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Phyllantus amarus (Schum. and *Thonn)*: An Antiplasmodial Plant

R. N. Nwaoguikpe[1], C. O. Ujowundu[1*], W. Braide[2] and J. Obi[1]

[1]Department of Biochemistry, Medical Biochemistry Unit, Federal University of Technology, P.M.B.1526 Owerri, Imo State, Nigeria.
[2]Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria.

Authors' contributions

All authors contributed to the success of the research work. Authors RNN and JO designed the work and carried out the animal studies. Author COU carried out the literature review and statistical analysis. Author WB carried out the phytochemical and all microscopic analyses. All authors sat together and compiled the work, read through and approved the work.

Original Research Article

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ABSTRACT

The incidence of malaria infestation has assumed epidemic status and treatment procedures have failed to yield required results due to resistance of most Plasmodial species to conventional antimalarial drugs especially in sub-Saharan Africa. *Phyllantus amarus (Schum and Thonn)* was screened for *in vivo* antiplasmodial activity in albino mice. Ethanol and aqueous extracts (ETOHE and AE) were used for the assays. Phytochemical screening of the ethanol and aqueous extracts of the aerial parts of the plant was carried out to ascertain the secondary metabolites present. Chloroquine resistant *Plasmodium berghei* (NK65) was injected into the mice by passaging. Forty-eight hours after inducing malaria infestation, the plant extracts were administered intraperitoneally for 4 days at a dose of 10mg/kg, same with the reference antimalarials. It was observed that the ethanol extract (ETOHE) showed the highest antiplasmodial activity comparable to that of Artemether (Group C) treated mice. There was no significant difference in antimalaria activity between the Artemether treated (Group C) and the ethanol treated (Group A) mice at p=0.01. The antiplasmodial activities of the extracts may be attributed to the presence of phytochemicals/secondary metabolites present in the aerial parts of the plant which include: alkaloids, saponins, flavonods and other secondary metabolites. The plant extracts were equally screened for their mineral

**Corresponding author: Email: coconacik@yahoo.com;*

contents, some of which were found to be abundant, such as Ca, Fe, Mn, K, Na, Cu and Zn. The plant extracts can be used for the treatment of malaria infestation as well the supplementation of mineral nutrients in health and disease.

Keywords: Antiplasmodials; Phyllantus amarus; albino mice; Plasmodium berghei; Plasmodium falciparum.

1. INTRODUCTION

Malaria is a mosquito borne infectious disease of humans caused by eukaryotic protists of the genus *Plasmodium*. It is widespread in tropical and subtropical regions of the world including much of the Sub-Saharan Africa. In Nigeria, malaria is mostly caused by *Plasmodium falciparum* and *Plasmodium malariae*. The female anopheles mosquito transmits these parasites to humans. Malaria has great morbidity and mortality than any other infectious disease of the world [1].

Despite advances in modern medicine, malaria remains a disease difficult to eradicate, therefore a major health problem [2]. Malaria is the most clinically investigated disease worldwide with an estimated 300 million to 500 million investigated cases annually. This according to the world health organization (WHO) results in approximately 1.5 million to 2.7 million deaths annually. Ninety percent of the deaths occur in children under five years of age living in Sub-Saharan Africa. In Africa, some 3000 children die of malaria daily, one every second [3]. In Nigeria, 60 million people experience malaria attack at least twice a year. The new report on Nigeria further reveals that 92% of pregnant women and children under 5 years of age are very susceptible because their resistance is low [4]. Malaria infestation is commonly associated with poverty and can indeed be a cause for poverty and a major hindrance to economic development. Human malaria likely originated in Africa and has co-evolved along with its hosts, mosquitoes and non-human primates. The first evidence of malaria parasite was found in mosquitoes preserved in amber from the Falacogne period that is approximately 30 million years ago [2]. Malaria parasites may have become human pathogen from the entire history of the species [5-8]. About 10,000 years ago, malaria started having a major impact on human survival which coincides with the start of Agriculture [9]; the consequence was natural selection for sickle cell disease (SCD), thalassemia, glucose 6-phosphate dehydrogenase deficiency, ovalocytosis, elliptocytosis, the loss of Garbich antigen (glycophorin C) and the duffy antigens on the erythrocytes; such blood disorders confer a selective advantage against malaria infection [10]. Throughout human history, the most critical factors in the spread and eradication of the disease have been human behavior (shifting population centers, changing farming methods and the like) and living standards. Poverty has been and remains a reason for the persistence of the disease, while it has undergone a decline in some other locations. Climate change is likely to affect future trends in malaria transmission, but the severity and geographical distribution of such effects is currently uncertain, though attracting scientific attention [11]. In humans, malaria is caused by *P.falciparum, P. malariae, P. ovale, P*. *vivax* and *P. knowlesi* [8]. Among these, infected *P. falciparum* is the most common species identified (75%) followed by *P.vivax* (20%). *P. falciparum* infestations account for the majority of deaths [12] and non-*falciparum* species have been found to be the cause of about 14% of cases of severe malaria in some human groups [12,13] *P. falciparum* infestation is proportionately more common outside Africa.

