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Research Article

In-Vivo Antimalarial Activity of the Aqueous Leaf Extract of *Phyllanthus amarus* Schum & Thonn. against *Plasmodium berghei* Infected Mice

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Abstract: The in-vivo antimalarial activity of the aqueous leaf extract of *Phyllanthus amarus* against quinine-sensitive strain of Plasmodium berghei-infected mice was investigated. The twenty-two (22) albino mice used were divided into six groups. Three groups were treated with different doses of *Phyllanthus amarus* extracts (100, 250 and 500mg/kg), another group was treated with the standard drug quinine (10mg/kg), the other groups were not treated. The extract, at all concentrations, produced considerable antiplasmodial activity by reducing the level of parasitaemia which was comparable to that the standard drug quinine, in a 4 day suppressive test. In an 8th day suppressive test, the 250mg/kg leaf extract had the highest curative activity (85%) as comparable to the standard drug quinine (81%). The mean survival time ranged from 11 to 15 days. The antimalarial effect of the aqueous extract of *Phyllanthus amarus* and its influence on hematological profiles in treated and untreated mice were assessed. The untreated group of mice infected with the quinine-sensitive strain of Plasmodium berghei recorded a significant ($P< 0.05$) reduction in PCV, RBC, MCV and neutrophils observably from day 4-8 post-infection while WBC and Platelets counts increased significantly ($p<0.05$) from day 4-8 post-infection. Administration of the aqueous extract of *Phyllanthus amarus* at different doses 100, 250, 500 mg/kg respectively to mice infected with *Plasmodium berghei* resulted in the normalization of hematological indices in the groups of mice infected and treated with the plant extract when compared with the data obtained for the experimental control groups of mice. Although, it was observed that the MCV and MCHC values did not vary significantly in the treated groups when compared with experiment control groups. Treatment with the 250 and 500mg/kg of the extract caused the serum AST, ALT and ALP levels of the mice to be within the normal range as comparable to mice treated with quinine. Phytochemical screening revealed the presence of saponin, carbohydrate, reducing sugar, anthraquinone, flavanoid, tannins, steroids, alkaloids, terpenoid and cardiac glycosides. These results consider the plant to possess antimalarial activity against malaria infection in mice.The immunostimulant function of the plant was also revealed in this study. The phytochemical constituents may further be analyzed to ascertain those with high antiplasmodial activity which may be exploited for use against human *Plasmodium* sp.

Keywords: *In vivo*; *Plasmodium berghei*; Aqueous extract, *Pyllanthus amarus*; Phytochemicals; Antimalarial

INTRODUCTION

Malaria has been a global threat to man, with Sub Saharan Africa, most endangered. According to the latest World malaria report (WHO, 2018), there were 228 million cases of malaria in 2018 compared to 231 million cases in 2017. The estimated number of malaria deaths stood at 405 000 in 2018, compared with 416 000 deaths in 2017.The WHO African Region continues to carry a disproportionately high share of the global malaria burden. In 2018, the region was home to 93% of malaria cases and 94% of malaria deaths. In 2018, six countries accounted for more than half of all malaria cases worldwide: Nigeria (25%), the Democratic Republic of the Congo (12%), Uganda (5%), and Côte d'Ivoire, Mozambique and Niger (4% each).

Children under 5 years of age are the most vulnerable group affected by malaria; in 2018, they accounted for 67% (272 000) of all malaria deaths worldwide. Malaria is a parasitic disease transmitted by the bites of female Anopheles mosquitoes infected with Plasmodium falciparum(most fatal), *P. vivax, P. malariae, P. ovale and P.knowlesi*. The disease primarily affects poor populations in tropical and sub-

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tropical areas especially those living in wood and mud houses as reported by Onyemaechi and Malan (2020).

Malaria due to P. falciparum is the leading cause of morbidity and mortality in Sub-Saharan Africa, especially in children under the age of five years, where a child dies every 30 seconds (WHO 2013).It has been recommended that to stem the tides of malaria related deaths among pregnant women and children aged under 5 years, key health stakeholders and authorities need to implement strategies and direct resources to improve the knowledge of mothers on malaria prevention and control (Oladimeji *et al.,* 2019).

