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In vivo antimalarial effects of ethanol and crude aqueous extracts of phyllantus amarus

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ABSTRACT: Phyllantus amarus was screened for in vivo antimalarial activity in albino mice. Ethanol and crude aqueous extracts were made and designated respectively as ethanol and crude aqueous extracts. Phytochemical screening of the entire plant except the roots were determined to ascertain the resident secondary metabolites which may be responsible for this antimalarial activity. The following phytochemicals were determined at varying concentrations: tannins, saponins, flavonoids, terpenoids, sterols, and alkaloids. The plant was equally screened for its mineral content, some of which were found to be abundant, such as Ca, Fe, Mn, Mg, K, Na, Cd, Pb, and Zn. Chloroquine resistant Plasmodium berghei (NK65) were injected into the animals. Forty eight hours after inducing malaria infestation, the plant extracts were administered intraperitoneally for 4 days at a dose of 10 mg/Kg body weight ; while Artemeter™ and Chloroquinine injections were used as standard antimalarials at a dose of 10 mg/Kg body wt respectively. Parasitemia was monitored microscopically in all groups for one week using thick and thin blood films obtained from the tail veins of each mouse. It was observed that the ethanol extract showed the highest antimalarial activity when compared with the aqueous extract treated group, the chloroquine treated group, the Artemeter treated group and the untreated group.The antimalarial activities of the extracts may be attributed to the phytochemicals/secondary metabolites resident in the ethnobotanical Phyllantus amarus. The plant can be used in the treatment of malarial infestation as well as the provision of essential minerals in health and nutrition.

Keywords: Antimalarials, *Phyllantus amarus,* albino mice, ethanol and crude aqueous extract, Plasmodium berghei

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 (WHO,2005). In humans, the parasite called sporozoites migrate to the liver, where they mature and release another form, the merozoites. These then enter the blood stream and infect the red blood cells.

Despite advances in modern medicine, malaria remains a disease difficult to eradicate, therefore a major health problem (Poinar,2005). 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 (Joy et al., 2003). In Nigeria, 60 million people experience malaria attack at least twice a year. The new data on Nigeria,further reveals that 92% of pregnant women and children under 5 years of age are very susceptible, because their resistance is low (Kahleen and Arthur,1999). Malaria infestation is commonly associated with poverty and can indeed be a cause of poverty and a major hindrance to economic development (Okwu et al., 2012).Human malaria likely originated in Africa and has co-evolved along with its hosts, mosquitoes and non-human primates. The first evidence of malaria parasites was found in mosquitoes preserved in amber from the Falacogne period, that is approximately 30 million years old (Poinar,2005) Malaria may have become a human pathogen from the entire history of the species (Zhongbua and Yau,1992;Hayakawa et al.,2008;WHO,2005).Humans may have originally caught Plasmodium falciparum from

gorillas (Neils et al.,2012).About 10,000 years ago, malaria started having a major impact on human survival which coincides with the start of Agriculture (Hempelmann,2009) ; 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, because, such blood disorders confer a selective advantage against malaria infection (Canil,2008). The three major types of inherited genetic resistance (Sickle cell disease, thalassemia and Glucose -6- phosphate dehydrogenase deficiency) were present in the Mediterranean countries. 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 (Perham et al.,2011). 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* accounts for the majority of deaths (David et al.,2011) non-falciparum species have been found to be the cause of about 14% of cases of severe malaria in some human groups (David et al.,2011) P. falciparum is proportionately more common outside Africa. There has been documented human infections with several species of Plasmodia from higher apes; however, with the exception of P. knowlesi- a zoonotic species that causes malaria in macaques (Hayakawa et al.,2009).

There are approximately, 156 named species of Plasmodia which infect various species of vertebrates. Four species are considered true parasites of humans, as they utilize humans almost exclusively as natural intermediate host. However, there are reports of Simian malaria parasites found in humans, most reports implicating P. knowlesi, as being naturally transmitted from human to human via the mosquito without the natural intermediate host (macaque monkeys). Therefore, P. knowlesi is still a zoonotic malaria parasite. The malaria parasite life cycle involves two hosts. During a blood meal, a malarial infested female anopheles mosquito inoculates sporozoites into the human host. Sporozoites infect liver cells and mature into schizonts which rupture and release merozoites. It is to be noted that P. vivax and P. ovale infections that release dormant schizonts (hypnozoites) which can persist in the liver and cause relapse by invading the blood stream weeks or even years later. After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells. The ring state trophozoites mature into schizonts which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease.

