

# Antiplasmodial Activity of the Methanol Extract and Aqueous Fraction of *Senna siamea*

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## Abstract

The purpose of this research was to evaluate the antiplasmodial activity of the aqueous fraction of the methanolic extract of *Senna siamea* at various dosage concentrations *in vivo* in experimental mice infected with chloroquine sensitive *Plasmodium berghei* NK65 strain. Groups A, B and C mice were infected and treated with 100, 200, and 300 mg extract/kg body weight of mouse respectively while group D was infected and treated with 200 mg chloroquine /kg body weight, group E was infected but not treated and group F was uninfected and untreated. The phytochemical constituents of the plant extract were evaluated giving 4 phytochemicals namely flavonoids and alkaloids, carbohydrates and saponins. In addition, the extract indicated a dose dependent decrease in the level of parasitaemia. The analysis showed that the extract has a decreasing effect on the Packed Cell Volume of the experimental mice. Also, some of the groups lived 10 days beyond the experimental period. Furthermore, the groups treated with 300 mg/kg extract, 200 mg/kg chloroquine and uninfected untreated increased in body weight compared to the negative control (infected untreated). Therefore, the aqueous fraction of the Methanol crude extract of *Senna siamea* leaves could serve as a possible source of antimalarial compounds.

**Keywords:** Plasmodium berghei, Senna siamea, Parasitemia, phytochemicals, Packed Cell Volume.

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## 1. INTRODUCTION

Malaria is one of the oldest recorded disease in the world. Each year, about 500 million people globally are diagnosed with the disease out of which about 1.5 million people die (Greenwood *et al.*, 2005). There is a rapid increase in the resistance of Plasmodium parasites to Antimalarial Combination Therapies (ACTs) and even resistance of the vectors to insecticides (Ridley, 2002).

The use of plants as medicines predates written human history. Ethnobotany (the study of traditional human use of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from “ethnomedical” plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant (Fabricant and Farnsworth, 2001).

*Senna siamea* is an important ornamental and medicinal plant which is readily available. Ethno

medically, it is used in the treatment of headaches, body aches, joint pains and typhoid and in the treatment of fevers. Thus, there’s a need for accessing it’s potentials in the treatment of malaria which could ease the burden caused by the increasing resistance to current anti malarial drugs.

The main aim of this research was to evaluate the antiplasmodial activity of the aqueous fraction and methanolic extract of the *Senna siamea* plant leaves.

## 2. MATERIALS AND METHODS

### Equipment

Separating funnel, cotton wool, beakers, evaporating dish, microscope, microscopic slides, Giemsa stain, test tubes.

### Chemicals/ reagents

Sodium hydroxide, Dragendorff’s reagent, Ferric chloride reagent, Acetic anhydride, Hydrochloric acid, Butanol, Methanol, Chloroform and N-Hexane.

**Materials for the infection of experimental mice**

Methanol, Cotton wool, Normal saline, Razor blade, Butterfly needle, 2 ml syringe, Beaker, Slides, Giemsa stain, Microscope.

**Preparation of methanol extract and aqueous fraction**

Fresh leaves of *Senna siamea* were collected from Mangu LGA in Plateau state and subsequently air-dried at room temperature for 18 days. The dried leaves were pounded into powdered form using a mortar and pestle. 100g of the leaf powder was measured with a weighing balance and constituted with 800 ml of methanol. The constitution was stirred properly for 1 hour and sieved using a silk sieve. The residue was rinsed several times and then filtered using fresh methanol to attain some level of exhaustive extraction. The filtrate was air-dried using an evaporating dish. The dried extract was harvested and weighed (18.6g) and stored in an air-tight container. About 15g of the extract was put into a separating funnel and mixed thoroughly with 100 ml of *n*-Hexane and left to stand for 24 hrs. The eluate was collected into a beaker and 100ml chloroform was added into the residue in the funnels and shaken vigorously. Mixture was allowed to stand for 24hrs again and the eluate collected into another beaker. 100mls of butanol was added into the funnel and thoroughly mixed with residue. Mixture was once again allowed to stand for another 24hrs then separated as before. Residue in the funnels with is the aqueous fraction was collected and stored in an airtight container pending usage.