Phyllanthus amarus is of the family Euphorbiaceae, it is commonly called "carry me seed", "stone breaker", "hurricane wind" or "gulf flower" (Bharatiya, 1992). In Nigeria, it is called Eyinolobe in Yoruba, Ubakofe in Igbo, Obukoiyeke in Urhobo in Delta State and Geeron-tsuntsayee in Hausa. Studies done on the efficicacy of herbal remedies used by herbalists in Oyo State Nigeria for the treatment of malaria infection have shown that majority of the rural dwellers depend on herbal remedies and *Phyllanthus amarus* was found to be the major component in the efficacious remedies. (Ajayeioba *et al*., 2004). In South-Eastern Nigeria, the roots are usually soaked in gin (alcoholic spirit distilled from grain or malt) and taken for the treatment of malaria (Chukwujekwu et al., 2005). It has been reported that Phenylpropanoids constitute the most prevalent class of compounds in the genus Phyllanthus (Nisar *et al*, 2018). A number of compounds have been identified in *P. amarus*, these include 5-demethoxy-niranthin, niranthin, phyllanthin, filtetralin, 5-demethoxy-nirtetralin, nirtetralin, hipophyllanthin, cinnamic acid, phenylalanine (Muthusamy *et al.,* 2016; Pereira *et al*, 2017, Pereira *et al*, 2016). However, studies on the toxicity of *Phyllanthus amarus* are scarce but available evidence shows no significant toxic effect for the dried water extract LD_{50}>5000mg/kg (Olabiyi*et al*., 2013).

In spite of various control strategies of malaria chemotherapy, the world over is confronted with the challenges of the emergence and spread of malaria parasite which are resistant to available drugs (Laufer, 2009). This makes the search for new anti-malaria drugs imperative. The aims of this study are to: demonstrate the *in vivo* antimalarial activity of the aqueous leaf extract of *Phyllanthus amarus* against *Plasmodium berghei*- infected mice; carry out the malaria parasite count in mice infected with *Plasmodium berghei*; determine the heamatological analysis on infected and uninfected mice used in the study; phytochemical analysis of *Phyllanthus amarus* aqueous leaf extract; evaluate the curative activity of the aqueous leaf extract of *Phyllanthus amarus*; and evaluate the effect of malaria parasite on the liver of Swiss albino mice.

MATERIALS AND METHODS

Equipment, Chemicals and Reagents

Syringes (2ml,5ml), Cotton wool, EDTA container, Hand gloves, Mice cages, feeding and water troughs, Universal container, Heparin tubes, Binocular Microscope, Microscope slides, test tube racks, test tubes, Giemsa stain (Sigma), Methylated spirit, electric blender, Rotary evaporator, Distilled water, Dissecting kit and board, Weighing balance, refrigerator, Cuvette, Capillary tube, PCV Reader, spectrophotometer.

Plant Preparation and Extraction

Fresh leaves of *Phyllanthus amarus* were collected from a natural habitat in Abraka, Ethiope East Local Government Area of Delta State, Nigeria. The plant was identified and authenticated by the Staff of Botanical Garden of University of Nigeria Nsukka. The leaves were rinsed, air-dried and ground to powder with an electric blender .The powder (100g) was dissolved in 500ml of distilled water (1:5) for 48-72 hours. Heating was done for 20mins as described by Patel *et al* (2011), followed by filtration of extract with a sterile handkerchief. The extract was further filtered with a sterile filter paper. A soxhlet extractor was used to concentrate and evaporate the filtrate to dryness in a hot water bath. The percentage yield of 3.8%, weighing 25g was placed in an air tight container and stored in a refrigerator. The dried extract was dissolved in distilled water.

Phytochemical Analysis

The phytochemical screening of *Phyllanthus amarus* was carried out to detect the presence of the following secondary metabolites; Alkaloids. flavanoids, tannins, saponins, reducing sugars, anthraquinones, steroids, terpenoid, cardiac glycosides and carbohydrate using standard procedures as described by Ukana *et al*., (2013).

