

The median lethal dose ($LD_{50}$) of the extract was estimated using albino mice by intraperitoneal (i.p) route using the method of Lorke (1983). This involved intraperitoneal administration of different doses of the extract (100 -1000 mg/kg) to groups of three mice each. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was recorded. The $LD_{50}$ was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

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

**Microorganism (Parasite)**

A chloroquine sensitive strain of *Plasmodium berghei* (ANKA) was obtained from the National Institute of Medical Research (NIMER), Yaba Lagos, Nigeria and was maintained by sub-passage in mice.

**Parasite Inoculation**

Each mouse used in the experiment was inoculated intraperitoneally with 0.2ml of infected blood containing about $1 \times 10^7$ *P. berghei berghei* parasitized erythrocytes. The inoculum consisted of $5 \times 10^7$ *P. berghei berghei* erythrocytes per ml. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations (Odetola and Basir, 1980).

**Drug administration**

The drugs, artesunate (Levers Pharmaceutical Company, Nigeria) and pyrimethamine (Sigma, USA), extract used in the antiplasmodial study were orally administered with the aid of a stainless metallic feeding cannula.

**Evaluation of anti-Plasmodial activity of ethanol crude leaf extract of *Solanum anomalum***

**Evaluation of suppressive activity of the extract (4-day test).**

This test was used to evaluate the schizontocidal activity of the extract and artesunate against early *P. berghei berghei* infection in mice. This was done as described by Knight and Peters (1980). Thirty mice were randomly divided into five groups of six mice each. On the first day ($D_O$), the Thirty mice were infected with the parasite and randomly divided into various groups. These were administered with the extract and artesunate. The mice in group 1 were administered with the 70 mg/kg, the group 2, 140 mg/kg and group 3, 210 mg/kg of crude extract , while group 4 was administered with 5 mg/kg of artesunate (positive control), and 10ml/kg of distilled water to group 5 (negative control) for four consecutive days ($D_0$ – $D_3$) between 8am and 9am. On the fifth day ($D_4$), thin blood film was made from tail blood. The film was then stained with leishman stain to reveal parasitized erythocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the controls as follows:

Average % parasitaemia=

Average % parasitaemia (in negative control- in Positive groups)
(Average % parasitaemia in negative control.)

**Evaluation of prophylactic or repository activities of extract**

The repository activity of the extract and artesunate was assessed by using the method described by Peters (1965). The mice were randomly divided into seven groups of six mice each. Groups 1 - 3 were administered with 70, 140 and 210 mg/kg/day of the extract respectively, Groups 4 and 5 were respectively administered with 1.2 mg/kg/day of pyrimethamine (positive control) and 10 ml/kg of distilled water (negative control). Administration of the extract/drug continued for three consecutive days ($D_0 - D_2$). On the fourth day ($D_3$) the mice were inoculated with *P. berghei berghei*. The parasitaemia level was assessed by blood smears seventy-two hours later.

**Evaluation of curative activities of extract (Rane's test)**

This was used to evaluate the schizontocidal activity of the extract, and artesunate in established infection. This was done as described by Ryley and Peters (1970). *P. berghei berghei* was injected intraperitoneally into another 30 mice on the first day ($D_O$). Seventy–two hours later ($D_3$), the mice was divided randomly into five groups of six mice each. Different doses of the extract, 70 mg/kg, 140 mg/kg and 210 mg/kg were orally administered respectively to mice in groups 1-3. 5 mg/kg/day of artesunate was administered to the group 4 (positive control) and group 5 was given 10 ml/kg of distilled water (negative control). The extract and drugs were administered once daily for 5 days. Leishman's stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days ($D_0 - D_{28}$).

$$MST = \frac{\text{No of days survived}}{\text{Total No. of days (29)}} \times 100$$

**Statistical analysis and data evaluation**

Data obtained from this work were analyzed statistically using Students' t-test and ANOVA (One- or Two- way) followed by a post test (Turkey-Kramer multiple comparison test). Differences between means was considered significant at 1% and 5% level of significance, that is $p \leq 0.01$and 0.05.

**RESULTS**

**Phytochemical screening**

Phytochemical screening of the crude leaf extract carried out employing standard procedures revealed the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, cardiac glycosides among others.

