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In Vivo Antiplasmodial Effect of the Ethyl Acetate Fraction of Crude Extract of Phyllanthus Niruri.

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Abstract
This research was designed to study the effect of ethyl acetate fraction of *Phyllanthus niruri* on *Plasmodium berghei* infected mice. *P.niruri* is known to have antimalaria properties. Crude extract was fractionated with ethyl acetate. 50, 100, 200 and 400mg/kg body weight of the ethyl acetate fraction was dosed to albino mice infected with *Plasmodium berghei*. The Plasmodium count, packed cell volume and body weight were measured before, during and after the experiment. Result showed that all doses of the extract exhibited parasitemia inhibition at 88.88%, 85.23%, 79.68%, and 82.54 % which was not statistically significant (P<0.05) when compared with the positive control chloroquine of (90.37%). The Packed cell volume was observed to reduce across group after treatment when compared to before the treatment. However, weight gain was observed in treated groups. Result shows that the ethyl acetate fraction of *P. niruri* has a curative effect on *Plasmodium berghei* and can be utilized to treat malaria after further purification to isolate the chemical responsible for the activity.

Keywords; Malaria, Ethyl acetate, Curative, Phyllanthus

INTRODUCTION

Malaria parasites have killed and sickened more people than any other eukaryotic illness (Chinsembu, 2015). Malaria caused around 411 000 deaths and 229 million infections worldwide in 2019, with children under the age of five continuing to be the most vulnerable population. (WHO, 2020). Despite the fact that malaria is curable, it continues to have a devastating impact on mankind. The poor and underprivileged are disproportionately afflicted by the disease since they have inadequate access to health care and cannot pay for the prescribed treatment in most nations. (WHO, 2020).

Artemisinin combination therapy has significantly aided in the reduction of malaria mortality. However, treatment failures and parasite resistance, as well as the fact that current antimalarial drugs are costly in disadvantaged areas, underscore the need for safe and affordable antimalarial therapies (Fairhurst & Dondorp, 2016).

Natural products play an important role in the treatment of malaria, as a source of lead compounds for the creation of new and effective antimalarial medications. (Boyom et al, 2011). Plant extractions are still used in Nigerian daily life to combat malaria and a range of other ailments, especially in areas where Western medications are unavailable.

The extensive and diverse flora of Nigeria is a potential source of powerful antiplasmodial natural compounds, and ethnopharmacological techniques to their study might be useful in the fight against malaria. In the exploration for new natural plant derived antimalaria, *invitro* and *invivo* antimalaria activity testing are required at the early stages.

Phyllanthus niruri is also known as kidney stone crusher (Micali et al., 2006). *P. niruri* is used to treat problems related to the gastrointestinal tracts (Karuna, Reddy, Baskar, & Saralakumari, 2009). *P. niruri* was also reported to display anticarcinogenic (Rajeshkumar et al., 2002), hepatoprotective (Amin, Alshawsh, Kassim, Ali, & Abdulla, 2013), and anti-inflammatory (Obidike, Salawu, Ndukuba, Okoli, & Osunkwo, 2010). *In vivo* and *in vitro* research have demonstrated that this herbal extract has antidiabetic (Okoli, Obidike, Ezike, Akah, & Salawu, 2011) and antioxidant effects (Colpo et al., 2014). In a bit to identify the active component of *P. niruri*, we investigated the effects of the ethyl acetate fraction of *P. niruri* plant extract on *Plasmodium berghei*-infected mice.

MATERIALS AND METHOD

Plant Collection and Identification

Phyllanthus niruri whole plant were collected from Bosso, Nigeria and was identified by a botanist at the Department of Biological Sciences, F. U. T. Minna.

Experimental Animals

For this investigation, 18 albino mice weighing 24-30g purchased from the National Institute for Pharmaceutical Research and Development Abuja, Nigeria. They were fed a conventional meal and given water, and they were kept in standard circumstances.