Preparation of Experimental Mice and Inoculation of *Plasmodium berghei*

Breeding of mice

Twenty-two albino mice of mixed sexes (15 females and 7 males), weighing between 22g to 27g and free from infection were obtained from the Laboratory Animal House, Facility of the Department of Pharmacology, Faculty of Basic Medical Sciences, Delta State University Abraka, Nigeria. They were acclimatized for 7 days and maintained in a well-ventilated room, with temperature of 28˚C±1 and fed on Top feed growers mash and sterile water *ad libitum* for the entire duration of the study. Good hygiene was maintained by constant cleaning of cages, replacement of their beddings and disinfecting the floor where the cages were placed (Aguiyi *et al*, 2002).

Parasite inoculation

The mice were infected by obtaining parasitized blood (3-4 drops) by pricking the tail of an

infected (donor) mouse. Then, 0.1ml of infected blood was diluted in 0.9ml of normal saline, and eighteen (18) mice were inoculated intraperitoneally, each with 0.1ml of the parasitized suspension which contained about hundred thousand (10^5) parasitized erythrocytes (Peters, 1965)

Evaluation of Curative Activities of Aqueous Leaf Extract of *Phyllanthus amarus* (Rane test) on Established Infection.

A modified method similar to Ryles and Peters was used. On the first day (day 0), standard inoculum of $1x10^3$ *Plasmodium berghei berghei* infected erythrocytes was injected intraperitoneally into the mice. Seventy-two hours later, the mice were divided into 6 groups. Groups 1, 2 and 3 were administered 100, 250 and 500mg/kg of *Phyllanthus amarus* leaf extracts respectively. Group 4 was given quinine drug while group 5 (positive control) was given sterile water and group 6 was the negative control. The quinine drug was given once daily for 3 days while the extracts were given once daily for 7 days. Thin smears stained with Giemsa stain were prepared from the blood collected from the cut tip of each mouse's tail to monitor the parasitaemia level by viewing under a microscope at x100 magnification. The mean survival time for each group was determined over a period of 15 days (Ryley *et al* 1970;National Institute of Health 1985).

Animal grouping and treatment

The animals (mice) were caged into six groups to allow free and easy movement and to avoid crowdedness. Group 1 mice were uninfected and untreated (normal control), Group 2 mice were infected with *Plasmodium berghei* and untreated (malaria control), Group 3 mice were infected and treated with quinine (10mg/ml/kg body weight), Group 4 mice were infected and treated with 100mg/ml/kg body weight of *Phyllanthus amarus* leaf extract, Group 5 mice were infected and treated with 250mg/ml/kg body weight of *Phyllanthus amarus* leaf extract and Group 6 mice were infected and treated with 500mg/ml/kg body weight of *Phyllanthus amarus* leaf extract. After 4 days of inoculation, mice in Groups 3, 4, 5 and 6 were administered different doses of *Phyllanthus amarus* aqueous leaf extracts and quinine once a day for a period of 7 days (National Institute of Health, 1985).

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Animal Sacrifice and Collection of Specimen

On the 8th day, the mice were fasted overnight and sacrificed. Whole blood was collected by ocular puncture via the capillary tube and dispensed into EDTA containers for haematological assay, and was centrifuged using bucket centrifuge for 10minutes at the rate of 4000 rpm to obtain serum for the biochemical analysis to determine Liver Function Test (LFT) .

Giemsa staining

Blood was collected by pricking the tails of the mice that were infected and thin blood films were prepared from the collected blood on different slides. The slides were air-dried and stained with 10% giemsa for 20 minutes. The slides were rinsed carefully and thoroughly under running tap water and left to stand in an upright position to dry. Prepared slides were viewed under the x100 objective (oil immersion) light microscope to give a good contrast. The number of infected erythrocytes in the whole area was counted. Malaria parasite counts were done before the commencement of treatment, after the 4^{th} day of treatment and after the 7^{th} day of treatment (Cheesbrough 2006).

Heamatological Analysis

Procedure for Packed Cell Volume (PCV)

Red blood was obtained using capillary tube via the ocular blood collection and then sealed using plasticin. The tubes were then centrifuged (spinned) using haematocrit centrifuge for 5minutes. After centrifuging, the percentage of parked cell volume was then read using haematocrit reader (PCV table reader) (Cheesebrough 2006).

