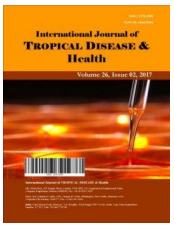

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Prophylactic and Curative Potency of *Alchornea laxiflora* Extract on *Plasmodium berghei* Infected Swiss Albino Mice

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Authors' contributions

This work was carried out in collaboration between both authors. Author OFO designed and supervised the study proofread the first draft of the manuscript and literature search. Author OAB did the literature search, carryout the laboratory analysis, source for all materials used and draft the first manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria is one of the most important infectious diseases in Nigeria and in Africa at large as everyone is at risk of the infection.
Objectives: This study was carried out to evaluate the antiplasmodial activity of *Alchornea laxiflora* leaf extracts against *Plasmodium berghei* infected mice.
Materials and Methods: *In vivo* antimalarial assay on chloroquine-sensitive *P. berghei*-infected mice was carried out by oral administration of graded doses (200 mg/kg, 400 mg/kg and 600 mg/kg) of methanolic and chloroform extracts using chloroquine and distilled water as positive and negative control respectively. Prophylactic potential in residual infection and curative assay against established infection were tested in *P. berghei*-infected mice. The assay was performed using 4-day suppressive standard test.
Results: The prophylactic efficacy of methanolic and chloroform leaf extacts showed percentage chemosuppression of 72.37% and 66.32% respectively at oral dose of 600 mg/kg. The methanol leaf extract of *A. laxiflora* displayed the highest curative activity of percentage chemosuppression of 98.36% at oral dose of 600 mg/kg. The extracts displayed dose-dependent significant ($p \leq 0.05$)

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antiplasmodial activity as compared to the control. Haematological analysis revealed an increase in packed cell volume, red blood cell, haemoglobin and white blood cell counts on dose-dependent manner in the treated mice compared to the negative control mice.
Conclusion: The high suppressive values obtained in this study show that the tested leaf extracts of *Alchornea laxiflora* might be a good alternative drug for the treatment of malaria infection in Nigeria.

Keywords: Antimalarial drugs; Alchornea laxiflora leaf extracts; prophylactic; curative.

1. INTRODUCTION

Despite the considerable progress made in the treatment of parasitic diseases, malaria remains a significant therapeutic challenge in the endemic area. There have been many control measures to combat the disease, although having shown some effectiveness, still have challenges of resistance to almost all conventional antimalarial drugs [1,2]. This condition constitutes a threat to malaria eradication among other solutions readily available therefore; there is a need for the search of new varieties of molecules derived from natural substances or sources through the study of traditional therapies [1].

Plants have been used for many years to treat diseases in local communities. The use of medicinal plants to treat illness can be traced back over five millennia [3]. Many plants, especially those used by traditional healers have numerous pharmacological activities. About, 85% of world population uses traditional medicine for primary health care and the demand is increasing in developed and developing countries [4,5].

In malaria endemic regions of Africa especially Nigeria, inaccessibility of health centers, the inequitable distribution of medical staff, socio-cultural attitudes and high cost of new antimalarial drugs lead people to use traditional medicines for their primary health care [6]. The usage of plants as antimalarial drugs is relative to its affordability, easier access regardless of socioeconomic status, safer level due to their long usage in the treatment of diseases according to knowledge accumulated over centuries and sometimes their better therapeutic value than synthetic drugs [7].

The inadequacy of new antimalarial drugs in circulation and it reduced *Plasmodium* sensitivity call for the urgent need to search for new plant-derived bioactive agents that can be explored to replace the currently used antimalarial or to broader their antimalarial drug [7]. The indisputable potentials of ethnomedicinal plants form the basic foundation of traditional medical practice worldwide; even, the World Health Organization (WHO) encouraged the inclusion of herbal medicine of proven safety and efficacy in health care delivery programmes in developing countries [5]. Plants have been considered invariably the sources of potential targets for research and development of alternative malarial drugs, with a safer mechanism of action (long usage over ages) as many pharmacological classes of drugs available today contain a natural product prototype [3,7].

All over the world, people have old tradition in the use of herbs for the treatment of several diseases, including malaria. *Alchornea laxiflora* (Benth) 'Ewe Iya, Pepe in Yoruba' leaves belongs to *Euphorbiaceae* family, it grows naturally in South-West Nigeria and are mostly used for packaging and preservation of kola nuts in Nigeria. The decoction of the leaves is taken to treat inflammatory and infectious diseases as reported by Osuntokun and Olajubu [8], and it is also a common ingredient in herbal antimalarial preparations [9].