The gametocytic male (microgametocytes) and the female (macrogametocytes) are ingested by anopheles mosquitoes during a blood meal. The parasite multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated, and invade the mid-gut wall of the mosquito, where they develop into oocytes. The oocytes grow, rupture and release sporozoites into a new human host, perpetuating the malaria or *plasmodium* life cycle. The malaria parasite requires amino acids for the synthesis of its protein, imported from host plasma and digested from host hemoglobin. Hemoglobin is an extremely abundant protein in the erythrocytes and cytoplasm, serves as the major source of amino acids for the parasites. The food vacuole is an acidic compartment (pH 5.0-5.4) that contain protease activities. In this regard, the food vacuole resembles a lysosome, except that other acid hydrolases are absent. It is assumed that other acid hydrolases are not needed since the micro-environment of the erythrocyte is almost exclusively protein and in particular, hemoglobin.

Several distinct protease activities representing three of the four major classes of proteases have been identified. The digestion of hemoglobin probably occurs by a semi-ordered process involving the sequential action of different proteases [14]. Many plasnespsin genes have been identified in the genome of *P*. *falciparum* and four of these appear to function in the food vacuole. Plasnespsin-1 and Plasnespsin-2 are the best characterized and both are capable of cleaving unwanted hemoglobin between phenylalanine and leucine residues located at positions 33 and 34 on the alpha(α) globin chains. These residues are located in a region known as “hinge region”, which is located in a conserved domain known to be very crucial to the stability of the overall structure of hemoglobin. Cleavage at this site presumably causes the globin subunits to dissociate and partially unfold. It has been suggested that falcipain 2 [15] and possibly falcipain 3 [16] are capable of digesting hemoglobin [16].

Over decades and centuries, man has been in search of drugs or medication for malaria treatment. These drugs known as antimalarials or antiplasmodials range from herbal or ethnobotanical compounds such as Quinine obtained from the bark of Cinchona tree to the synthetic antimalarials like chloroquine and the 4-aminoquinolones [17]. As a result of drug resistance in the treatment of malaria, a new conventional approach has been at interplay to design drugs on the concept of combination therapy (CT). The main aim being to minimize drug resistance and to potentiate the activities of antimalarials that have found use in the therapy of malaria by prompt parasitological confirmation using microscopy or other current diagnostic methods such as those employing Lactate dehydrogenase (LDH) diagnostic kits [18]. Quinine has a long history, stretching from Peru and the discovery of Cinchona tree, the potential uses of its bark to the current day collection of derivatives that are still frequently used in the treatment of malaria.

Chloroquine was until recently, the most widely used antimalarial. It was the original prototype from which most methods of treatment are derived. It is also the least expensive, best tested and safest of all available drugs. The emergence of drug resistant parasitic strains is rapidly decreasing the effectiveness, however; it is still the first line drug of choice in most Sub-Saharan African countries. Chloroquine is a 4-aminoquinolone compound with a complicated and still unclear mechanism of action. It is believed to reach high concentrations in the vacuoles of the parasites, which is due to the alkaline nature of the vacoule, which raises the internal pH. The most significant level of activity found is against all forms of schizonts. Apart from chloroquine, a wide array of antimalarials synthetically produced have been used over the years for the prophylaxis and chemotherapy of malaria. These include- Amodiaquine, Pyrimethamine, Artemisinin and its derivates, Proguanil, Sulfonamide,

Mefloquine, Atovaquine and Primaquine. Artemisinin was derived from a Chinese herb (Qinghaosu) that has been used in the treatment of fever for over 1600 years, thus predating the use of Quinine in the western world. It is derived from the plant *Artemisia annica* and the active compound was isolated first in 1971 and named Artemisinin. It is a sesquiterpene lactone with a chemically rare peroxide bridge linkage. It is this linkage that is thought to be responsible for the majority of its antimalarial actions, although the target within the parasite remains controversial.

Artemisinin has a very rapid action and the vast majority of patients treated show significant improvement within 1-3 days of receiving treatment. It has demonstrated the fastest clearance of all antimalarials currently used and acts primarily on the trophozoite phase, thus preventing the progression of the disease. Semi-synthetic Artemisinin derivatives such as Artesunate and Artemether are easier to use than the parent compound and are converted rapidly in the body to the active compound Dehydroartemisinin [19,20].