Procedure for determination of White Blood Count (WBC)

Two drops of the whole blood were obtained from the EDTA container with a pasture pipette and dispensed into a plain container. Turk's solution (0.3ml) was added and mixed gently. It was allowed to stay for 3minutes and the Neubauer chamber was flushed with 2-3 drops of the diluted blood, and allowed to settle for 5minutes undisturbed. After 5mins, it was focused with ×40 0bjective lens of the microscope. White blood cells were counted by means of the Neubauer chamber (Cheesebrough 2006).

Procedure for Determination of Differential Cell Count

A drop of blood was made on the edge of a glass slide; it was slide downwards using a cover slide to make a thin film. The blood was allowed to air dry and stained with Leishman stain for 2 minutes and washed with phosphate buffer for 2 minutes. Slides were incubated for 10 – 15 minutes at 37^0C, to stain blood cells. Slides were rinsed with phosphate buffer for 2 minutes and air-dried in a tilted position for a few hours. Slides were observed under oil immersion objective lens of the microscope. The following cells were counted (neutrophils, monocytes, lymphocytes, eosinophil, basophils) (Cheesebrough 2006).

Procedure for determination of Liver Function Test (LFT)

Blood was collected from the mice by the ocular puncture into a capillary tube, which was then centrifuged for 10munites to obtain the serum needed for the test (LFT). Alkaline phosphate (0.5ml) was dispensed into the labeled test tubes and equilibrated to 37 ˚C for 3minutes. After which, 0.005ml (50µl) was added to respective test tubes for 1 minute, and mixed

gently. Deionized water was used as blank and 2.5ml of alkaline phosphatase colour developer was added at timed intervals (1-3 minutes). The reading scale was set to zero with deionized water which served as blank at a wavelength range of 500-630nm. Reading was done in presence of ultra violet visible spectrum set at a wavelength of 590nm. The results were recorded (Cheesebrough 2006).

Statistical Analysis

The results were analyzed using a Statistical Software Package (SPSS) version 12. Experimental data were expressed as mean ± standard error of the mean (mean ± SEM) and subjected to using one way analysis of variance (ANOVA) to determine the differences between groups. Differences between means at $p < 0.05$ were considered significant.

RESULTS

The result of phytochemical screening of the aqueous leaf extract of Phyllanthus amarus revealed secondary metabolites which include: steroids, tannins, reducing sugar, alkaloid, saponin, flavanoid, cardiac glycosides, terpenoid, carbohydrate and anthraquinone (Table1).

Table 1: Phytochemical Analysis of the Aqueous Extract of *Phyllanthus amarus*

Constituent	Inference
Steroids	Present
Tannins	Present
Reducing sugar	Present
Alkaloid	Present
Saponin	
Frothing test	Present
Emulsion	Present
Flavanoid	Present
Cardiac glycosides	Present
Terpenoid	Present
Carbohydrate	Present
Anthraquinone	Present

From the result in Table 1, the mean±SEM number of parasites counted for mice treated with 100, 250 and 500mg/kg leaf extracts of *Phyllanthus amarus* and quinine drug were 12.50±2.35, 11.25±3.47, 9.25±1.49 and 10.75±3.61 respectively. Of the extracts used the 500mg/kg concentration showed the highest curative activity (60%) followed by the 250mg/kg (51%) which is comparable with the quinine standard (53%), the 100mg/kg leaf extract was also curative but least among the concentrations.

Table 2: Effect of *Phyllanthus amarus*on parasitaemia in *Plasmodium berghei*-infected mice in a 4-day suppression test

Treatment	Dose	Parasitaemia	Chemosuppression (%)
Sterile water	Nil	Nil	Nil
Infected but not treated	Nil	23.00±8.59	Increased malaria parasite
Extract	100mg/kg	12.50±2.35	46%
Extract	250mg/kg	11.25±3.47	51%
Extract	500mg/kg	9.25±1.49	60%
Quinine	10mg/kg	10.75±3.61	53%

Values are expressed as mean±SEM. ANOVA followed by LSD's multiple range tests.