In most parts of Nigeria, plant extracts are still being used in their crude forms to treat malaria and in most cases, therapeutic effects and other purported benefits derived from the use of these extracts are yet to be scientifically validated [10]. Therefore, search for new antimalarial drugs through the evaluation and validation of traditional medicines offers a good opportunity for the discovery and development of better medicines. This study therefore provide a valid scientific proof for the *in vivo* prophylactic and curative efficacy of crude extracts of *Alchornea laxiflora* on *Plasmodium berghei* infected Swiss albino mice.

2. MATERIALS AND METHODS

This study was carried out at the Department of Microbiology, Federal University of Technology, Akure between November, 2015 and August, 2016.

2.1 Collection of Plant Leaf

The fresh leaves of plant were collected from their natural habitats in Eriti Akoko, Akoko North West Local Government Area Ondo State, Nigeria during the day time and authenticated by the expert in the Department of Crop, Soil and Pest Management, School of Agricultural Technology, Federal University of Technology, Akure, Ondo State, Nigeria as *Alchornea laxiflora.*

2.2 Extraction of Plant

Extraction of plant material was performed by soxhlet apparatus (Sigma-Aldrich, USA) using two solvents; 95% chloroform and methanol. Using the method of Wabo et al. [11] modified. A dry weight of 200 g of powdered plant leaf sample was macerated in the 1.0 liter of solvents to extract the bioactive components of plants. The mixture was daily stirred for 72 hours. The resultant mixtures were filtered with muslin cloth followed by No. 1. Whatman filter paper of pore size 2.5µm. Solvents were removed from the filtrate under reduced pressure in a rotary evaporator (R110) at 40°C to obtained the extracts.

2.3 Phytochemical Analysis for the Plant Extracts

The chloroform and methanol extracts of leave were subjected to qualitative and quantitative phytochemical screening according to the method of Trease and Evans[12], to detect the presence or absence of plant secondary metabolites such as; saponins, tannins, alkaloids, flavonoids, steroids, anthraquinones, and cardiac glycosides according to the method of Trease and Evans [12].

2.4 Acclimatization of Experimental Mice

Healthy adult Swiss albino mice (both sexes) weighing 18 – 30 g at eight weeks were procured from the Animal facility of Afe Babalola University Ado Ekiti, Nigeria. The mice were housed in clean dry cages and fed with growers mash. They were acclimatized for seven (7) days with free access to food and water *ad libitum*. The mice were maintained and cared for according to the international guidelines for the use and maintenance of experimental animals [13].

2.5 Preparation of Inoculum

Donor chloroquine-sensitive *Plasmodium berghei* infected mice were subjected to chloroform in a container and the blood was collected by heart puncture. Heparinized blood was taken from a donor mouse with approximately 30% parasitemia. The blood was diluted with 5 ml of phosphate buffer solution (PBS) at pH 7.2 so that each 0.2 ml contained approximately 1×10^7 infected red cells [14]. An aliquot of 0.2 ml ($2x10^7$ parasitized erythrocytes) of this suspension was injected intraperitoneally into experimental mice. Each animal received inoculums of about 10 million parasites per kilogram body weight, which is expected to produce a steadily rising infection in mice.

2.6 Experimental Design

2.61 *In vivo* prophylactic assay

Prophylactic test was carried out according to Ettebong et al. [15], was used. After 3 days of treating with the extracts through orogastric administration (mg/Kg per body weight), they were infected with *Plasmodium berghei* intraperitoneally, the mice were then examined for another 5 days for parasitemia load and percentage chemo-suppression of the extract in experimental mice. The mice were grouped randomly into 8 of 3 mice per group as follows:

Group A : group of mice treated with 0.2 ml of methanol extract (200 mg/kg) before infection
Group B : group of mice treated with 0.2 ml of methanol extract (400 mg/kg) before infection
Group C : group of mice treated with 0.2 ml of methanol extract (600 mg/kg) before infection
Group D : group of mice treated with 0.2 ml of chloroform extract (200 mg/kg) before infection
Group E : group of mice treated with 0.2 ml of chloroform extract (400 mg/kg) before infection
Group F : group of mice treated with 0.2 ml of chloroform extract (600 mg/kg) before infection
PC : group of mice treated with 0.2ml of chloroquine solution (25 mg/kg) before infection
NC : group of mice treated with 0.2 ml of saline water before infection

2.6.2 *In vivo* curative assay

Rane's test of established infection was adopted in this study with modified method of Rukayyah

et al., [16]. The mice were infected and left for 72 hours before treatment. All the mice were fed once daily and treated with extract for five days. The mice were then examined every day for parasitemia load and percentage chemo-suppression of the extract in experimental mice.