**Determination of median lethal dose ($LD_{50}$)**

Administration of the leaf extract (100 -1000 mg/kg, i.p) produced various degrees of toxicity and mortality. The physical signs of toxicity included excitation paw licking increased respiratory rate, decreased motor activity, gasping and coma which was followed by death. Doses of extract, 500 mg/kg and below did not cause any mortality in the treatment groups. While 1000 mg/kg of the extract caused 100 % mortality. The Median Lethal Dose ($LD_{50}$) was calculated to be 701.10 mg/kg

**Effect on suppressive activity of ethanolic leaf extract of *Solanum anomalum***

The extract showed a dose-dependent chemosuppressive effect on the parasitaemia. These effects were statistically significant relative to the control ($p<0.05$ - 0.001). The chemoinhibitory percentages ranged from 9.60 to 5.32 (Table 1). However, the effect of the extract was incomparable to that of the standard drug, artesunate, with a chemosuppression of 98.82%. (Table 1).

Table 1: Suppressive Activities of leaf extract of *Solanum anomalum* (4-day test).

| Drug/extract | Dose (mg/kg) | Parasitaemia | % chemosuppression |
|---|---|---|---|
| Distilled water | 10ml/kg | 41.33 ± 6.67 | _ |
| Extract | 70 | 37.33 ±7.12 | 9.60 |
| | 140 | 32.66 ± 3.84[a] | 20.97 |
| | 210 | 14.33 ± 4.63[b] | 65.32 |
| Artesunate | 5 | 0.52 ± 0.01[b] | 98.82 |

Values are expressed as mean ± S.E.M. Significance relative to control: [a]$p < 0.05$;[b]$p<0.001$: n=6

**Effect on repository activity of ethanol leaf extract of *Solanum anomalum***

The ethanol leaf extract of *Solanum anomalum* showed a dose-dependent chemosuppressive effect (6.82 – 65.89%) on the parasitaemia during prophylactic studies. These effects were statistically significant relative to the control ($p<0.001$). However, these effects were incomparable to that of the standard drug, artesuante, with chemosuppression of 90.92 %. (Table 2).

Table 2: Repository/Prophylactic activity of leaf extract of *Solanum anomalum* on *Plasmodium berghei* infection in mice

| Treatments | Dose(mg/kg) | Parasitaemia | % Chemosuppression |
|---|---|---|---|
| Normal saline | 10ml/kg | 14.66±0.66 | - |
| Crude extract | 70 | 12.32± 2.63 | 18.99 |
| | 140 | 10.73±1.57 | 26.80 |
| | 210 | 8.10 ±0.10 [a] | 44.74 |
| Pyrimethamine | 1.2 | 1.33±0.66 [b] | 90.92 |

Values are expressed as mean ± SEM. Significance relative to control [a]$p<0.05$; b$p<0.001$ n = 6.

Table 3: Mean Survival Time (MST) of Mice receiving different doses of leaf extract of *Solanum anomalum* during established infection

| Drug/extract | Dose(mg/kg) | MST (days) |
|---|---|---|
| Distilled water | 10ml/kg | 12.66±0.88 |
| extract | 70 | 13.66 ± 0.33 |
| | 140 | 14.66 ±0.66 |
| | 210 | 15.66± 0.33 [a] |
| Artesunate | 5 | 30.00 ± 0.00 [b] |

Values are expressed as mean ±S.E.M. Significance relative to control: a$p<0.05$; b$p<0.001$, n=6

Table 4: Antiplasmodial activity of leaf extract of *Solanum anomalum* (Curative test).

| DRUG/Extract | DOSE mg/kg | PERCENTAGE MEAN REDUCTION IN PARASITAEMIA PER DAY 3 | 4 | 5 | 7 |
|---|---|---|---|---|---|
| Distilled water | 10ml/kg | 28.6 ±2.33 | 39.50 ± 4.35 | 41.33 ±9.60 | 50.66 ±3.52 |
| Extract | 70 | 30.33 ± 5.23 | 35.66± 9.66 | 27.0 ±4.35 [b] | 25.66 ±7.21 [c] |
| | 140 | 32.66 ±2.60 | 47.66±3.34 | 24.66 ±5.20 [b] | 15.06 ± 2.90 [c] |
| | 210 | 32.15 ± 6.24 | 39.0±5.85 | 23.33 ±3.55 [c] | 15.00 ± 4.97 [c] |
| Artesunate | 5 | 30.0± 4.04 | 24.33± 1.68 [a] | 15.23±1.76 [c] | 2.43 ±0.60 [c] |

Values are expressed as mean ± S.E.M. Significance related to control: a$p<0.05$; b$p<0.01$ c$p<0.001$, n=6.

