

## PHYTOCHEMICAL ANALYSIS AND ANTIMALARIAL ACTIVITY OF THE AQUEOUS EXTRACT OF *Senna alata* LEAF.

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### ABSTRACT

Leaf aqueous extract of *Senna alata* was investigated for its phytochemical constituents and antimalarial activity. Thirty (30) albino mice were infected by intraperitoneal injection of standard inoculum chloroquine-sensitive strain of *Plasmodium berghei* (NK-65). The animals were randomly divided into 6 groups of 5 mice each. Groups (1 to 4) were administered orally with 0.5ml of the extract at 50, 100, 200 and 400 mg/kg body weight respectively. Group 5 were given 0.5ml of 5mg/kg body weight of chloroquine and Group 6 which served as the control group were not treated. The results of the phytochemical analysis showed the presence of alkaloid, glycoside, phenolic, steroid and tannin. There was 100 per cent parasite inhibition in the chloroquine group and 99.07 per cent parasite clearance in the group which received 400mg/kg body weight of the extract. The Mean Survival time (MST), for the control group was 12.5 days; and 27.9 days for chloroquine-treated group, while the groups that received 200 and 400 mg/kg body weight of the extract were 27.7 and 27.5 respectively. The aqueous leaf extract of *Senna alata* thus has promising antiplasmodial activity which can be harnessed in malaria therapy.

**Key Words:** Antimalarial bioactivity, aqueous extract, *Senna alata*, Phytochemicals.

### INTRODUCTION

Malaria is a life threatening parasitic disease transmitted from person to person by female anopheles mosquitoes. It remains the major cause of morbidity and mortality in the tropical regions of the world with over 300 million new cases reported annually (WHO, 2005). This vector-borne infectious disease is a classic example of one that affects the productivity of individuals, families and the whole society, since it causes more energy loss, more debilitation, more loss of work capacity and more economic damage than any other human parasitic diseases (Sachs, 2002). Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development. It is widespread in tropical and subtropical regions, including parts of the Americas, Asia and Africa. A total of 109 countries were endemic for malaria in 2008, 45 within the WHO African region (Batista *et al.*, 2009).

Current anti-protozoal drugs are inadequate due to parasite resistance, toxicity, lack of efficacy and inability to eliminate all stages of parasites from the host (Tasdemir *et al.*, 2005). Moreover, with the

increased cases of antimalarial drug resistance and prevalence of sub-quality drugs in developing countries, the need for new drugs or drug combinations for the treatment of malaria these days becomes important, including the exploitation of traditional medicine (Philipson and Wright, 1991).

Rodent plasmodia such as *Plasmodium berghei* are commonly used as malaria models in mice and have tremendous impact on the investigations of antimalarial activities. The need to search and develop more effective drugs that are readily available and affordable to wide populace in developing countries like Nigeria has necessitated this study. Medicinal plants have been the focus of many anti-infective drugs and alternative sources of antimalarial agents in various parts of the world since time immemorial (Schuster, 2001). One of such plants claimed to possess antimalarial activities is the leaf of *Senna alata*.

*Senna alata* formerly known as *Cassia alata*, is an important medicinal tree as well as an ornamental flowering plant in the subfamily Caesalpinioideae. The plant *Senna alata* grows all year round, and flowers during November to January. It grows well in the forest areas of West Africa and it is identified by various names in Nigeria, such as "asuwon oyinbo" (Yoruba), Ogalu (Igbo), and "zowaginidi" (Hausa). The plant is ethno medically reputed to be useful in the treatment of several infections, which include ringworm, parasitic skin diseases (Palanichamy and Nagorajon, 1990). The leaves are reported to be useful in treating convulsion, gonorrhoea, heart failure, abdominal pains, oedema and are also used as a purgative (Ogunti and Elujobi, 1993). Because of the use in Nigeria of *Senna alata* as an antimalarial plant in traditional medicine, the present experiment was carried out to scientifically evaluate the *in vivo* antimalarial activity of aqueous leaf extract of *Senna alata* against *Plasmodium berghei* in mice possibly for development as an orthodox antimalarial agent.