Group A : group of mice treated with 0.2 ml of methanol extract (200 mg/kg) after infection
Group B : group of mice treated with 0.2 ml of methanol extract (400 mg/kg) after infection
Group C : group of mice treated with 0.2 ml of methanol extract (600 mg/kg) after infection
Group D : group of mice treated with 0.2 ml of chloroform extract (200 mg/kg) after infection
Group E : group of mice treated with 0.2 ml of chloroform extract (400 mg/kg) after infection
Group F : group of mice treated with 0.2 ml of chloroform extract (600 mg/kg) after infection
PC : group of mice treated with 0.2ml of chloroquine solution (25 mg/kg) after infection
NC : group of mice treated with 0.2 ml of saline water only after infection

2.7 Parasitaemia Count

During prophylactic and curative assay, a drop of blood was collected from each infected mouse for parasitemia screening by tail nip. The blood collected was placed on a slide and smeared to make a thick film, fixed with methanol and stained with Giemsa stain. When dried, the film was microscopically viewed by adding a drop of immersion oil and viewing it under x100 magnification of the microscope. The parasitemia density was examined by counting the parasitized red blood cell.

Percentage parasitaemia suppression was calculated according to the following formula

$$\%\text{Parasitaemia} = \frac{\text{total number of Parasitized red blood cells}}{\text{Parasitized red blood cells}} X\ 100$$

$$\%\text{chemo suppression} = \frac{(\text{Parasitaemia in control group} - \text{parasitaemia in study group})\ \text{X}\ 100}{\text{Parasitaemia in control group}}$$

2.8 Determination of Body Weight (g) and Temperature (°C) Change

The body weight and temperature of the mice during prophylactic and curative assay were determined as described by Dada and Oloruntola[17]. Rectal temperatures of mice were obtained using a digital thermometer (BIOSEB-BIO9882). The body weight (BW) of each mouse in all groups was measured every day by using a sensitive digital weighing balance and mean BW per group was calculated using the formula:

$$\text{Mean BW} = \frac{\text{Body Weight of mice in a group}}{\text{Total number of mice in that group}}$$

2.9 Haematological Parameters

Blood were collected by anaesthetization method and collected through cardio-vascular puncture into EDTA bottles for haematological analysis into labelled bottles. Haematological tests such as PCV, HB, RBC, ESR and WBC differential count were done according to Cheesbrough [17].

2.10 Statistical Analysis

Statistical analysis of data was done using one way analysis of variance (ANOVA) and Means were compared by Duncan's new multiple range test at 95% confidence level and level of significance was ($p<0.05$) using SPSS version 20.0.

3. RESULTS

3.1 Percent of Yield of the Plant Extract and Phytochemical Screening

The extract yields as shown in Table 1, it was revealed that from 200 grams of *Alchornea laxiflora,* methanol and chloroform used as solvent yielded 15.33 and 11.15 grams extract respectively. In Table 2, qualitative phytochemical screening of the methanolic and chloroform crude extracts revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, cardiac glycosides and phenol. Saponnis, terpernoids, tannins, alkaloids and cardiac glycoside were present in all the extracts. However, quantitative phytochemical screening of the extracts revealed that saponin (13.68 mg/g) was highest follow by phenols (6.38 mg/g) in chloroform extracts Fig. 1.

Table 1. Percentage (%) yield of crude extracts obtained from leaves powders of *Alchornea laxiflora*

Solvents	Weight of plants used (grams)	Yields (grams)	Percentage yield (%)
Methanol	200	15.33	7.67
Chloroform	200	11.15	5.58

Table 2. Qualitative phytochemical screening of crude extracts of *Alchornea laxiflora* leaves

Phytochemical constituents	Methanol	Chloroform	Raw sample
Tannins	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Terpernoids	+	+	+
Cardiac Glycoside	+	+	+
Alkaloids	+	+	+
Phenols	-	+	+
Distilled H_2O	-	-	-

Key: + Shows the presence of photochemical constituent, – shows the absence of phytochemical constituent.