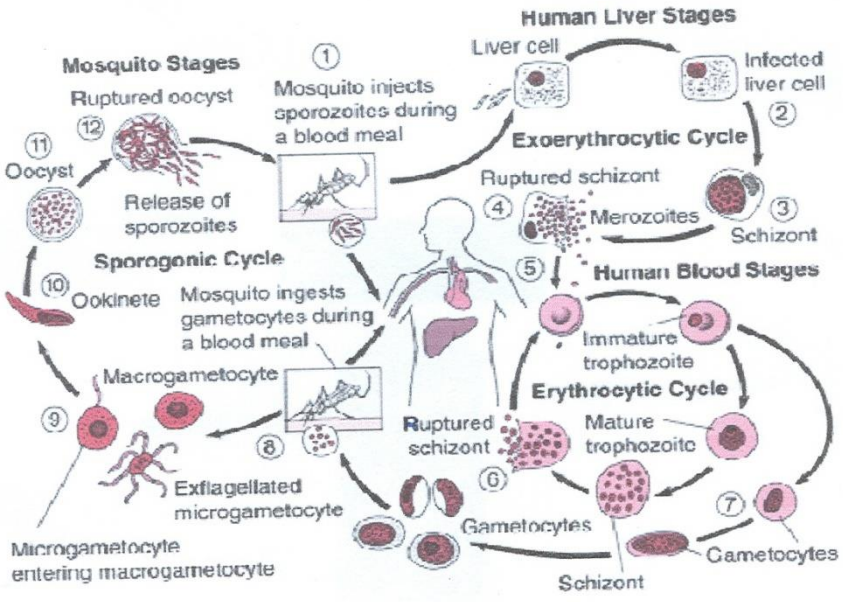

Figure 1. Life cycle of Plasmodium Species

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, cytoplasm and 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 probably by a semi-ordered process involving the sequential action of different proteases (Goldberg,2012). 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 position 33 and 34 on the alpha(α) globin chain. These residues are located in a conserved 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 (Subramananian et al,, 2009) and possibly falcipain 3(Babu and Larry,2005) were capable of digesting either native hemoglobin and therefore may also participate in the initial cleavage of hemoglobin digestion and releases heme. Free heme is toxic due to its ability to destabilize and lyze membranes, as well as inhibiting the activity of many enzymes. Three and possibly four mechanisms are involved in the detoxification of heme (a) the sequestration of the free heme into hemozoin or the natural pigment. (b) a degradation facilitated by hydrogen peroxide with the food vacuole (c) a glutathione dependent degradation which occurs in the parasite cytoplasm (d) a heme oxygenase, which has been found in P. berghei (rodent parasite) and P.knowlesi (Simian parasite), but not P. falciparum..Both the hemozoin formation pathway and the degradative pathway possibly function simultaneously with 25-50% of the free heme reconverted into hemozoin and the remainder degraded. However, some studies suggest that up to 955 molecules of free iron released during hemoglobin digestion is found in hemozoin (Babu and Larry,2005) .Over decades and centuries, man has been in search of drugs for malarial treatment .These drugs known as antimalarials range from herbal or ethnobotanical compounds such as Quinine from the bark of Cinchona tree to the synthetic antimalarials like chloroquine and the 4-aminoquinolones (Ene et al,, 2008c). 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 .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 by microscopy or other current diagnostic methods like using LDH kits (Nwaoguikpe, 2012). Quinine has a long history, stretching from Peru and the discovery of Cinchona tree, the potential uses of its bark to the current day, and a collection of derivatives that are still frequently used in the treatment of malaria.

Quinine is an alkaloid that acts as a blood schizonticide and weak gametocide against Plasmodium vivax and Plasmodium malariae. As an alkaloid, it is accumulated in the food vacuole of Plasmodium species especially P.falciparum. It acts by inhibiting the hemozoin biocrystallization, thus facilitating the aggregation of cytotoxic heme .Quinine is less toxic as a blood schizonticidal agent than chloroquine; however, it is still very effective and widely used in the treatment of acute cases of severe P. falciparum. Quinine and quinidine are the two most commonly used alkaloids related to quinine in the treatment and prevention of malaria. Quinimax is a combination of four alkaloids (quinine, quinidine, cinchoine and cinchonidine).This combination has been more effective than quinine, supposedly due to synergism. 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 its alkaline nature, 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 and Artemisinin and its derivates, Proguanil, Sulfonamide, Mefloquine ,Atovaquine, 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 ,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, arthemeter are easier to use than the parent compound and are converted rapidly once in the body to the active compound dehydroartemisinin.