From the result in Table 2 the mean±SEMnumber of parasites counted for the mice treated with 100, 250 and 500mg/kg leaf extracts of *Phyllanthus amarus* and the standard quinine were 6.50±4.79, 3.50±1.26, 4.50±0.29 and 5.50±0.82 respectively. The % chemosuppression was calculated based on the initial parasiteamia after 72 hours of inoculation and the parasiteamia after termination of treatment.

Table 3: Effect of *Phyllanthus amarus*on parasitaemia in *Plasmodium berghei*-infected mice in an 8-day suppression test

Treatment	Dose	Parasitaemia	chemosuppression
Sterile water	Nil	Nil	
Infected but not treated	Nil	nil (died)	
Extract	100mg/kg	6.50±4.79	75%
Extract	250mg/kg	3.50±1.26	85%
Extract	500mg/kg	4.50±0.29	79%
Quinine	10mg/kg	5.50±0.82	81%

Values are expressed as mean±SEM. ANOVA followed by LSD's multiple range tests.

Table 3 showed that the result of mean survival time of mice receiving aqueous leaf extract of *Phyllanthus amarus.* The mice that were administered mean ± SEM number of parasites counted for the mice were infected and not treated had a mean survival time of 9 days, those treated with 100, 250 and 500mg/kg aqueous leaf extract of *Phyllanthus amarus* had mean survival times of 12.5, 13.5 and 15 days while those treated with the standard drug (10mg/kg Quinine) also had mean survival time of 15 days.

Table 4: Mean survival time of mice receiving aqueous leaf extract of *Phyllanthus amarus*during established infection

Treatment	Dose (mg/kg)	Mean survival time
Sterile water	Nil	15 days
Infected but not treated	Nil	9 days
Extract	100mg/kg	12.5 days
Extract	250mg/kg	13.5 days
Extract	500mg/kg	15 days
Quinine	10mg/kg	15 days

The result in Table 4 showed that the PCV of the negative control mice and those treated with 100mg/kg leaf extract (24.75±1.60 and 26.00±0.71) are significantly different from mice treated with the standard drug quinine (38.25±1.11). However the PCV of the positive control mice (40.50±0.87) and mice treated with 250 and 500mg/kg (38.50±0.96 and 39.00±0.58) leaf extracts of *Phyllanthus amarus*are comparable with mice treated with the standard drug quinine.

The RBC of the negative control mice and mice treated with 100mg/kg (2.10±0.27 and 2.35±0.12) leaf extract are significantly different from mice treated with the quinine drug (4.35±0.18). However the PCV of the mice treated with 500mg/kg (4.50±0.12) leaf extract is comparable with the positive control mice and mice treated with the standard quinine (5.03±0.27 and 4.35±0.18) respectively.

The MCH level of the negative control mice and mice treated with 100mg/kg (40.80±3.61 and 36.93±0.83) leaf extract are significantly different from mice treated with the standard quinine (27.01±1.26). However the MCH of those treated with 250 and 500mg/kg (31.30±0.33 and 28.92±0.36) leaf extracts are comparable with mice treated with the quinine drug and that of the positive control mice. For the MCHC level there is no significant difference in the groups. This implies that both the leaf extracts and standard drug quinine di not cause any significant change in the MCHC level of the mice.

For the WBC parameters, the negative control mice and mice treated with 100mg/kg leaf extract are significantly different from the mice treated with quinine while those treated with 250 and 500mg/kg leaf extracts are comparable with the mice treated with quinine.