Phyllantus amarus (Schumach and Thonn) is a plant of the family Euphorbiaceae. It is a wild herb of the Amazonian forest, though widely distributed in the tropics and sub-tropics across Nigeria [21,22]. It is known by several local names, but commonly called (Enyikwonwa) in Igbo language and regarded as a plant of general medicinal application. Traditional local uses in Nigeria include: the treatment of diarrhea and gastrointestinal disorders. It is also a food additive for puerperal and lactating/breastfeeding mothers and used in South-Eastern Nigeria, to treat malaria related syndromes; effective against fungi and antiviral (hepatitis B). It binds hepatitis B surface antigen (HBsAg) and inhibits HIV DNA polymerase activity as well as other hepatitis viral DNAs [23]. The parts of the plant normally used include- the dried leaves and stem. *Phyllantus amarus* extract have been reported by many workers to possess hepatoprotective activity [24]. The plant extract has equally been found to exhibit hypoglycemic effect in diabetes induced animals, thus useful for the management of diabetes mellitus [24-26]. It also possess antimicrobial properties and has been found to interfere with the formation of kidney stones, therefore, a useful alternative for protective treatment [27]. Possible antispasmolytic effects of the extracts on smooth muscles have been reported to contribute to its effect on urolithiasis. Recent reports on studies of the antibacterial, anti-inflammatory and anti-malarial activities of some Nigerian medicinal plants did not include *Phyllantus amarus* [28], irrespective of the widespread use of the plant parts for the treatment of fevers in South-Eastern Nigeria. This study aims at unveiling the antiplasmodial effects of aqueous and ethanol extracts (AE and ETOHE) of the aerial parts of *Phyllantus amarus* and the comparison of the antiplasmodial effects of its extracts with some reference antimalarials such as Chloroquine and Dihydroartemisinin (Artemether) on *Plasmodium* infected Albino mice.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Sample

Phyllantus amarus was collected from the herbarium of the Department of Forestry and Wildlife of the School of Agriculture and Technology, Federal University of Technology, Owerri, Nigeria with voucher no PX231WLFDO. The plant was authenticated by a plant taxonomist Mr. Francis Iwunze, as being the best variety. One hundred and fifty grams (150g) of the aerial parts were collected from the plants, washed under running water and dried at room temperature of 27ºC. The dried aerial parts were blended into powder using an electric grinder. The blended sample was sieved to get fine powder. Fifty grams (50g) of the

powdered sample were soaked in 300ml each of distilled water and ethanol of analytical grades for 48h. Each sample was filtered using Whatman No 1 filter paper. The aqueous extract (AE) and ethanolic extract (ETOHE) were concentrated using rotor evaporator maintained at room temperature of 27ºC. The extracts were then stored in a refrigerator at 8ºC until used. The percentage yield of each extract was calculated, expressed in grams and standardized.

2.2 Phytochemical Analysis

Phytochemical screening of aqueous and ethanolic (AE and ETOHE) extracts of the plant *Phyllantus amarus* were carried out using the standard procedures of the analytical methods of the Association of Official Analytical Chemists [29].

2.3 Experimental Animals

Twenty-five Swiss albino mice of either sex were obtained from National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. The mice were acclimatized for a period of 10 days and fed standard mouse cubes and clean drinking water. The mice were caged in five (5) groups of separate cages of five mice in each.

2.4 Collection of Malaria Parasite

The Chloroquine resistant *Plasmodium berghei (*NK65) used for the study was obtained from NIPRD (National Institute for Pharmaceutical Research and Development, Idu Abuja, where five animals (4 males and 1 female) weighing 19g to 22g, were selected and infested with *Plasmodium berghei* through passaging.

2.5 *In vivo* Culture of the *Plasmodium berghei* Using Albino Mice

The infested/passaged mice were brought back to the Department of Biochemistry Research Laboratory (FUTO) from Abuja. The mice were allowed to stay for 4 days for the incubation of the parasites until the parasite infestation was established. After 4 days, the passaged mice were sacrificed and their blood used to passage/infect the 25 healthy mice via the intraperitoneal route. The *P. berghei* infested blood cells were injected intraperitoneally into the mice from blood diluted with phosphate buffered saline (pH 7.2), such that each 0.2ml of blood had approximately $10X10^7$ infested cells (parasite/kg). The mice (both the infected and co-infected) have free access to standard laboratory mice foodstuff (vital starter) and water; the mice being kept under standard laboratory conditions of light, air and water with frequent checks. Five different cages were made to contain five mice in each, making up the 5 groups based on their similar weights and project design.