**Phytochemical Analysis of the Aqueous Fraction**

The aqueous fraction was subjected to phytochemical screening to check for the presence or absence of plant secondary metabolites such as: Saponins, Tannins, Alkaloids, Flavonoids, Steroids and Terpens, Cardiac Glycosides, Balsam, Carbohydrates, Phenols and Resins. Trease and Evans (1996) method was used.

**Test for alkaloids**

To 2mls of fraction, few drops of Dragendorff's reagent was added. An orange colouration would indicate the presence of alkaloids.

**Test for flavonoids**

To 2mls of the fraction, few drops of 5% lead acetate was added. A cream light colour would indicate the presence of flavonoids.

**Test for tannins**

To 2mls of the fraction, few drops of 10% ferric chloride was added. A deep bluish or greenish colour would indicate the presence of tannins.

**Test for Saponins**

To 1ml of the fraction, 4mls of distilled water was added and shaken vigorously. Formation of froth would indicate the presence of Saponins.

**Test for terpens and steroids**

To 1ml of the fraction, 2mls of concentrated Sulphuric acid ( $H_2SO_4$ ) was added along the sides of the test tube. Formation of reddish brown ring at the interphase would indicate the presence of terpenes and steroids.

**Test for cardiac glycosides (Salkowski's test)**

Two (2) ml of the fraction was dissolved in 2mls of chloroform and Sulphuric acid ( $H_2SO_4$ ) was carefully added to form a lower layer. A reddish brown colour at the interphase would indicate the presence of cardiac glycosides.

**General test for balsam**

Three drops of alcoholic ferric chloride was added to 2mls of the fraction. A dark green colour formation would indicate the presence of balsam.

**Test carbohydrates**

Five drops of the extract was added to 2mls of Benedict's reagent, the mixture was heated in a hot water bath for 5 minutes and was observed for the formation of brick red precipitate which would indicate the presence of carbohydrates.

**Test for phenol**

To 2mls of the fraction, 2mls of ferric chloride was added and observed for the formation of a deep bluish-green colouration which would indicate the presence of phenol.

**Test for resins**

To 2mls of the fraction, 2mls of acetic anhydride was added and drops of concentrated Sulphuric acid was added to observe for a violet colour which would indicate the presence of resins.

**Acute toxicity testing**

LD<sub>50</sub> determination was done using the Lorker's method (Lorke D, 1983). Three groups containing three mice in each was administered the fraction orally at a dose of 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. The mice was observed for morbid signs as well as mortality for 24hrs. In the second phase, another three animals were divided into three groups with one animal each and administered 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the extract respectively and then observed for 24hrs for behavior as well as mortality.

**Preparation of Experimental Mice (Infection)**

The experimental mice were separated into six groups with three mice in each group and was fed and cared for, for 5 days to allow animals gain more weight

and acclimatize to the new environment. Three *Plasmodium berghei* infected mice were purchased from the animal farm and was used as donors to infect the mice with the parasite. About 10mls of blood from from the 3 infected mice was collected in a beaker and mixed with 30mls of normal saline. The uninfected mice were inoculated intraperitoneally with 0.5ml parasite suspension.

### Experimental Design

The 18 mice were grouped into six (6) groups. Three treatment groups and three control groups (Positive, negative and normal control groups). Each group had three mice. The three treatments groups and the negative and positive control groups were infected with *Plasmodium berghei* parasite. The treatment groups were treated with 100 mg/kg, 200 mg/kg and 300 mg/kg body weight of extracts respectively. While the negative groups were left untreated.

1. **GROUP A:** 3 infected mice administered 100 mg/kg of extract per body weight.
2. **GROUP B:** 3 infected mice administered 200 mg/kg of extract per body weight.
3. **GROUP C:** 3 infected mice administered 300 mg/kg of extract per body weight.
4. **GROUP D:** 3 infected mice administered 200 mg/kg of standard drug (chloroquine) per body weight (Positive control).
5. **GROUP E:** 3 infected mice with no treatment (Negative control).
6. **GROUP F:** 3 uninfected mice without treatment (Normal control)

### Administration of fraction and standard chloroquine drug

After 24, 48 and 72 hours of infection, blood samples were collected from the infected mice to check for the presence of the parasites. Cutting the tip of the tails of the mice caused blood to flow which was collected on slides and smeared to make a thin blood film and dried under the shade for about 30 minutes and was fixed with methanol. After the methanol dried completely, the blood was stained with giemsa stain for 15 minutes and rinsed with water which was taken for microscopic examination after drying. Immersion oil was added to the stained slides and each of the slides were viewed under the X100 objective lens. The *Plasmodium berghei* were seen in the infected blood samples as spherical, curved or clustered images in the stained samples. Parasitemia was counted based on giemsa positive bodies which represents the parasitized red blood cells. The parasitemia count was done and recorded 72 hours after infection and done after every 48 hours throughout the period of administration.