## MATERIALS AND METHODS

### Plant Materials:

The leaf of *Senna alata* (family Caesalpinioideae), was obtained from Ilorin, and was authenticated at the Department of Botany, University of Ilorin. A voucher sample was deposited in the herbarium.

### Aqueous Extraction:

The leaves were cleaned, air-dried at room temperature until constant weight was achieved and ground into powder using an electric milling machine. Aqueous extract was prepared by soaking 200g of the powdered leaf in 1.5 liters of distilled water for 24 hours with constant shaking using the GFL shaker (No. 3017MbH, Germany). The contents were filtered with Whatman no. 1 filter paper and the filtrate obtained was concentrated to dryness in a vacuum evaporator at 40°C. the dried extract was weighed and reconstituted into desired doses.

### Phytochemical Analysis:

Crude aqueous extract was subjected to phytochemical analysis, using standard chemical procedure as described by (Trease and Evans, 1989).

### Experimental Animals:

Albino mice (*Mus musculus*), weighing 20-25g were obtained from the small animal holding unit of the Department of Biochemistry, University of Ilorin, Nigeria. The animals were housed in standard wired cages with free access to pelleted Feed and tap water. The study was conducted in accordance with the recommendations from the declaration of Helsinki on guiding principles in the care and use of animals.

**Drugs and Reagents:**

The chloroquine used was obtained from May & Baker Pharmaceutical Company Limited, Nigeria. Other reagents were of analytical grade.

**Malaria Parasite:**

The chloroquine sensitive, *Plasmodium berghei* (NK 65 strain) was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria. The parasites were maintained by weekly blood passage in mice.

**Inoculation of experimental Mice:**

Albino mice were infected intraperitoneally with standard inoculum (0.2ml of  $1 \times 10^7$  parasitized red blood cells). The inoculum was from a single donor mouse previously infected with *Plasmodium berghei* (33 per cent parasitaemia).

**2.8. Animal Grouping and Extract Administration.** The animals were randomly divided into 6 groups of 5 mice each, after confirmation of parasitaemia 72h post-inoculation. Groups (1, 2, 3 and 4) were administered orally with 0.5ml of aqueous crude extract of *Senna alata* leaf at 50, 100, 200 and 400 mg/kg body weight respectively for five days. Group 5 received 5mg/kg body weight of chloroquine daily for 5 days. Group 6 (control) was left untreated but administered appropriate volume of distilled water.

**Estimation of Percentage Parasitaemia:**

Percentage parasitaemia was estimated at the end of the observational period of 28 days using the formula:

$$\% \text{ Parasitaemia} = \frac{\text{Parasitized RBC}}{\text{Parasitized RBC} + \text{Non-parasitized RBC}} \times 100$$

**Estimation of Percentage Chemosuppression:**

The percentage chemosuppression was calculated using the formula:

$$\% \text{ Chemosuppression} = \frac{B - C}{C} \times 100$$

Where B = parasitaemia in study group, C = parasitaemia in control.

**2.11. Estimation of Mean Survival Time (MST).** The number of days each animal survived was recorded for the animals in each group and the mean survival time calculated using the formula:

$$\text{MST} = \frac{\text{Sum of days of survival of animals / group}}{\text{Total number of animals in the group}}$$

**Statistical Analysis:**

Values are expressed as mean  $\pm$  SEM. Data from the test were compared with their respective controls and differences at  $p < 0.05$  were considered statistically significant using analysis of variance (ANOVA).

