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THERAPEUTIC ACTIVITY OF AQUEOUS LEAF EXTRACT OF *Phyllanthus niruri* AGAINST *Plasmodium falciparum*

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ABSTRACT

Therapeutic activity of aqueous extract of *Phyllanthus niruri* against *Plasmodium falciparum* malarial parasite was examined *In vivo.* Total of 24 mice were used to test for acute toxicity of the plant extract, 4 days suppressive test and curative ability of the leaf extract. Average percentage parasitaemia of each group of mice was calculated and student's t-test statistical package was used to analyse the data obtained. Aqueous extract of *P. niruri* show no any sign of toxicity neither mortality observed, the plant extract exhibited high percentage chemosuppression of 76.1% at 100 mg/kg followed by 59.0% at 80 mg/kg in suppressive test. High chemosuppression was obtained at 200 mg/kg 59.09% and 100 mg/kg with 42.04% in the curative test. There was a significant ($P \leq 0.05$) reduction in the number of parasitized red blood cells relative to the negative control, hence the result shows that the aqueous extracts of *P. niruri* possess promising antimalarial activities which can be exploited for malaria therapy, and also justifies the traditional use of the plant in malaria treatment.

Keywords: Therapeutic, Aqueous Extract, *Phyllanthus niruri, Plasmodium falciparum, In vivo*

INTRODUCTION

Malaria is one of the major public health problems in tropical and subtropical regions where it account for over one million death annually (World Malaria Report, 2005) There are about five species of *Plasmodium* known to transmit malaria to humans, with *P. falciparum* as the most virulent form of malaria while the other species *P. vivax, P. ovale,* and *P. malariae* generally cause a milder form of malaria (WHO, 2014). In Nigeria, malaria is endemic throughout the country. World Health Organization (WHO, 2018) estimated malaria mortality rate for children under five in Nigeria at 729 per 100, 000. The Ministry of Health reported in April 2004 that malaria is responsible for one out of ten deaths in pregnant women and has caused the Federal Government of Nigeria over one billion Naira annually in treating malaria (Government in Action, 2005) Malaria exert an adverse effects on pregnant women according to Nigeria Centre for diseases control (NCDC) where both mother and the foetus suffer from maternal anaemia, miscarriage, premature delivery, intrauterine growth retardation, delivery of low weight infants of less than 2.5 kg. In children, it causes high fever, chills, impaired consciousness, convulsion, difficult breathing and respiratory distress, jaundice and vital organ dysfunction. Malaria account for 25% of infants' mortality, 11% of maternal mortality and 30% mortality of children under the age of five Nas *et al*. (2017). . In the light of this statistical data, malaria is a major public health problem currently and multi-drug resistance has become one of the most important problems impeding malaria control efforts (Htut *et al.,* 2009, Sendagire *et al*., 2005). In malaria endemic countries, traditional medicinal plants are frequently used to treat malaria especially in rural and sub-urban settings (Nondo *et al*., 2016). The analyses of traditional medicines that are employed for the treatment of malaria represent a potential in the global efforts at discovery of lead molecules for development of antimalarial drugs. This has led to the attempts to discover other antimalarial agents, mainly from plant sources. *Phyllanthus niruri* is commonly known as Seed-under-leaf, it belong to the family of Phyllanthaceae. It has being used to treat malaria in Nigeria among the locals, it has also been used traditionally to treat various illness such as renal stones, gastrointestinal disturbances, cough, hepatitis, gonorrhoea, fever as well as antioxidant properties Giribabu *et al*. (2014). Some of the medicinal uses have been supported in experimental models, suggestive that the plant extracts possess various pharmacological properties and hence the biotic basis of most pharmacopias (Patel *et al*., 2011; Vermad *et al*., 2014). It is essential that the plant *Phyllanthus niruri* which have folklore reputation for anti-malarial properties is investigated hence this work to determine the therapeutic property of aqueous leaf extract of *Phyllanthus niruri* against *Plasmodium falciparum.*

MATERIALS AND METHOD

Plant Collection

The plant, *Phyllanthus niruri* was collected from its natural habitat in Unguwan Rimi Kaduna and around Kaduna State House of Assembly Complex and taken to the Botanical Laboratory of the Department of Biological Sciences Kaduna State University(10°31`N, 007°26`E) for identification.