2.6 *In vivo* Treatment of the Infested Albino Mice

Tests were performed using a 4 day curative standard test [30], employing the rodent malaria parasite, *P. berghei (*NK 65*)*. Five mice were used in each of the groups, labeled A to E and regarded as test/treatment groups. Forty-eight hours after infestation with the malaria species, the plant extracts (AE and ETOHE) were administered to the experimental groups (Groups A, B) at a dose of 10mg/kg for 4 days. Groups C and D mice were administered Artemether and Chloroquine respectively. The negative control group (Group E) mice were not treated after infestation. All drug administration was performed via the

intraperitoneal route. The extracts were dissolved to the indicated suitable dose level in solution and suspension; the later requires dissolution in 3% w/v Tween 80. Treatments were performed daily for 4 days starting 48 hours after infection; each mouse receiving a total of 4 intraperitoneal doses of the reference drugs [30,31]. Two reference antimalarial drugs used included: Dihydroartemisinin and Chloroquine as injections were purchased from Eva Pharmacy Ltd Elele, Port Harcourt, and administered at a dose of 10mg/kg respectively for 4 days. Thick and thin blood smears were prepared from blood obtained from the tail veins of the mice , fixed with methanol, stained with Geimsa at pH 7.2 and examined under microscope usingX100 (under oil immersion) to assess the level of parasitemia. The percentage parasitemia was calculated according to the relationship outlined [32] as follows:

$$\% \text{ Parasitemia} = \frac{\text{No of parasites in treatedX100}}{\text{No of parasites in control}}$$

This is normally assumed to be:

$$\% \text{ Parasitemia} = \frac{\text{No of parasites in treated x100}}{500}$$

2.7 Preparation of Samples for Mineral Determination

Two grams (2g) of the dried powdered samples were weighed into separate beakers using micro-weighing balance treated with 20ml of concentrated HNO_3, heated to reflux on an electric hot plate at 80ºC-100ºC until digestion was complete. 20ml of conc. HNO_3 was also added to an empty beaker which served as control. The content of the beaker was allowed to cool, filtered through Whatman filter paper No. 42 into a volumetric flask and the volume made up to 100ml with de-ionized water. The flasks were then covered.

2.7.1 Determination of minerals

The mineral content of the sample was analyzed using the hydrolysate prepared above in 2.7 by the methods of the Association of Official Analytical Chemists [29] employing Atomic Absorption Spectrophotometer (AAS 969 Unicam Solar 32); but Na and K were determined by flame photometry (JENWAY PF7). All reagents and chemicals used were of analytical grade. Acid washed glass wares as well as de-ionized water were used throughout the analyses. The digested samples were made up to 100ml and stored in polypropylene containers. Four working standards (1000ppm) and a blank were prepared for each of the mineral elements: Ca, Fe, Mn, Mg, K, Na, Cd, Pb, and Zn in concentrated HNO_3. Absorbance values were noted for standard solutions of each element in the sample using Atomic Absorption Spectrophotometer (AAS). Calibration curve was obtained for concentration versus absorbance and data were statistically analyzed by fitting of straight line by least square method. A blank reading was also taken and the concentrations of various elements were calculated in mg. All values are means of three replicate determinations. The standard deviation from the mean of the mineral content were determined, computed and recorded.

2.8 Staining Techniques

The staining technique is aimed at identifying chloroquine resistant malaria parasites of *P. berghei*. The procedure is as follows:

(a) Using sterile scissors, blood was collected from the tail veins of infested albino mice into a glass slide.
(b) Placing another slide on the blood, a thin film smear was made and allowed to dry.
(c) The thin film smear was now flooded with methanol for about 2-3 minutes. This is to fix the blood parasites permanently on the slide.
(d) The film smear was flooded with Giemsa stain and allowed for about 15-20 minutes.
(e) Sterile water was now used to wash the Giemsa stain and allowed to dry.
(f) Immersion oil was dropped on the slide (film) and viewed under the microscope at X100 eye piece.

2.9 Statistical Analysis

Each analysis of the parasite level was carried out in triplicate. Data obtained were analyzed using student's t-test and ANOVA. Values for $P \leq 0.01$ were taken as significant.

3. RESULTS AND DISCUSSION

Results are presented in (Tables 1-5) and (Figs. 1, 2 and 3) respectively.

The table above shows the result of different phytochemicals identified qualitatively; those with (+) were present in the plant sample. The table equally shows the abundance of the secondary metabolites.

The negative values in the table show decrease in body weight of parasitized mice, while positive values indicate increase in body weight. There is significant difference at $p \leq 0.01$ in percent body weights of groups A, B and C mice when compared with groups D and E mice.