### Packed Cell Volume determination

After administration, mice that survived were sacrificed and blood was collected from the jugular vein into an E.D.T.A containing tube. Capillary tubes were

filled to two-third of its capacity and one end was sealed with plastacine. The capillary tubes were carefully placed in a hematocrit centrifuge and spun at 3,000 RPM for 5 minutes. After spinning, the capillary tubes were placed on a specially designed hematocrit reader and the PCV was read in percentage.

A reduced PCV is caused by loss of blood or red blood cells from the circulating blood. PCV is the percentage of red blood cells in the whole blood. Low PCV can be due to vitamins and mineral deficiencies, recent or long term blood loss, an infection or a white blood cell disorder such as leukemia and lymphoma. High PCV can be caused by dehydration or an abnormal increase in production of white blood cells.

### Weight determination

The mice was weighed before and after the administration to check for change in weight.

## 3. RESULTS AND DISCUSSION

### Percentage yield and phytochemical screening

The percentage yield was calculated to be 18.6% and the phytochemical screening gave a positive result for Alkaloids, Flavonoids, Saponins and Carbohydrates. The other secondary metabolites were seen to be absent (Table 1).

### Parasitemia for mice treated with aqueous fraction

The effects of the fraction on the parasitemia level was tested. The fractions had a decreasing effect on the parasitemia level but the group treated with 200 mg chloroquine had a more decreasing effect. The group that was infected but not treated had a continually increasing parasitemia level. Among the three test groups, the group treated with 300 mg had a more decreasing effect on the parasitemia count. Figure 1 gives a detailed result.

### Parasitemia for mice treated with methanol extract fraction

The effects of the extract on the parasitemia was tested. The extract had a decreasing effect on the level of parasite but the 200 mg/kg had a more decreasing effect than the lower dosage of 100 mg/kg and the high dosage of 200 mg/kg. The positive control had a more decreasing effect and the negative control parasitemia level had a continually increasing level of parasites.

### Weight

After the administration, the percentage weight of the Normal control, Positive control and the 300 mg test group increased by 48%, 3.7% and 5.3% respectively while the weights of the Negative control, 100mg and 200mg reduced by 22%, 6% and 1.3% respectively.

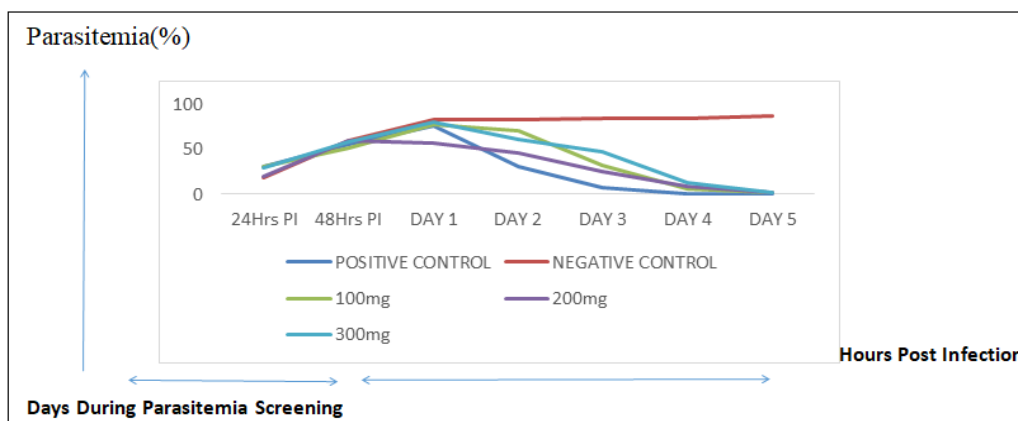
**Table 1: Phytochemical constituents of the aqueous fraction of the methanol extract of *Senna siamea* leaves**