The leaves were dried at room temperature, then pulverized using mortar and pestle and macerated for 24 hours then filtered with Whatman No. 1 filter paper size. The filtrate was subsequently concentrated using a water bath. The weight of residue obtained was 37g.

Plate 1: *Phyllanthus niruri* in its natural habitat

Experimental Animals and Parasites

Mice of both sexes with a body weight of 26g - 29g were purchased from the parasitology Laboratory of Nigerian Institute for Tryponosomiasis Research (NITR) Kaduna for this study. They were allowed for two weeks for acclimatization to their environment, and were maintained on a standard mouse cubes and water *ad libitum*. Infected blood with *Plasmodium falcifarum* parasites was collected from Primal Diagnostic Laboratory Kaduna.

Acute Toxicity Test

Acute toxicity test was carried out according to the method of Lorke (1983). Eight rats used were divided into four groups and were acclimatized for 7 days before the onset of the experiment. The rats were dosed orally with 100, 200, 300, 400 mg/kg body weight of the plant extract. The rats were observed for signs of toxicity such as tremors, weakness and refusal to feed, falling off of hair, coma, or even death after 24 hours.

Test on Early Malaria Infection (4-Days Suppressive Test)

The 4-days suppressive test was used to measure the schizonticidal activity of *P. niruri* leaf extract, Kweyamba (2019) whose method was a modification of Peter and Robinson (1992) 4-days Suppressive test of plasmodium parasite was adopted. Eight mice were divided into 4 groups containing 2 mice each; all were inoculated intra-peritoneally with 0.1ml of infected blood (parasite) at the first day (Day 1). The first two groups were administered 80mg/kg and 100mg/kg body weight respectively of the leaf extract on the first day (Day 1), while the third and fourth group served as positive control and negative control respectively. The positive control was administered 5mg/kg of Artemether and Lumefantrine while the negative control receives 0.5ml of distilled water. On the fifth day (Day 5) 1 drop of blood sample from the animal's caudal vein was collected and transferred to microscope slides, thus, making a thin film from each mouse, fixed with methanol and staining with Giemsa stain of 10%, each stained slide was examined systematically under the microscope with oil immersion objectives of 100X magnification in order to evaluate percent of suppression of each extract dose with respect to positive control and negative control groups, so that the average percentage parasitaemia could be calculated as follows -

Average % suppression = 100[(A-B)/A]

Where A= Average parasitaemia of the negative control group and B=average parasitaemia of treated group (Kweyamba, 2019).

Test on Established Infection (Curative Test)

A total of eight mice were divided into 4 groups containing 2 mice each were inoculated intra-peritoneally with 0.1ml of infected blood (parasite) at the first day (Day 1). The mice were not treated until the parasitaemia was established. On day 4 after the animals were infected. The first 2 groups were administered 100mg/kg, and 200mg/kg body weight of the leaf extract per day for 4 days i.p, while the third group which serves as the positive control received 5mg/kg of Artemether and Lumefantrine and the fourth group received 0.5ml of distilled water and serves as the negative control. On the fifth day (day 5) 1drop of blood sample from each animal's caudal vein was collected and transferred on slides, making thin film from each mouse, fixed with methanol and stained with 10% Giemsa stain, for systematic microscopy with oil immersion objectives of 100X magnification. The average percent parasitaemia was determined for each mouse using the above formula.

Statistical Analysis

Data obtained in this study were analysed using Student's t-test. Differences between means at 5% level ($P \leq 0.05$) were considered significant.

RESULTS

Toxicity Test

The oral administration of aqueous leaf extracts of *P. niruri* in the doses of 100, 200, 300 and 400 mg/kg body weight did not cause any sign of acute toxicity. No deaths of mice were recorded 24 h after oral administration. All other parameters such as tremors, urination, refusal to feed, falling of hair, and coma were not observed.