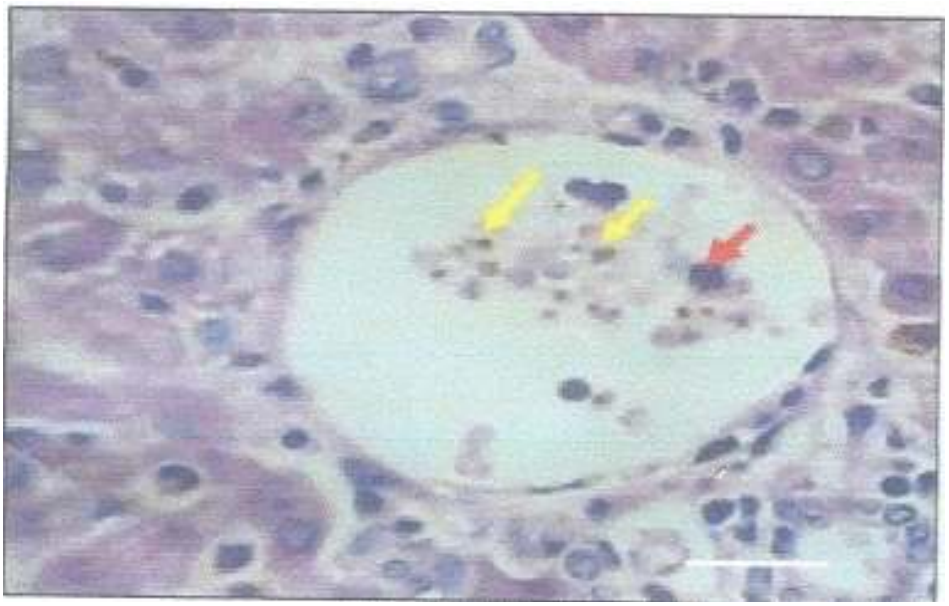

Fig. 1. Thin blood film smear showing red cells infected by *P. berghei* at X100 magnification, treated with ethanolic extract (ETOHE) of *Phyllantus amarus* at day 4. Red arrows show parasitized zones; yellow arrows show cleared zones

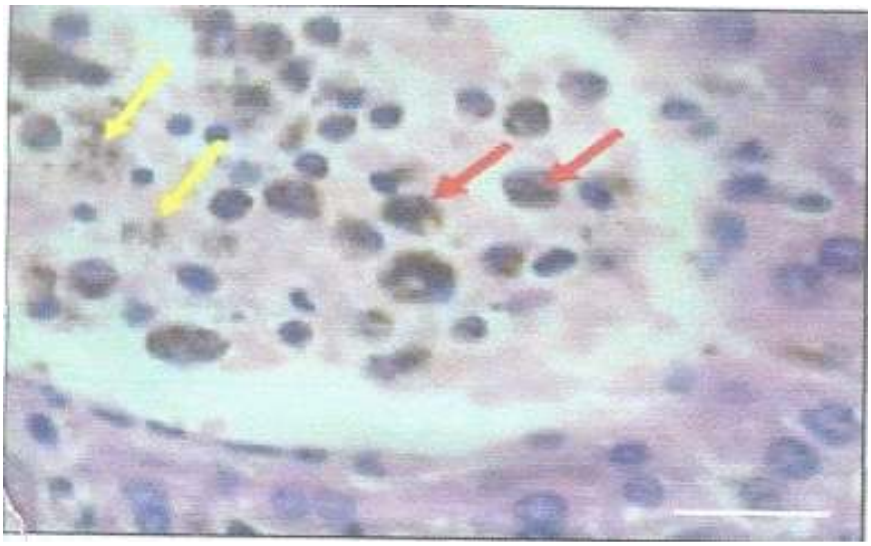

Fig. 2. Thin blood film showing red cells of *Plasmodium berghei* from infected mice at X100 magnification, not treated at day 4. Red arrows show parasitized zones. There are no cleared zones

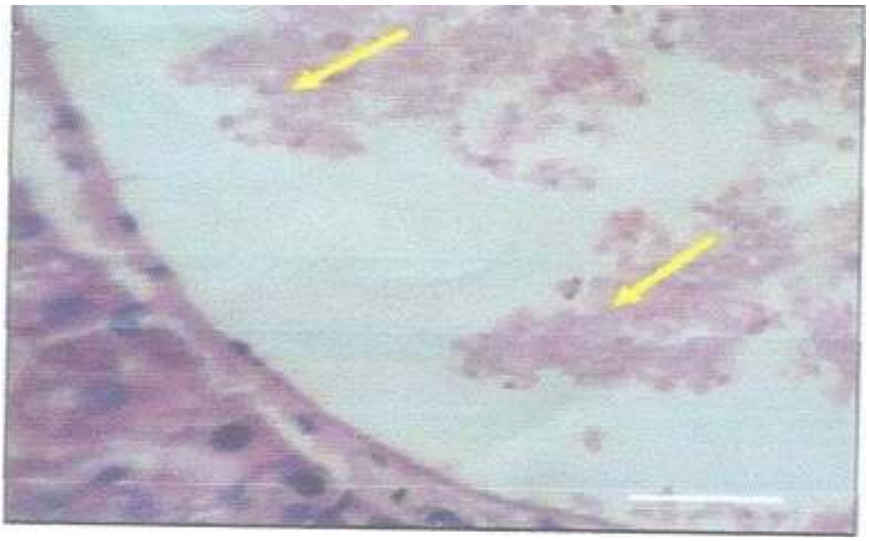

Fig. 3. Thin blood film smear showing, red cells of *P. berghei* of infested mice at X100 magnification, treated with Artemether at day 4. Red arrows are virtually absent, while yellow arrows show cleared zones