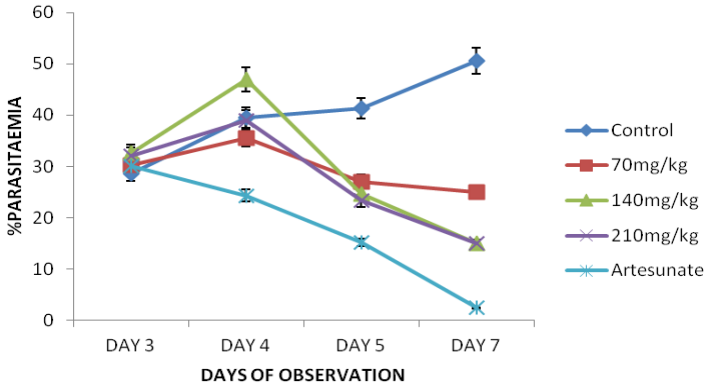


Figure 1: Antiplasmodial activity of leaf extract of *Solanum anomalum* (Curative test).

**Antiplasmodial effect of ethanol leaf extract of *Solanum anomalum* on established infection**

The extract showed a dose- dependent schizonticidal effect on the parasitaemia. There were reductions in the percentage parasitaemia of the extract/ artesunate-treated groups compared to that of the control in which prominent increases were recorded. These reductions were statistically significant relative to the control ($p<0.05$ - 0.001) (Table 4 and Figure 1). Though the extract showed a significant ($p<0.05$ - 0.001), dose -dependent mean survival time on established infection, the effect of the extract(70 -210 mg/kg) was incomparable to that of the standard drug, artesunate.(Table 3).

## DISCUSSION

In this work, median lethal dose ($LD_{50}$) was determined to be 701.10 mg/kg, and the extract was found to be relatively safe with moderate toxicity (Homburger, 1989).

The antiplasmodial activity of leaf extract of *Solanum anomalum* was also investigated using standard models. It was found that the extract significantly reduced the parasitaemia in prophylactic, suppressive and curative models in a dose - dependent fashion. Some secondary metabolites of plants have been reported to have antiplasmodial activity. Among these metabolites are flavonoids and triterpenoids (Philipson and Wright,1991; Christensen and Kharazmi,2001; Kirby *et al*., 1989).

The leaf extract of *S. anomalum* have been found to contain alkaloids, saponins, tannins, phlabatannins, flavonoids and cardiac glycosides among others. Flavonoids are known to exert antiplasmodial activity by chelating with nucleic acid base pairing of the parasite (Lui *et al*., 1992), thereby producing plasmocidal effect and triterpenes like quassinoids are potent protein inhibitors (Liao *et al*., 1976). These compounds (flavonoids and triterpenoids) present in this plant extract may in part have contributed to the plasmocidal activity of this extract and therefore explained the mechanism of antiplasmodial effect of the extract.

Phytochemical compounds such as terpenes and their derivatives such as monoterpenes and sesquiterpenes have been implicated in antiplasmodial activity of many plants (Philipson and Wright, 1991; Christensen and Kharazmi, 2001). Monoterpenes such as limonene have been implicated in endoperoxidation leading to plasmocidal activity (Hatzakis et al., 2000). These could have also contributed to the antiplasmodial activity of this extract

## CONCLUSION

The study was carried out to evaluate the antiplasmodial activity of ethanol crude leaf extract of *Solanum anomalum* in mice. The result obtained in this study indicated that the leaf of *Solanum anomalum* possesses a moderate antiplasmodial activity which justify and confirm the usage of this plant in the treatment of malaria.

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