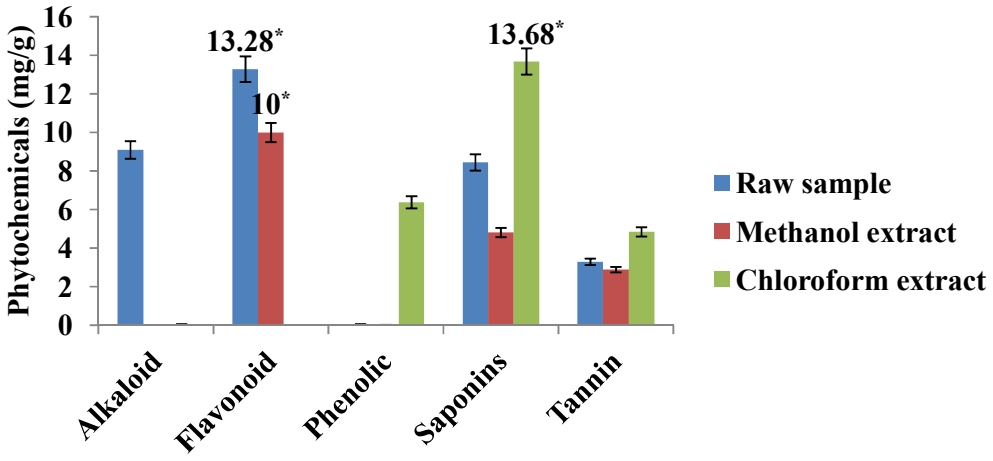

Fig. 1. Quantitative phytochemical screening crude extracts of *Alchornea laxiflora* leaves

3.2 Effects of Extracts on Body Weight of Infected Mice in Prophylactic and Curative Assay

Effects of extracts on body weight of infected mice for prophylactic assay were revealed in Fig. 2. The result showed that there was no significant ($p \geq 0.05$) weight reduction in all the treated groups except the negative control (infected not treated) and the group treated with 200 mg/kg of chloroform extract had reduction in body weight (from 27.33±0.88 to 24.67±0.33 g) while the effects of extracts on body weight for curative assay were shown in Fig. 3. It was observed that only the negative control (infected not treated) showed a significant ($p \geq 0.05$) reduction in body weight.

3.3 Effects of Extracts on Body Temperature of Infected Mice in Prophylactic and Curative Assay

Effects of extracts on body temperature of infected mice for prophylactic and curative treatment are shown in Figs. 4 and 5 respectively. The result of prophylactic treatment revealed that there was significant ($p \geq 0.05$) reduction in body temperature of negative control group and group treated with 200 mg/kg of chloroform extract at day 5. For curative treatment, the initial reduction of body temperature was observed at day 1 however, for all the treatment group except negative control group, there was significant increase in the body temperature at day 5.

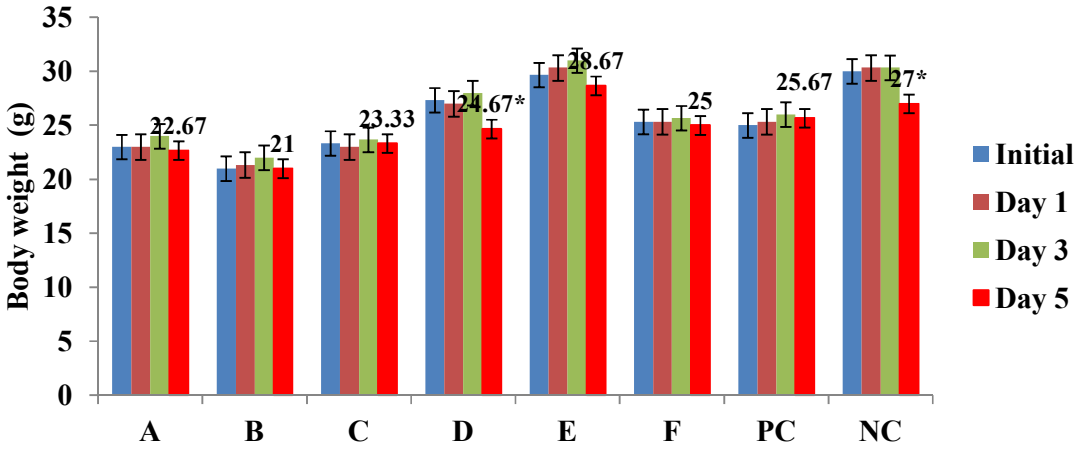

Fig. 2. Effects of extracts on body weight of infected mice for prophylactic assay
Values with (*) showed a significant (p ≥ 0.05) weight reduction