The values in the table are the Mean±SD from triplicate determinations. Cadmium and lead were not detected in the plant sample.Ca is very outstanding in its value. This can be very valuable for the fortification of meals both for infants and adults, preventing rickets in children and osteoporosis in adults. The value of Fe is worth mentioning. As a component of blood, the plant extract would now play a dual role; thus, as an antiplasmodial agent and a source of Fe for the replenishment and synthesis of hemoglobin especially during convalescence.

Table 1. Results of qualitative phytochemical screening

Sample	Alkaloid	Flavonoid	Anthraquinone	Glycoside	Phlobatamin	Saponins	Tannin	Sterol	Terpenoid
Phyllantus amarus	++	++	+	_	+	+++	+++	+++	+++

Table 2. Yields of aqueous (AE) and ethanolic extracts (ETOHE) of *Phyllantus amarus*

Sample	Fraction	Vol. of extract(ml)	Sample weight(g)	Weight of extract(g)
P. amarus	AE	350.0	50.0	11.0
P. amarus	ETOHE	300.0	50.0	10.0

The yields in the two media were almost equal, no significant difference at p≤0.01, since both solvents exhibit similar polarity

Table 3. Percentage (%) increase/decrease in body weight (g) of parasitized animals

Groups	No. of mice in group	Drug dose (mg/kg)	Initial wt. of mice(g)	Final wt. of mice(g)	% increase in wt. of mice
ETOHE(A)	5.0	10.0	21.0	22.00	4.76
AE(B)	5.0	10.0	21.0	21.50	2.38
Artemether(C)	5.0	10.0	21.0	23.00	9.52
Chloroquine(D)	5.0	10.0	21.0	19.00	-5.26
Untreated(E)	5.0	----	21.0	18.50	-7.50

Table 4. Mineral content of the hydrolysate of powdered aerial parts of *Phyllantus amarus*

Mineral	Ca	K	Na	Mn	Mg	Fe	Cu	Zn	Cd and Pb
Conc.(mg/g)	157.42±1.42	11.34±0.10	14.33±0.2	7.92±0 .27	59.62±0.15	34.55±0.15	3.08±0.67	3.30±0.24	----------

Table 5. Antiplasmodial effects of ethanolic and aqueous extracts of *Phyllantus amarus*, compared with reference antiplasmodials and levels of parasitemia in malaria infested albino mice

Sample	No of mice	Dosage of drug mg/kg	Day 0	Day 1	Day 2	Day 3	Day 4	Day5	Day 6	Day 7	Day 8	Day 9	Day10	Day 11
Phyllantus aqueous extract	5.0	10.oo	1.53±0.b2^{b}	1.60±0.0^{b}	1.46±0.1^{b}	1.30±0.1^{b}	0.86±0.1^{b}	0.73±±0.1^{b}	0.53±0.2^{b}	0.60±0.1^{b}	1.06±0.2^{a}	1.13±0.1^{a}	1.13±0.1^{a}	1.00±0.0^{a}
EtOHE	5.0	10.0	1.60±0.1^{b}	1.30±0.1^{b}	1.20±0.1^{a}	1.13±0.2^{a}	0.53±0.2^{b}	0.46±0.2^{b}	0.40±0.2^{b}	0.40±0.0^{b}	0.67±0.1^{b}	0.60±0.2^{b}	0.45±0.2^{b}	0.40±0.2^{b}
Artemeter reference control	5.0	10.0	1.60±0.1^{b}	1.20±0.1^{a}	1.20±0.1^{a}	0.80±0.2^{b}	0.53±0.2^{b}	0.33±0.1^{b}	0.20±0.1^{b}	0.40±0.2^{b}	0.40±0.01^{b}	0.26±0.2^{b}	0.06±0.2^{b}	0.40±0.1^{b}
Chloroquinine reference control	5.0	10.0	1.46±0.10^{b}	1.66±0.1^{b}	1.73±0.1^{b}	2.06±0.2^{c}	1.93±0.1^{c}	2.13±0.2c	2.08±0.2^{c}	2.13±0.2^{c}	2.08±0.2^{c}	3.30±0.1^{c}	3.50±0.0^{c}	4.00±0.0^{c}
Untreated	5.0	====	1.53±0.1^{b}	1.66±0.1^{b}	1.86±0.2^{b}	2.66±0.2^{c}	2.60±0.2^{c}	2.60±0.0^{c}	2.80±0.2^{c}	3.10±0.1^{c}	3.40±0.0^{c}	4.00±0.0^{c}	4.05±0.2^{c}	4.00±0.0^{c}