**RESULTS AND DISCUSSION**

The results (Table 1) show the presence of alkaloids, flavonoids, glycosides, tannins, steroids and phenolics all of which may be responsible for the pharmacologic activity of the extract. These compounds have been variously implicated in antiparasmodial activities of many plants (Miliken, 1997; Abo *et al.*, 1999) and maybe responsible for the observed antiparasmodial activity of this extract. Although, the mechanism of action of this extract has not been elucidated, some plants are known to exert antiparasmodial activity by causing elevation of red cell oxidation (Etkin, 1997) or by inhibiting protein synthesis (Kirby *et al.*, 1989). The extract could have elicited its action through either of the two mechanisms or by some other unknown mechanism. Earlier workers have shown that isolated alkaloid, 9-methoxycanthin-6-one displayed higher antimalarial activity against *Plasmodium falciparum* Gombak isolate, when compared with chloroquine (Chan, *et al.*, 2004). In addition, potent antimalarial agents, raphidecurperoxin and polysyphorin, were isolated from the Vietnamese medicinal plants, *Rhaphidophora decursiva* (Zhang, *et al.*, 2004). The extracts of *Nigella sativa* (Black seed), contained different classes of alkaloids that were believed to block protein synthesis in *Plasmodium falciparum* (Abdulelah and Zainal, 2007). Consistent with this concept, different extracts of *Senna alata* have been reported to exert antimicrobial activities, including antibacterial and antifungal (Sule *et al.*, 2010). The antioxidant effect of plant alkaloids may represent another mechanism that contributed to its antimalarial activity.

Results also showed that at the end of 28 days, there was no parasitaemia in the blood of mice in the chloroquine treated group and the groups that received 400mg/kg body weight of crude extract. Less than 2 per cent parasitaemia was found in the blood of mice that received 200mg/kg and 100mg/kg body weight of the extract. However, there was high parasitaemia of 43 per cent in the infected untreated group (Fig. 1). The leaf extract also exerted significant curative effect in the Rane test established infection and this property is comparable to that of the standard drug (chloroquine) as shown in the percentage parasitaemia (Fig. 1) of the mice in both extract and chloroquine treated groups. Rane test which is a standard test commonly used in antimalarial screening relies on the ability of standard inoculum of *P. berghei* to kill the recipient mouse thereafter.

The percentage chemosuppression achieved by the administration of *Senna alata* leaf extract at different concentrations of 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg body weight respectively of over a period of twenty-eight (28) days (Fig. 2) showed a significant percentage increase in chemosuppression. 100% chemosuppression was obtained on day 28 post infection in the group that received 5mg/kg body weight chloroquine which was significantly different ( $p < 0.05$ ) from all extract treated groups. The initial low percentage chemosuppression observed in the extract-treated group and the chloroquine group (Fig. 2) may be due to the fact that the extract at the dose administered had not accumulated sufficiently to bring about considerable chemosuppression (Adebayo *et al.*, 2003). However, the prolonged administration of the extract led to the total clearance of the parasites, this result from accumulation of enough active compounds to effect total clearance of the parasites.

The control group (infected-untreated) had the least MST of 12.5 days while the highest MST of 27.9 days was obtained in the chloroquine treated group (C). The MST of 27.6 and 27.5 days were obtained respectively for the groups that received 200mg/kg body weight and 400mg/kg body weight of the extract, while the MST of 16.2 days was obtained in the group that received 50mg/kg body weight. The mean survival time of 27.6 days in the group that received 200mg/kg body weight of the extract was comparable to 27.9 days for the chloroquine group. Survival of experimental animals beyond 12 days is regarded as significant activity (Abosi and Raseroka, 2003; Ajaiyeoba *et al.*, 2006).

The determination of percentage reduction in parasitaemia level is the most reliable parameter in antimalarial screening. It is clear from the results (Fig. 1) that the *P. berghei* - infected mice treated with the aqueous leaf

extract of *S. alata* reduced percentage parasitaemia compared to those of the infected untreated animals. The result from this study therefore, lent scientific credence to the folkloric use of the leaf extract of *S. alata* for the treatment of malaria in Africa.

**Table 1: Phytochemical constituents of the aqueous leaf extract of *Senna alata*.**

| Phytochemicals | Status |
|----------------|--------|
| Alkaloids      | +      |
| Tannins        | +      |
| Glycosides     | ++     |
| Steroids       | ++     |
| Phenolics      | ++     |