Table 5: Effect of *Phyllanthus amarus* on Haematological Parameters (RBC + WBC) after termination of treatment

Treatment Groups

Phyllanthus amarus						
Parameter	**Positive control**	**Negative control**	**Quinine**	**100 mg/kg**	**250mg/kg**	**500mg/kg**
PCV(%)	40.50±0.87	24.75±1.60	38.25±1.11	26.00±0.71	38.50±0.96	39.00±0.58
RBC(cu.mm)	5.03±0.27	2.10±0.27	4.35±0.18	2.35±0.12	3.58±0.14	4.50±0.12
MCH(pg)	27.01±1.26	40.80±3.61	29.37±0.47	36.93±0.83	31.30±0.33	28.92±0.36
MCHC(%)	33.27±0.02	33.44±0.04	33.34±0.04	33.27±0.06	33.35±0.05	33.34±0.05
MCV(F1)	81.16±3.76	121.98±10.69	88.10±1.34	111.01±2.44	93.84±1.13	86.74±0.94
TWBC(cu.Mm)	4.06±0.19	6.10±0.53	4.40±0.07	5.55±0.12	4.69±0.06	4.34±0.05
Platelete(cu.Mm)	269.75±12.54	406.00±35.53	293.00±4.67	369.50±8.19	312.50±3.57	288.50±3.18
Neutrophil(%)	6.50±3.18	3.54±0.23	5.46±0.16	3.71±0.10	4.79±0.14	5.57±0.08
Eosinophil(%)	2.72±0.06	1.72±0.06	2.55±0.09	1.73±0.06	2.23±0.05	2.57±0.05
Basophil(%)	0.45±0.03	0.18±0.03	0.38±0.03	0.20±0.00	0.30±0.00	0.40±0.00
Lymphocytes(%)	8.10±0.17	4.95±0.32	7.95±0.32	5.20±0.14	6.70±0.19	7.80±0.12
Monocytes(%)	0.22±0.00	0.18±0.02	0.21±0.01	0.16±0.03	0.32±0.02	0.24±0.03

Values are expressed as mean±SEM. ANOVA followed by LSD's multiple range tests.

Key word:

MCH: mean corpuscular haemoglobinpg: pictogram
MCHC: mean corpuscular haemoglobin concentration Cu.Mm: cubic millimeter
MCV: mean corpuscular volume
TWBC: total white blood cell
PCV: packed cell volume
RBC: red blood cell

Table 5 showed the level of serum AST and ALT and ALP in normal control and aqueous extract of *Phyllanthus amarus* (100 mg, 250mg and 500 mg/kg) at day 8. Decrease was observed with the negative control and 100mg/kg aqueous extract of *Phyllanthus amarus.* There was a significant increase of serum AST and ALT and ALP level in *Plasmodium berghei* infected and treated with Quinine (10 mg/kg) and aqueous extract of *Phyllanthus amarus*(250, 500mg/kg/).

TABLE 6: Liver Function Test of mice on termination of treatment

				Treatment Groups		
Phyllanthus amarus						
PARAMETER	**Positive Control**	**Negative control**	**Quinine**	**100mg/kg**	**250mg/kg**	**500mg/kg**
AST (U/L)	31.33±2.23	19.71±1.01	26.61±1.86	24.15±1.49	25.29±0.50	26.62±2.26
ALT (U/L)	49.99±4.18	16.29±0.84	47.81±6.62	30.76±5.68	41.86±6.84	45.97±4.35
ALP (U/L)	47.81±4.53	23.71±3.15	36.40±1.99	28.32±2.14	31.85±1.86	33.50±1.82

Values are expressed as mean±SEM. n=6, ANOVA followed by LSD's multiple range tests.

KEY: (AST) = Aspartate transaminase, (ALT) = Alanine tranferase, (ALP) = Alanine phosphatase.

DISCUSSION

Malaria is an infectious disease that has continued to cause morbidity and mortality especially among the highly vulnerable groups, young children and pregnant women in tropical countries (Uhegbu *et al*., 2009). Resistant strains of malaria parasite seem to be on the increase despite the presence of many antimalarial drugs. This makes the search for new anti-malaria drugs imperative. Phytochemical analysis of the aqueous leaf extract of *Phyllanthus amarus* as shown in Table 6 revealed the presence of saponins, tannins, flavanoids, carbohydrate, terpenoids, alkaloids ,steroids, glycosides, reducing sugars and anthraquinone. This is similar to the research findings of an initial phytochemical exploration of *Phyllanthus* species showing the occurence of terpenoids, alkaloids, glycosides, flavonoids, tannins, and saponins(Mao *et al.,* 2016; Lee *et al.,* 2016). Alkaloids, saponins and flavanoids are suggested as being responsible for the antimalarial activities of the plant (Shigemori *et al.,* 2003). These secondary metabolites could have elicited the observed antiplasmodial activity either singly or in synergy with each other. Alkaloids have been implicated in the antimalarial activity of many plants (Kharazmi *et al.,* 2001). It has been shown to mediate its antimalarial properties by blocking protein synthesis in *Plasmodium falciparum.* (Abdulelah and