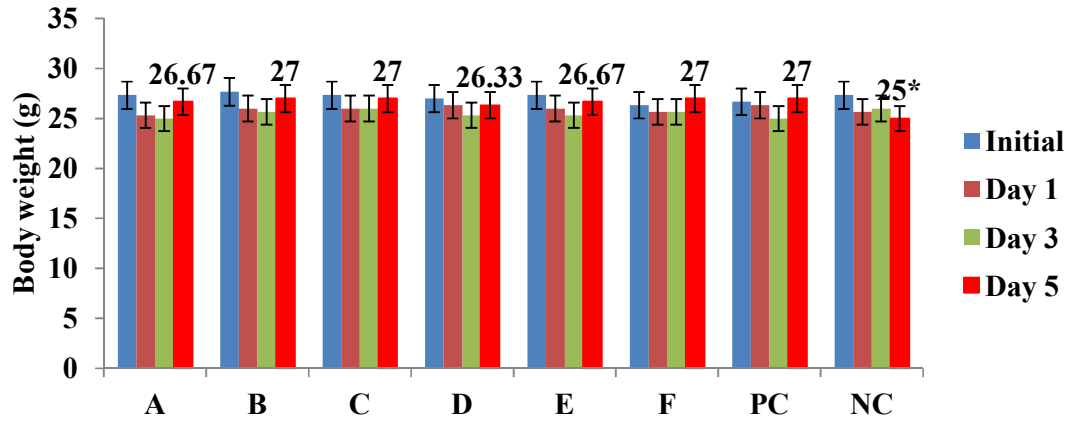

Fig. 3. Effects of extracts on body weight of infected mice for curative assay
Values with (*) showed a significant (p ≥ 0.05) weight reduction

3.4 Chemo-suppressive Efficacy of *A. laxiflora* Leaf Extracts on *P. berghei* Infected Swiss Albino Mice

Percentage chemo-suppression was observed to be dose dependent because there was increase in chemo-suppressive activity of the extracts as extracts concentration increases. The result of prophylactic treatment revealed that methanol extract of showed significant (p ≥ 0.05) 46.61%, 63.89% and 72.37% chemo-suppression at the doses of 200, 400 and 600 mg/kg respectively while the highest chemo-suppression (66.32%) showed by chloroform extract was observed at 600 mg/kg dosage. However the extracts showed higher chemo-suppression at curative treatment, 600 mg/kg of methanol and chloroform extracts showed 98.38% and 94.16% chemo-suppression respectively (Fig. 6).

3.5 Effects of *A. laxiflora* Leave Extracts on Haematological Parameters of *P. berghei* Infected Swiss Albino Mice

The prophylactic result as revealed in Fig. 7 showed that the values of heamatological parameters at concentration of 200 and 400 mg/kg chloroform extracts were higher than methanol extract at respective concentrations. However, at 600 mg/kg concentration, heamatological parameters of group treated with methanol extract were significantly higher (p ≥ 0.05). In curative assay shown in Fig. 8, PCV values were generally higher than those observed in prophylactic treatment.