Values in the table are the Mean±SD from triplicate determinations. Values having the same superscript are significantly the same at P≤0.01 along the rows and different from others along the columns

4. DISCUSSION

From various analyses, it was discovered that the plant extracts contain various phytochemicals like tannins, alkaloids, saponins, flavonoids, sterols and terpenoids as shown in (Table 1). The table also shows the level of abundance of the various secondary metabolites. Alkaloids are a group of secondary metabolites found both in plants and animals and exhibit remarkable physiological effects in the human body. Some can function as drugs. Flavonoids are antioxidants, functioning as free radical scavengers, protecting the human body against peroxidation and cancer development [33]. Saponins were equally identified in the extracts. They possess high polarity and soluble in aqueous media. Steroidal saponins are of great pharmaceutical relevance and are of great relationship to compounds such as sex hormones, diuretic steroids, vitamin D and cardiac glycosides [34]. (Table 2) shows the yield of extracts from various solvents. There is no significant difference in the yield from the two solvents at $p \leq 0.01$. (Table 3) depicts the percent (%) increase or decrease in weight of the parasitized mice. Groups A, B and C mice experienced increase in weight while Groups D and E exhibited decrease in body weights. This decrease in weight could result from the invasive nature of the parasitemia, moreso; the malaria parasite feeds on blood. The mechanism of protein synthesis was still on with the groups that increased in weight. Apart from that, there was net utilization of nitrogen in the diets as the therapy proceeds, leading to massive reduction of the parasitemia, leading to positive nitrogen balance. The converse is true for groups D and E mice. It has also been reported by many workers that Chloroquine inhibits protein synthesizing machinery in malaria patients treated with the drug. For the group E mice that were infested and not treated, the parasitemic level was very high, which finally led to drastic loss of weight and finally death. The mice were in negative nitrogen balance and could not synthesize the basic nutrients for growth and repair, which are needed for survival. (Table 4) shows the mineral element composition of the aerial parts of the plants. The plant is really a rich source of mineral element and can be of importance for convalescence, since most of these are important for metabolism of some hormones and drugs. For example, zinc is very important for insulin metabolism. More than 300 enzymes are zinc dependent and also an antioxidant [35]. Magnesium and manganese are required by some antioxidant enzymes like catalase and carbonic anhydrase for their activities. Magnesium within the cells is bound to proteins and negatively charged molecules. Eighty percent of cytosolic magnesium is bound to ATP and as MgATP, a substrate to many enzymes before catalysis [36]. Sodium and potassium are required for nervous transmission along neurons and axons.

The normal Swiss albino mice infested with *Plasmodium berghei* but not treated (Group E) and Group D mice treated with Chloroquine became weak after day 6; while the infested Group C treated with Dihydroartemisinin (Artemether 10mg/kg) survived the infestation, and the parasitemia cleared in day 4. However, comparing day 0 and day 11 treatments with the ethanol extract of *P. amarus*; the level of parasitemia greatly reduced from (1.60±0.2 to 0.4±0.2) which is significant at $P \leq 0.01$). The ethanolic extract was more efficacious than the aqueous extract (AE) in reducing the parasitemia (1.53±0.2 to 1.0±0.2) as compared to the level of parasitemia (1.53±0.1 to 4.0±0.1) in the untreated group. Comparisons made between day 0, day 4, day 8 and day 11, imply that the parasitemic level of the infested animals treated with ethanolic and aqueous extracts of the plant showed significant difference ($P \leq 0.01$) when compared with other treatment groups like the infested untreated group and the Chloroquine treated group. There was no significant difference ($P \leq 0.01$) observed when Group D mice, treated with chloroquine and Group E (untreated animals) were compared at $P \leq 0.01$). The difference in the efficacy of the ethanolic and aqueous extracts may be due to the concentration of the active metabolites in the two extracts.

Although the parasitemia was not completely cleared in groups A and B mice, there was dramatic reduction as shown in (Table 5). The antIplasmodial effect of extracts from *Phyllantus embelica* have been reported [32,37]. Apart from the treatment of malaria, the plant extracts have been reported by many workers to be effective in the treatment of hepatitis B and C [38,39] and even urolithiasis as reported by some other workers [27].