Zainal., 2007). Triterpenoids, steroids and saponins have been found to be detrimental to several infectious protozoans such as *Plasmodium falciparum (Delmas et al.,* 2000). Flavanoids are known to chelate nucleic acid base pairing of malaria parasite (Agrawal, 1989). While tannins are phenolic compounds that can act as primary antioxidants or free radical scavengers. So these phenolic compounds can contribute to the protection of the liver and kidney arising from malaria parasite infection.

Studies have also been carried out on the phytochemical analysis of *Phyllanthus amarus*. When the phytochemical screening of the methanolic leaf extracts of *Phyllanthus amarus* were investigated, it revealed the presence of flavanoids, tannins, alkaloids, terpenoids, steroids, and cardiac glycosides (Obianime and Uche, 2007). Another study carried out by Igwe et al, (2007), on the phytochemical screening of the aqueous extracts of pulverized whole plant of *Phyllanthus amarus* showed that it contains saponins, tannins, oxalates, as well as moisture, ash, fats, fibres and carbohydrates.

The result in Table 1 shows the effect of the aqueous leaf extract of *Phyllanthus amarus* on parasiteamia in *Plasmodium berghei* infected mice in a 4-day suppressive test in which the mice that were infected but not treated (malaria control) had an increase in the number of parasites count. While mice treated with the 100, 250 and 500mg/kg leaf extracts and the standard drug quinine (10mg/kg), had parasites counts of 12.50±2.35, 11.25±3.47, 9.25±1.49 and 10.75±3.61 respectively. The 500mg/kg leaf extract had the highest curative activity (59.80%), followed by the 250mg/kg extract (51.10%) which was comparable to the standard quinine (53.26%). The 100mg/kg extract (45.70%) was also curative but was the least among the concentrations. This means that the plant extract at the concentration of 500mg/kg has the highest level of antiplasmodial activity than the standard drug quinine. This result is similar to the findings of Kabiru et al, (2013), although the standard drug had the highest curative activity of 72.32% with the aqueous leaf extracts of the plant (200, 400 and 500mg/kg) having their curative activities of 56%, 61% and 68% respectively. Similarly, in the work of Onyesom *et al.* (2015), the extract yielded 94.5% inhibition at a concentration of 200mg/kg as against 94.7% for quinine.

The result for the mice treated with the aqueous leaf extract of *Phyllanthus amarus* on parasitaemiain an 8-day suppressive test as shown in Table 2, indicated that the parasites counts of mice treated with 100, 250 and 500mg/kg leaf extracts and the standard drug quinine were 6.50±4.79, 3.50±1.26, 4.50±0.29 and 5.50±0.82 respectively. As at this time the negative control had died, so the % chemosuppression was calculated based on the initial parasiteamia after 72 hours of inoculation and the parasitaemia after the termination of treatment. Of all the extaracts used the 250mg/kg concentration had the highest curative activity of 85% which was comparable to the standard quinine (81%), followed by the 500mg/kg extracts having its curative activity of 79%. Although the 100mg/kg leaf extract was also curative (75%) but was the least among the concentrations. This implies that the leaf extract inhibited the malaria parasite best at 250mg/kg.

The result of the mean survival time of mice receiving the aqueous extract of *Phyllanthus amarus* during established infection in Table 3, indicated that the mice administered sterile water (positive control) had a mean survival time of 15 days because they were not infected with *Plasmodium berghei*. The malaria control had a mean survival time of 9 days, they died due to the increase in the level of parasitaemia. The mice treated with 100, 250, and 500mg/kg extracts and 10mg/kg of quinine had mean survival times of 12, 13.5 and 15 days respectively before they were sacrificed. This was due to the reduction of parasitaemia as regard to their % chemosuppressions. This result is similar to the reports of Aziagba *et al* (2007), in which the mice treated with different concentrations of aqueous leaf extracts of *Phyllanthus amarus* (200, 400 and 600mg/kg) had mean survival time of 11, 14 and 17days respectively which was comparable to the standard drug quinine (5mg/kg) having a mean survival time of 17days.