Extract Preparation

The plant was thoroughly washed, dried, and ground into powder, and was soaked in 80% methanol of 24hours. This was filtered through Whatman filter paper grade 1. The filtrate was evaporated with a rotary evaporator and concentrated using a water bath. The crude extract was placed into a separating funnel, and dissolved in 50ml of distilled water. In order of polarity, n-hexane, chloroform, and Ethyl Acetate were used to partition the methanol extract. The Ethyl Acetate fraction was collected and used for this investigation.

Parasite Inoculation

Plasmodium berghei was received from the National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria, where it was kept alive in mice for a week. On day 0, each mouse was given 0.2ml of contaminated blood containing *P. berghei* parasitised red blood cells intraperitoneally. In addition, the freshly injected animals were checked on a daily basis for parasitemia.

Evaluation of the Curative Effect

Six groups of three mice were formed from the eighteen animals. To collect the blood, a mouse infected with *P. berghei* was anesthetised with chloroform and slaughtered. The blood was diluted with normal saline to provide a volume of 0.2ml. Each of the eighteen mice was given 0.2ml diluted blood intraperitoneally. The extract was given orally once daily for six days after 72 hours of inoculation at dosage levels of 50, 100, 200, and 400mg/kgbw, respectively (D0, D1, D2, D3, D4 and D5). In the fifth group, a similar test with Chloroquine (5 mg/kgbw) acts as a positive control. The sixth group was not given any treatment and functioned as a control group. Thin films were produced from D0-D5 tail blood, treated with methanol, and stained for 20 minutes with 4 percent Giemsa (PH 7.2) before being studied under the microscope. On each slide, five fields were inspected, and the number of infected red blood cells (RBC) was counted and the mean was calculated. (Kabiru et al., 2013).

$$\%\text{Parasitemia Inhibition} = \frac{A-B}{A} \times 100$$

A= Basal Parasitemia count,

B = Day 5 Parasitemia count

Estimation of Packed Cell volume.

By capillary action, blood was collected from each mouse's tail with a capillary tube. One end of the tube was plastestrine-sealed. After that, the closed capillary tubes were placed on the hematocrit centrifuge and spun for five minutes at 1200 rev per min. The PCV was measured and recorded using a hematocrit reader.

Data Analysis

The data was analysed using Graph Pad Prism6. Data are presented as mean ± SD, One-way Anova were carried out to determine significant difference in parasitemia count across groups.

RESULT AND DISCUSSION

Table 1: Curative test result of ethyl acetate fraction of *P. niruri*.

Groups	Treatment	Basal Parasitemia	DAY5 Parasitemia	% Parasitemia inhibition
1	50 mg/kg PnEtF	12.6 ±2.27	1.4 ±0.53*	88.88
2	100 mg/kg PnEtF	11.5 ±1.60	1.7 ±1.13*	85.23
3	200 mg/kg PnEtF	12.3 ±2.31	2.5 ±0.50*	79.68
4	400 mg/kg PnEtF	12.6 ±3.86	2.2 ±0.28*	82.54
5	5 mg/kg Chq	13.5 ±0.83	1.3 ±1.33*	90.37
6	NC	11.3 ±1.17	12.3 ±0.31	-8.85

* There is significant difference at P < 0.05 Compared to Negative Control group

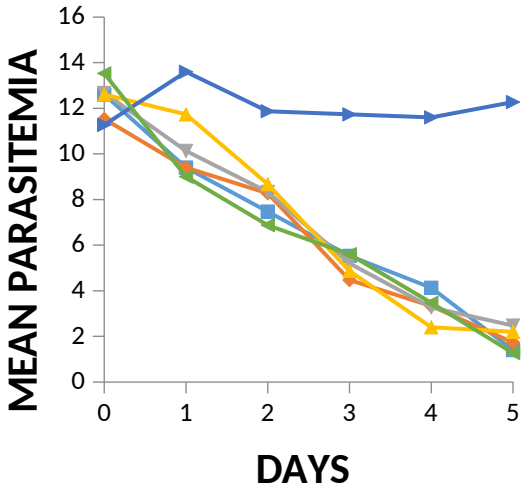

Figure 1: Effect of ethyl acetate fraction of *P.niruri* on the parasitemia of infected mice.