Phytochemicals	Result
ALKALOIDS	+
FLAVONOIDS	+
TANINS	-
SAPONINS	+
TERPENS AND STEROIDS	-
CARDIAC GLYCOSIDES	-
BALSAM	-
CARBOHYDRATE	+
PHENOL	-
RESINS	-

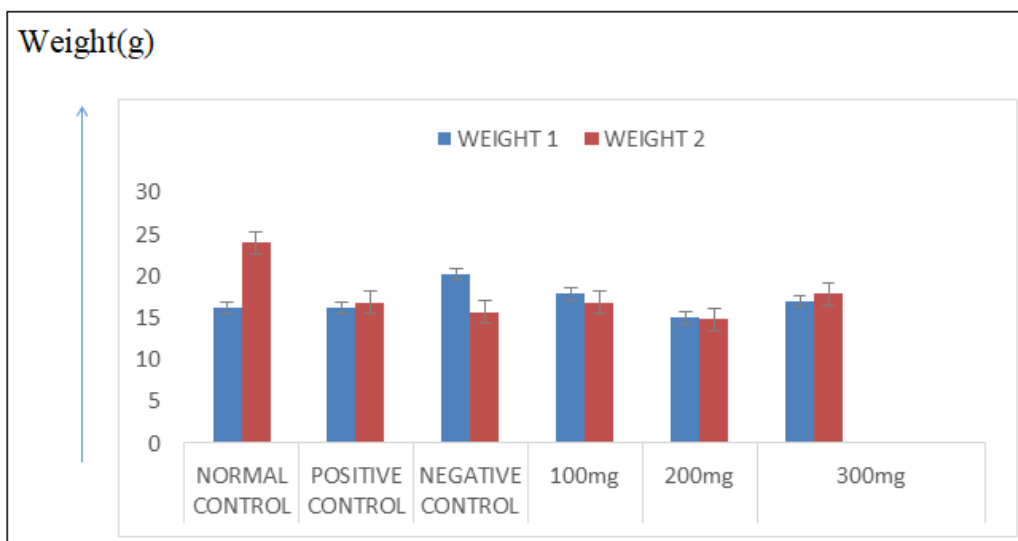
Keys: - = Absent, + = Present

**Table 2: Acute toxicity testing (LD<sub>50</sub> determination)**

Phase	Dose (mg/kg)	No. of Animals	Mortality
1	10	3	0
1	100	3	0
1	1000	3	0
2	1600	1	0
2	2900	1	0
2	5000	1	0



**Figure 1: Graph showing average mean parasitemia count from day one of administration of the aqueous fraction**



**Figure 2: Bar chart showing average mean weight of mice before inoculation of parasite and after administration**

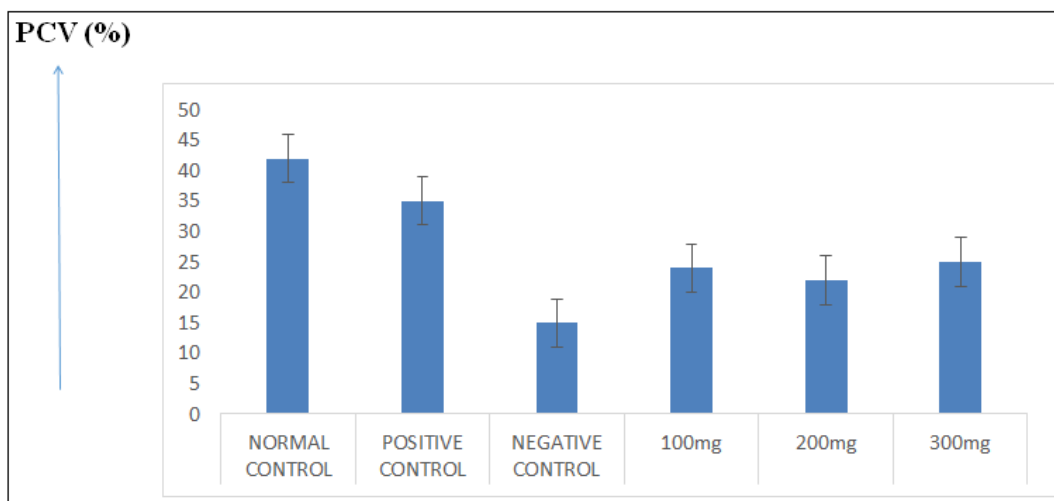


Figure 3: Bar chart showing the Packed Cell Volume (PCV) of mice after administration