The result obtained on the hematological effects of the aqueous leaf extract of *Phyllanthus amarus* after termination of treatment is shown in Table 4. The red blood cell count (RBC) and packed cell volume (PCV), in mice treated with 250mg/kg and 500mg/kg extracts of *Phyllanthus amarus* increased and were comparable to that of mice treated with quinine. However those treated with 100mg/kg extract of *Phyllanthus amarus* were significantly different from those treated with standard drug Quinine. This implies that the plant extract at the concentrations of 250 and 500mg/kg had a positive effect on the PCV and RBC levels of the mice. There was also an increase in the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and total white blood cell level (TWC) in the mice treated with 250 and 500mg/kg extract of *Phyllanthus amarus* which was comparable to the mice treated with standard drug Quinine. Increase in platelets count in mice treated with 250 and 500mg/kg extract of *Phyllanthus amarus* are comparable with the mice treated with Quinine but differs significantly from mice with 100mg/kg extract of *Phyllanthus amarus*. While for the lymphocytes the mice treated with 100mg/kg extract of *Phyllanthus amarus* was comparable to the mice treated standard drug Quinine but differs significantly ($P<0.05$) from mice treated with 250 and 500mg/kg extract of

Phyllanthus amarus. This finding validates that of Ilangkovan and colleagues (2016), who reported that P. *amarus* strongly inhibits the phagocytic activity of human neutrophils and reduces cellular immune responses in rats. Our finding suggests that the extract of this plant may contain agents that stimulate production of leucocytes. The crucial role of white blood cell in defending the body against infection and tissue damage is well known. Thus, the implication that *Phyllanthus amarus* is a potent immunostimulant used for the treatment of immune related diseases can be justified.

The result of the Liver Function Test in Table 4, showed that the level of serum for ALT, AST and ALP were within normal range. Although the AST level of the mice treated with 100mg/kg, 250mg/kg and 500mg/kg extract of *Phyllanthus amarus* were not significantly different ($P<0.05$) from mice treated with standard drug Quinine. For the ALT level the negative control mice and the mice treated with 100mg/kg extract of *Phyllanthus amarus* differ significantly from the mice treated with standard drug Quinine. For the ALP level a decrease from the normal range was observed with the negative control mice and mice treated with 100mg/kg extract of *Phyllanthus amarus*, which differs significantly from the mice treated with standard drug Quinine. However, the mice treated with 250 and 500mg/kg extract of *Phyllanthus amarus* are comparable to the mice treated with standard drug Quinine. This suggests that *Phyllanthus amarus* is hepatotoxic at the lower dose of 100mg/kg/ml which led to the death of the mice treated with the dose. Accordingly, serum levels of AST, ALT and ALP of the experimental subjects were within corresponding reference intervals at doses of 250 and 500 mg/kg/ml extracts according to Henry, (1974). This was an indication that moderate malarial infection did not cause profound hepatic dysfunction. Hepatic dysfunction in *Plasmodium falciparum* malaria manifested in form of jaundice is one of the important features of malaria.

CONCLUSION

On the whole *Phyllanthus amarus* showed that it has the ability to treat malaria in *Plasmodium berghei* infected mice. This ability may be due to the bioactivities of identified phytochemicals which displayed significant *in vivo* plasmodial inhibition. The mechanism of *Phyllanthus amarus* potency needs to be understood. Therefore the phytochemicals should be purified and used to assess the stage in the life of the parasite in which the active ingredient(s) of the plant is/are most potent.

Recommendation

If these results are applicable to man, *Phyllanthus amarus* can be used as an antimalarial drug. It can also be used as immune booster in herbal medicine. There is, however, the need to exercise caution on excessive and prolonged use of this plant.

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