Suppressive Test

Early malaria infection or Peters four days chemosuppressive activity test for the aqueous extract of *P. niruri* produced a dose dependent chemosuppression activity as shown in the table 1. The highest suppression of parasitaemia was observed at the dose of 100mg/kg body weight of mice. Percentage suppression was observed to increase as extract concentration increased. After four days treatment with the different extract dose, the mean parasitaemia of the test groups ranged from 10.5% to 18.0% while the corresponding value of the negative control group being 44.0%. The mice treated with Artemether and Lumefantrine is 8.5% on 4 days suppression. The therapeutic activity against malarial parasites produced by the plant extract was statistically significant ($P \leq 0.05$) when compared to the negative control.

Table 1: Effects of aqueous extract of *P. niruri* on early malaria infection (Suppressive Test)

S/N	Treatment	Dose/day mg/kg	Average parasitaemia in Percent (%)	Chemosuppression in percent (%)	Significance
1	Extract	80	18	59.0	P<0.005
2	Extract	100	10.5	76.1	P<0.005
3	+ve Control	5	8.5	80.6	
4	Distilled water	0.5ml	44	0	

Key: +ve = positive control (Artemether and Lumefantrine).

Curative Test

The result of the *in vivo* examination of the *P. niruri* extract on established infection showed an increase in chemo-suppressive activity with increased in concentration. The extract was marginally active at 100 mg/kg per day (42.04%) and highest activity at 200mg/kg per day (59.09%) Table 2. The mice that received 5mg/kg of Artemether and Lumefantrine showed (72.72%) chemosuppression. The antimalarial activity produced by the extract was statistically significant (P≤0.05) when related to the negative control.

Table 2: Effects of aqueous extract of *P. niruri* on established malaria infection (Curative Test)

S/N	Treatment	Dose/day Mg/kg	Average parasitemia In percent (%)	(%)percent Chemosuppresssion	Level of Significance
1	Extract	100	12.75	42.04	P<0.05
2	Extract	200	9.0	59.09	P<0.05
3	+ve	5	6.0	72.72	
4	Distilled water	0.5ml	22	0	

Key: +ve = positive control (Artemether and Lumefantrine).

DISCUSSION

Acute systemic toxicity is usually the first test conducted for every chemical before other tests are carried out, it estimates the dose of a test substance that produces 50% death in a given species of animals. The result obtained in the acute toxicity test implies that aqueous leaf extracts of *P. niruri* in the doses of 100, 200, 300 and 400 mg/kg body weight (orally) were not toxic to the mice, therefore the extract is considered safe for use. This is similar with the finding of Adebisi *et al.,* 2021 who reported safety of the extract at studied doses of 200, 400 and 800 mg/kg body weight. This safety has also been demonstrated in various studies where extracts of Phyllantus family were generally administered (Ansari *et al*., 2017)

The various doses of aqueous leaf extract of *P. niruri* utilized in this study demonstrated a potent antimalaria activities against *P. falciparium* infection. The extract at doses of 100 and 200mg/kg were effective at suppressing the induced mice malaria compared to Artemether and Lumefantrine which is the standard drug and the response was dose dependent. This observed therapeutic efficacy is consistent with evidence in literature. Aarthi and Murugan (2011) investigated the antimalarial activities during early and established infections of the ethanolic extract of *P. niruri* against *Plasmodium berghei* infections in mice and reported that *P. niruri* exhibit significant antimalarial property. Adebisi *et al.* (2021) demonstrated in vivo antiplasmodial potency of aqueous leaf extract of *P. niruri* used in malaria naturopathy. Ajala *et al.* (2011) also demonstrated antiplasmodial effect of extracts and formulated capsules of *Phyllanthus amarus* on a resistant malaria parasite strain, *P. yoelii* induced malaria infection in Swiss albino mice. These authors employed both aqueous and ethanolic extracts of whole plant at daily doses of 200 mg/kg – 1600mg/kg in prophylactic and therapeutic settings. Aarthi and Murugan (2011) reported that the antimalarial activity exhibited by this plant might be attributed to the presence of alkaloids or flavonoids or even a combined action of more than one metabolite.

CONCLUSION

In conclusion, the present study has demonstrated, in an animal model, the antimalarial efficacy of the leaf extracts of *Phyllanthus niruri,* traditionally used in chemotherapy of *Plasmodium falciparum* infection in humans. So the traditional use of these plants to treat malaria is based on a real antiparasitic activity. It would therefore be worthwhile to extract the active components which can serve as a template for the production of cheap anti-malaria drug from indigenous plant in Nigeria.

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