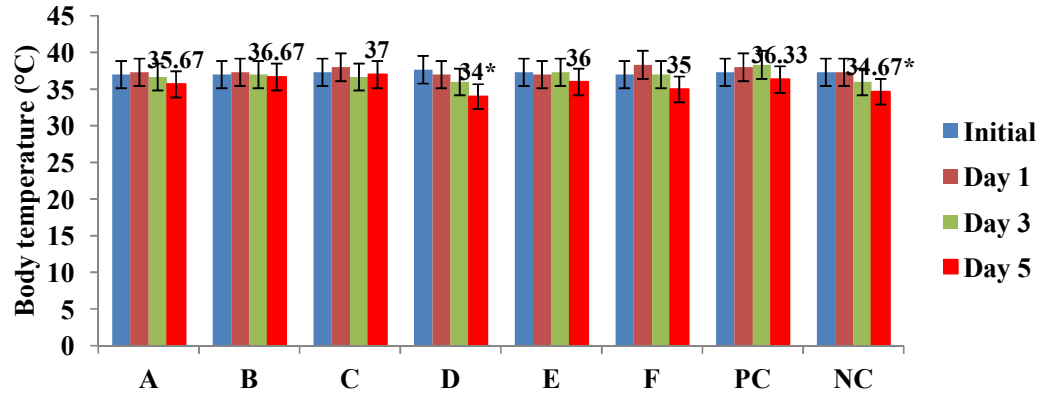

Fig. 4. Effects of extracts on body temperature of infected mice for prophylactic assay
Values with (*) showed a significant (p ≥ 0.05) weight reduction

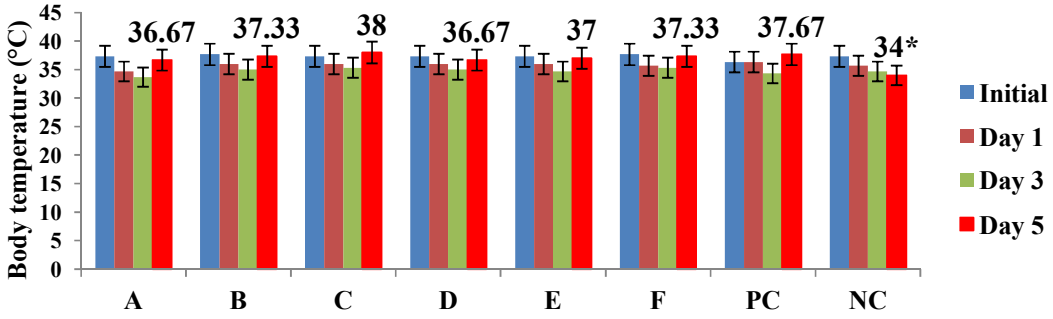

Fig. 5. Effects of extracts on body temperature of infected mice for curative assay
Values with (*) showed a significant (p ≥ 0.05) weight reduction

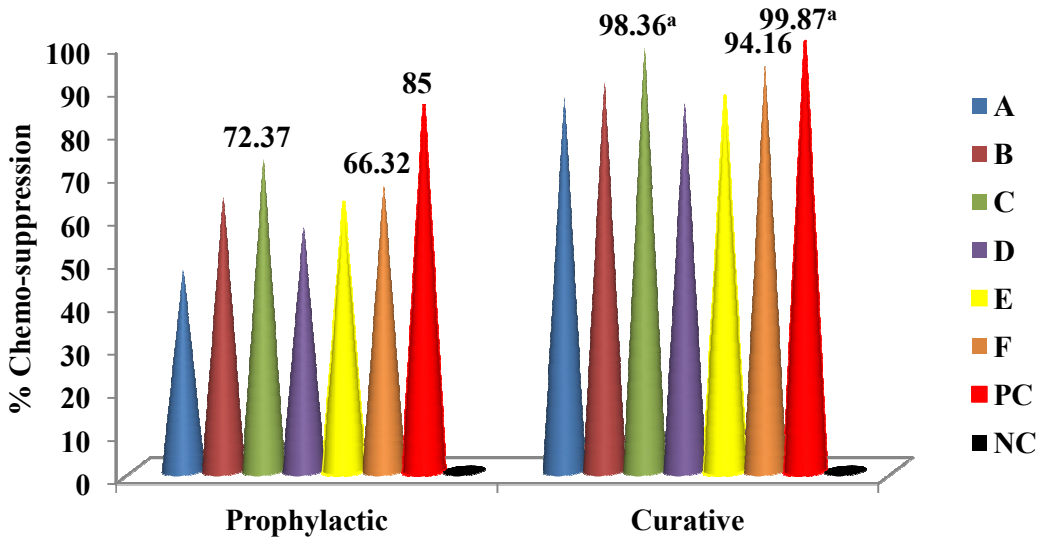

Fig. 6. Chemo-suppressive efficacy of *A. laxiflora* leaf extracts on *P. berghei* infected Swiss albino mice
There is no significant (p ≥ 0.05) difference between the values with same superscript.