The use of ethanol and water as solvents for the extraction of the active metabolites is in consonance with folkloric procedure for treatment using decoctions and ethanolic extracts. The percentage (%) yield of the extracts shown in (Table 2) varied probably due to the solvent medium. The methods employed required no heat, thus preserving most of the thermo-labile metabolites in their active forms. The aqueous extract had more yield suggesting more solubility of most hydrophilic components of the metabolites in that medium like the saponins that have been referred as very polar. In the study, the ethanolic extract demonstrated higher antiplasmodial activity than the aqueous extract because like most antimalarial herbal extracts, possess outstanding inhibitory effect on the multiplication of *Plasmodium berghei* used in the study .Ethanol is less dense than water and possess greater diffusibility in the same medium than water, this may also account for its bioavailability in cells and tissues. This might also account for the greater efficacy exhibited by the ethanolic extract over the aqueous extract. It might also be possible that the most active metabolites were more soluble in ethanol than water, for example, the presence of alkaloids, flavonoids and tannins, conferring this advantage on the ethanolic extract. There was no significant ($p \leq 0.01$) percent increase in body weight of the experimental animals treated with ethanolic and aqueous extracts. It may be that the active metabolites and other components of the plant extracts possess lipid lowering effects by increasing the metabolic activity of the mice. The phytochemical composition of the plant is notable, containing tannins, terpenoids, flavonoids, steroids, and alkaloids. The high preponderance of these metabolites and phytochemicals may be responsible for the antiplasmodial activity exhibited by the plant extracts [40,41,42]. Also present in the plant extract are some nutrients and micro elements which may be responsible for the activity of the active principles acting as cofactors, coenzymes and catalysts. For example, many metabolic enzymes like the kinases, require the presence of magnesium (Mg) ions for catalysis. Most antioxidant enzymes require manganese (Mn) Some of the mineral elements are cofactors to many metabolic enzymes, drugs, metabolic intermediates and participate in carbohydrates, lipids , protein metabolism, as well as in many biosynthetic and transformation reactions. For decades and centuries, man has been in search for the cure of malaria which seems intractable. Many herbal preparations have been in vogue and new combinations of drugs developed. Many theories emanating from the tropical localization of this syndrome [43-45] exist, but these have not yielded the desired effects. Conventional synthetic drugs have failed to ameliorate the malaria syndrome and this explains while traditional Africans resort to herbal remedies which have exhibited novel therapeutic applications for treatment of acute malarial infestations and for which malarial species have failed to gain resistance [46,47]. Apart from its role as an antiplasmodial plant, a lot of literature have cited many roles for the plant extracts such as hepatoprotectiveness, anti-cancer, anti-HIV 1 and 2 [48-50]. Others include dyslipidemic, antimicrobial, anti-hepatitis B and C [51-53]. As a result of the endemic nature of the malaria parasite, arising from more than 200 species of Plasmodia and of which more than 11 species use man as their secondary host; malaria remains a sickness with the highest rate of mortality and morbidity [54,55]. We suggest that research be intensified in the discovery of phytomedicines as panacea for the therapy of most tropical diseases such as malaria and sickle cell disease. There is no doubt that the antiplasmodial activity of the plant extracts of *Phyllantus amarus* compared favorably with that of the most current antimalarial, Artemeter, that has overcome Chloroquine resistance

Plasmodium falciparum malaria infections. This plant endowed with preponderance of phytochemicals, nutrients, vitamins (some antioxidants) and mineral elements, would nonetheless provide radical cure for all types of benign malaria attacks and their accompanying sequale when administered at appropriate dosages.

5. CONCLUSION

Extracts of the aerial parts of the plant *Phyllantus amarus* (Schum and Thonn) were assayed for antiplasmodial activity. The ethanol extract demonstrated profound antiplasmodial activity comparable to Artemether, a reference antimalarial agent. The plant was found to possess high content of phytochemicals such as flavonoids, saponins, tannins and alkaloids. The aerial part of the plant was equally found to be a rich source of mineral nutrients such as- Ca, K, Na, Mn, Mg, Fe, Cu and Zn. The plant extracts can be used for nutrient supplementation and the treatment of malaria infestations.

CONSENT

As a fundamental condition for researches involving animal and blood samples. The ethical committee of the Federal Medical Centre, Owerri, Nigeria in collaboration with the Hematology Unit of the Hospital, got the expressed consent of the patients who attend sickle cell clinic of the hospital after explaining to them the benefits of the research.

ETHICAL APPROVAL

All authors hereby declare that the “Principles of laboratory animal care (NIH publication No.85-23, revised 1985) were followed as well as specific national laws where appropriate. All experiments have been examined and approved by the Federal University of Technology Owerri Ethics Committee on the handling of laboratory animals for research.

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COMPETING INTERESTS

Authors have declared that no competing interests existed.

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