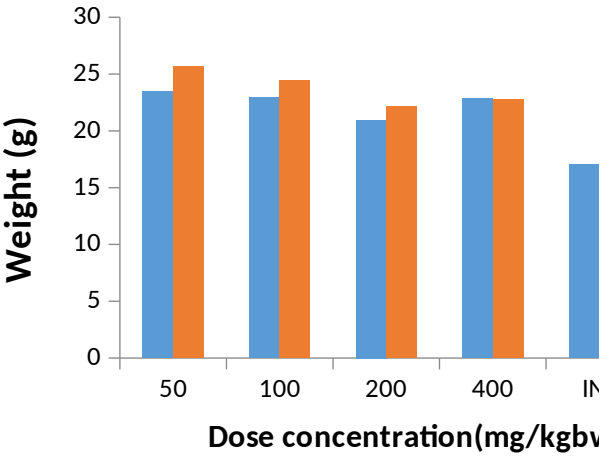

Figure 2: Effect of ethyl acetate fraction of *P.niruri* on the weight.

Data Presented as Mean ± SD, n=3

* There is significant difference at P < 0.05 Between Day 0 and Day 5

Int(Infected not treated), Chq(Chloroquine)

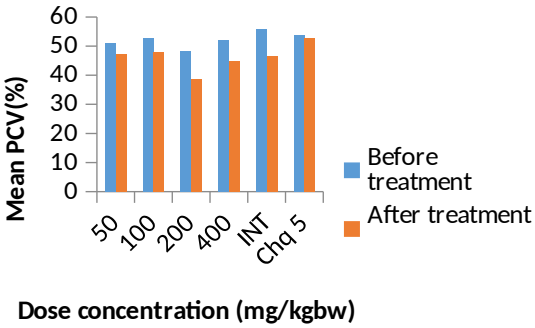

Figure 3: Effect of ethyl acetate fraction of *P.niruri* on Packed cell volume.

Data Presented as Mean ± SD, n=3

* There is significant difference at P < 0.05 Between Day 0 and Day 5

Int(Infected not treated), Chq(Chloroquine).

DISCUSSION

The phytochemicals found in the crude extract of *P. niruri* in a literature study indicate that it has pharmacological activities (Kabiru et al.,2013). The mice infected with *plasmodium burgei* and dosed with the ethyl acetate fraction from the result in Fig 1 showed a strong curative effect when compared with the positive control group treated with chloroquine.

The effects of ethyl acetate extracts on percent PCV (percentage Packed Cell Volume) after therapy was found to decrease though not statistically significant, these corroborates the findings of (Kabiru et al., 2013 and Nardos and Makonnen, 2017). This is a common occurrence owing to the extract's action on infected blood cells, particularly red blood cells (RBCs). Days following therapy, the cells continue to progressively divide, and this situation is generally transient.

The impact of the ethyl acetate extracts on weight was also considered, and it was discovered that, as shown in figure 3, there was weight gain after treatment, and progressively increased days following treatment. The Initial weight loss might be due to the disease's (malaria) signs and symptoms, which include weight loss. The plant extract however during and after treatment improved on the weight.

CONCLUSION

The ethyl acetate fraction of *P. niruri* has a curative effect on *Plasmodium burgei* and can be utilized to

treat malaria after further purification to isolate the active compound responsible for the activity.

It is also recommended that the ethyl acetate fraction be subjected to further fractionation, such as vacuum liquid chromatography and column chromatography, in order to better exploit the active components in the fraction, increase yields, and serve as templates for the development of therapeutically more effective antimalarial drugs.

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