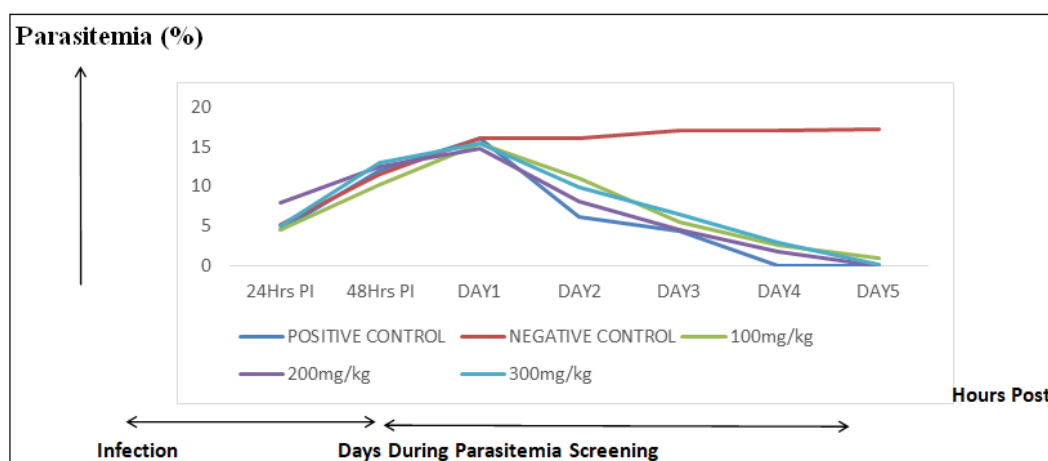


Figure 4: Graph showing mean parasitemia level when administered with the Methanol extract

## CONCLUSION

The work tested the effects of the aqueous fraction of methanol extract of *Senna siamea* leaves. Preliminary phytochemical screening gave a positive result for Alkaloids, flavonoids, saponins and carbohydrates. Alkaloids have been said to block protein synthesis in plasmodium species (Abdulelah *et al.*, 2007). Equally, flavonoids have been shown to inhibit the growth of the malaria parasite through the L-glutamine pathway although their exact mechanism of action is yet to be clarified (Zhou *et al.*, 2011). Triterpenoid and steroid saponins have been found to be detrimental to several infectious protozoans such as the *Plasmodium falciparum* (Elias *et al.*, 2000). Saponins inhibit the intestinal permeability of glucose and may consequently inhibit the growth of *Plasmodium falciparum* which uses glucose as food (Lutgen, 2015).

The plant was seen to be non toxic even up to a high dose of 5000 mg/kg body weight of mice. According to the Food and Agricultural Organization of the United Nations, a six months toxicity study of the leaves was performed and was found to be non toxic

even at a high dose of 2000 mg/kg/body weight per day (Pranee *et al.*, 2003).

The fraction was subjected to *in vivo* anti-plasmodial studies compared to chloroquine, a standard anti-malarial drug showed different antiplasmodial activities depending on the dose, against the *Plasmodium berghei* species in experimental mice. The various dosage concentration of 100 mg/kg, 200 mg/kg and 300 mg/kg per body weight all showed curative activity of the aqueous fraction of the methanol extract of *Senna siamea* and chloroquine to different extents. However, the 200 mg/kg of chloroquine and the 300 mg/kg dosage concentration of the aqueous fraction of the methanol extract of *Senna siamea* showed more curative activities compared to the lower dosage concentration of 100 mg/kg and 200 mg/kg per body weight. Also the animals treated with 300 mg/kg of fraction and 200 mg/kg of chloroquin increased in weight just like the group that was uninfected and untreated. The other groups experienced a decline in weight. However, a significant difference was seen in the packed cell volume of the mice treated with the fractions and the infected and untreated mice as

compared to the normal control. This study showed that the fraction and the crude methanol extract possess antiplasmodial activities and can therefore be used in the treatment of malaria.

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