++ Strongly present, + fairly present

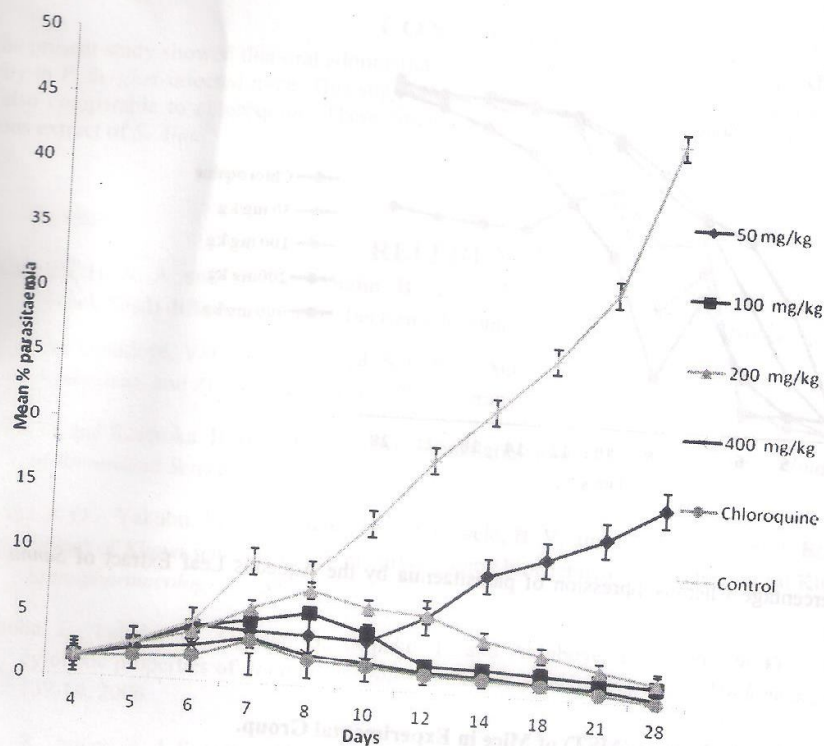


**Fig. 1:** Percentage Parasitaemia of *Plasmodium berghei* infected mice treated with aqueous extract of *Senna alata* leaf and chloroquine. Each point is a percentage average count from five infected mice. Values are expressed as mean  $\pm$  S.E.M

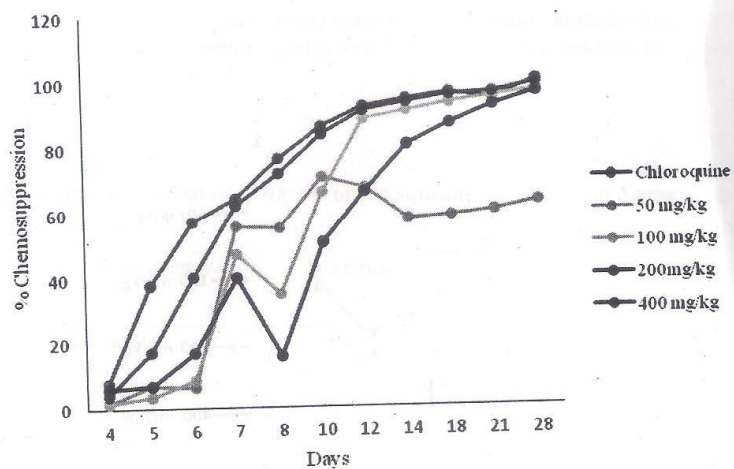


**Figure 2:** Percentage Chemosuppression of parasitaemia by the Aqueous Leaf Extract of *Senna alata* and Chloroquine.

**Table 2:** Mean Survival Times (MST) of Mice in Experimental Group.

| GROUP       | Mean survival Time (days) |
|-------------|---------------------------|
| Control     | 12.5                      |
| 50 mg/kg    | 16.2                      |
| 100 mg/kg   | 21.4                      |
| 200 mg/kg   | 27.6                      |
| 400 mg/kg   | 27.5                      |
| Chloroquine | 27.9                      |

## CONCLUSION

The present study showed that oral administration of aqueous leaf extract of *S. alata* exhibited antimalarial activity in *P. berghesi*-infected mice. This suggests the beneficial effects of the herb. The effect of the extract was also comparable to chloroquine. These findings thus give a scientific credence to the folkloric use of aqueous extract of *S. Alata*.

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