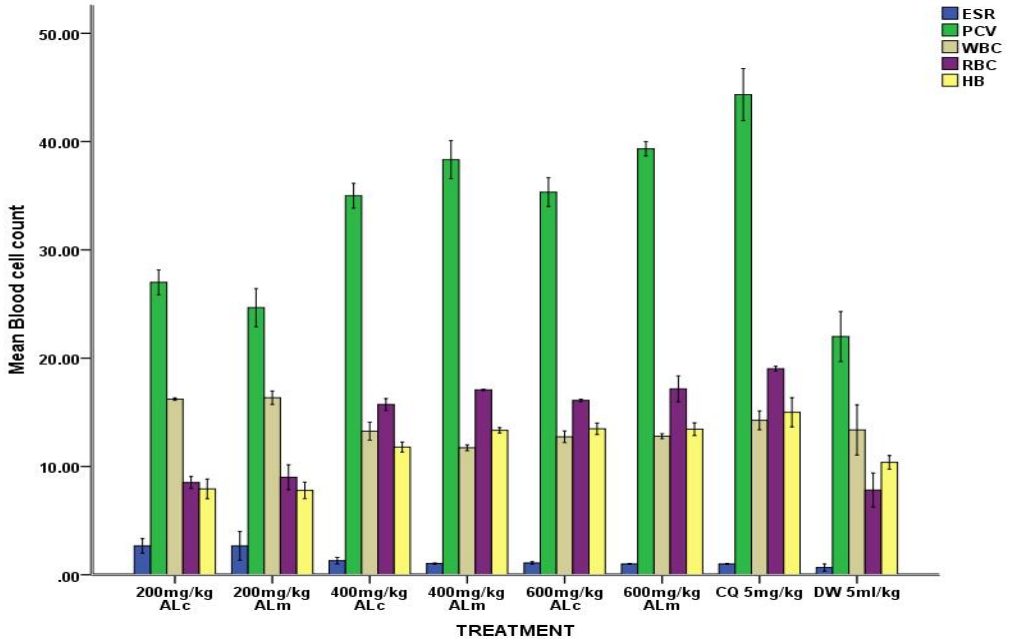

Fig. 7. Effects of *A. laxiflora* leaves extracts on haematological parameters of mice in prophylactic assay

Keys: ALc = Chloroform extracts of A. laxiflora; ALm = Methanol extracts of A. laxiflora; CQ = Chloroquine; DW = Distilled water; ESR = Erythrocyte Sedimentation Rate; PCV = Packed cell volume; Hb = Haemoglobin concentration; RBC = Red Blood Cell; WBC = White Blood Cell.

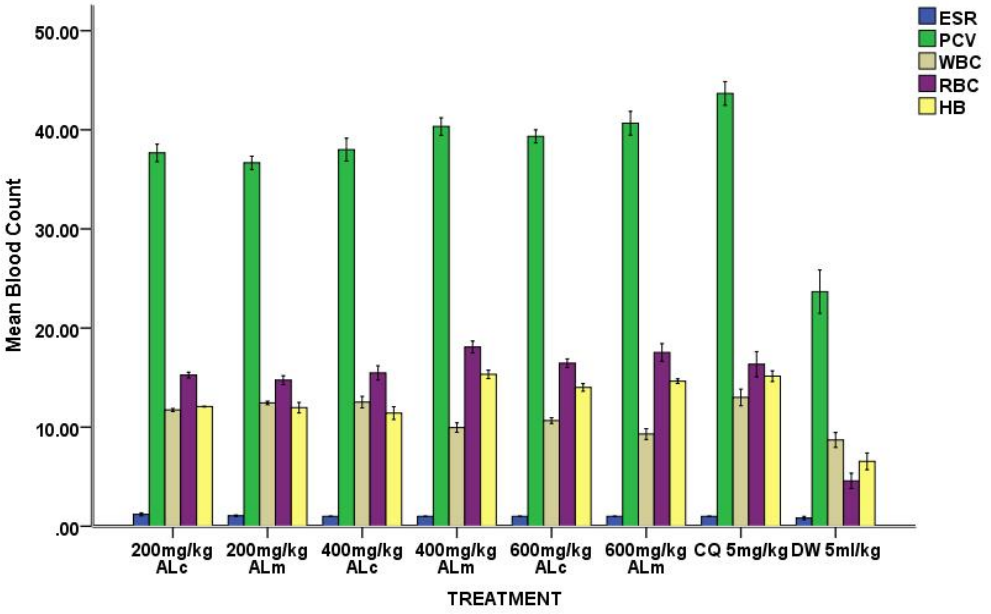

Fig. 8. Effects of *A. laxiflora* leaves extracts on haematological parameters of mice in curative model

ALc = Chloroform extracts of A. laxiflora; ALm = Methanol extracts of A. laxiflora; CQ = Chloroquine; DW = Distilled water; ESR = Erythrocyte Sedimentation Rate; PCV = Packed cell volume; Hb = Haemoglobin concentration; RBC = Red Blood Cell; WBC = White Blood Cell.

4. DISCUSSION

The high prevalence of malaria and resistance developed by *Plasmodium* and it vector (mosquito) posed a challenge to the disease control and has necessitated the hunt for new and effective therapeutic options [18]. The variations in the yield of the methanolic and chloroform extracts is due to affinity of the chemical composition likewise, variation might be due to high concentration of less polar compounds in the leaves species, which are capable of dissolving in relatively less polar solvents. Compounds have been found to be more soluble in polar solvent such as methanol when compared with chloroform which is more of non-polar solvent [19].

The qualitative and quantitative phytochemicals extracted from the plant differ and this could be depending on the solvent used for the extraction. Some reports show that methanol extracts more number and types of compounds in plant materials than other extraction solvents such as acetone, chloroform, ether, water and ethanol[4]. Antimalarial and therapeutic properties of medicinal plants have been attributed to the presence of different classes of secondary metabolites or phytochemicals compounds contained in them. Phytochemicals such as alkaloids, terpenes, saponins, flavonoids, cardiac glycoside among others have been reported to exhibit antiplasmodial activity through various mechanisms of actions [20,19].

The prophylactic and curative model showed no significant ($p \geq 0.05$) loss of body weight except groups treated with 200 mg/kg for prophylactic assay. The loss of body weight in this group might be due to decrease in appetite and reduced suppressive ability of the crude extract on infected mice since it was given before infection this scenario has previously reported by Bantie et al. [21].

The body temperature ideally decreases as the parasite level escalates. Bioactive compounds are known to prevent the rapid dropping of the body temperature, the extracts at all doses except 200 mg/kg at prophylactic treatment have a protective effect against temperature reduction in a dose-dependent manner, prevention of body temperature showed by this extract could be attributed to the effect of plant extract as it may have less amount of hypothermic effect on the extracts treated mice [1].

Methanol extracts of *A. laxiflora* showed higher chemo-suppression than chloroform extract. The fact that methanol extracts showed the highest chemo-suppressive effect when compared with others suggests that the pharmacological active ingredients of these plants responsible for its antimalarial activities may be localized in the extract. Chloroquine treatment group showed highest chemo-suppression in the infected mice based on the fact that the parasite is sensitive to chloroquine and it has been purified compared with the crude extracts that were used [19]. This current study showed an increment chemo-suppressive effect than the previous reports, which might be as a result of solvent used by the present study being a good extractive solvent and plant environment has been proven to contribute to the chemotherapeutic efficacy [19,22].

The white blood cell (WBC) counts increased significantly ($p \geq 0.05$) and the increase in the WBC can be attributed to the role it plays during infection. Also, at high dose, extract showed higher PCV and this corroborates the report on *V. amygdalina,* which indicated that the mice treated with the highest dose of the extract (600mg/kg) and the group administered standard drug (chloroquine) showed a high PCV on the fifth day [23]. This might be due to the fact that extracts at high dose can reverse the manifestation of the low PCV that occurred during the early stage of malaria infection of the mice as the treatment continues [24]. The significant decrease in the PCV of the normal control can be an indication of anaemic condition which may be attributed to the malaria infection [24].

5. CONCLUSION

The antiplasmodial action of the plant may be attributed to the presence of active secondary metabolites in their leaf and this affirms their use in ethno medicine in the treatment of malaria in Nigeria local communities. The methanol extracts showed a relative higher activity over the chloroform extracts. *A. laxiflora* extracts are relatively safe as they help in maintaining the blood volume during the infection period. The plant also demonstrated a better antiplasmodial efficacy when used as curative treatment.

This study discovered that extract of *A. laxiflora* can be beneficial for the treatment of malaria and could be best used for curative treatment. This study will help the researchers to uncover the

specific bioactive component of the extract that exerted the antiplasmodial efficacy and the developmental stage of parasite that was targeted by the extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All assays were in accordance with the method of Association for the Study of Animal Behavior [25].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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