Malaria caused by parasites of the genus:"plasmodia" is one of the leading infectious diseases in many tropical regions including, Nigeria, where transmission occurs all year round. The high cost of malaria treatment has left the poor masses of Nigeria heavily reliant on traditional medicine practitioners and ethno-medicinal plants as remedies against fever and other symptoms of malaria. Surprisingly, a review of studies into medicinal plants used in treating malaria across all ethnic and cultural groups in Nigeria, showed that there are more than 110 plant species employed in the treatment of malaria and other fevers. The review entitled" Medicinal plants used in Nigeria was documented (Adebayo and Kareth,2010), such plants include: the leaves of Newboulden (Akokola) in Yoruba, *Emilia chlorantha* (African yellow). Other plants include: *Eupartorium odorantum (*Ogbogbo in Yoruba),*Cajanus cajan (*Pigeon pea in English), *Pisidium guajava* (guava),*Mangifera indica* (mango), A, *Cymbopogon gigante* as leaf and *Uvaria Chamal* bark. Another multi-herbal combination is the mixture *Alchornea cordifolia* (baushe in Hausa) .Most of these antimalarial plants are used as monotherapy and only a few of these plants are taken together In combined therapy pattern. An example is the multi-herbal extract referred to as " Agbo-Iba" , made up of *Cajanus cajan* (pigeon pea), *Anacardium occidentale, Euphorbia lateriflora* leaf, *Mangifera indica* leaf, and *Nauclea latifolia* bark, *Cassia alata* leafof *Carica papaya* leaves, Cymbopogon leaves and Neem leaves. Pharmacological studies have demonstrated under laboratory conditions, antimalarial effects of extracts from plant species used in Nigerian folkloric medicine out of 51 species tested (Adekunle,2008). Previous studies on Neem leaves attributed its antimalarial to it affecting all stages of the malaria parasite in the body unlike artemisinin and primaquine that is seen to affect the inactive stages of the malaria parasite.*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 (Ajala et al., 2011;Iranloye et al.,2011) across Nigeria. 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 and also as a food additive for puerperal and lactating/breastfeeding mothers. It is also used in Southeastern Nigeria, to treat malaria related syndromes. It is effective against fungi, antiviral (hepatitis B). It binds hepatitis B surface antigen (HBsAg) and inhibits HIV DNA polymerase activity as well as other hepatitis viral DNA (Patel et al.,2011). The parts of the plant normally used include- the dried leaves and stem. *Phyllantus amarus* extract have been found to possess hepatoprotective activity (Patel et al.,2011). It has equally been found to exhibit hypoglycemia effect in diabetic induced animals and thus useful for the management of diabetes mellitus(Karuma et al., 2011;Ravikumar et al.,2011) and also possess antimicrobial properties and found to interfere with the formation of kidney stones, therefore, a useful alternative for protective treatment (Xavier et al.,2012). 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* (Samuel et al., 2011), irrespective of the widespread use of the plant for the treatment of fevers in Southeastern, Nigeria. This study aims at unveiling the antimalarial effects of the leaves and stem extracts of the plant and the comparison of its antimalarial effect with standard antimalarials on malaria infested Albino mice.

MATERIALS AND METHODS

Phytochemical analysis

Phytochemical screening of aqueous and methanol extracts of the *Phyllanthus amarus* was carried out using standard procedures outlined in the Analytical methods of the Association of Official Analytical Chemists (AOAC, 1990)

Preparation of Samples for Mineral Determination

The antimalarial plant "*Phyllantus amarus*" was collected from the herbarium of the Department of Forestry and Wildlife of the School of Agriculture and Technology, of the Federal University of Technology, Owerri , Nigeria. The plant was authenticated by a plant taxonomist Mr Francis Iwunze, attached to the department as being of the best variety. One hundred and fifty grams (150 g) of the plant leaves were plucked from the plants washed under running water and dried at room temperature of 27^0 C until completely dried. The dried leaves were blended into powder using an electric grinder. The blended sample was sieved to get fine powder. Fifty grams (50.g) of the powdered sample were soaked in 300.ml of distilled water and ethanol of analytical grade for 48 hr. Each sample was filtered using Whatman No 1 filter paper. The crude aqueous (CAE) and ethanol extracts were concentrated using rotor evaporator. The extracts were then stored in a refrigerator at 8^0 C until used. The percentage yield of each extract was calculated and standardized. Two standard antimalaria drugs were used which include-Paluta Artemeter injection purchased from Eva Pharmacy PLC, PortHarcourt, prepared at a concentration of 10 mg/kg/bw and chloroquine phosphate injection was prepared at the same concentration of 10 mg/bd wt for administration.

Animals

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

Malaria parasite

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

METHODS

In vivo culture of the Plasmodium berghei using Albino Mice

The infested/passaged mice were brought back to the Biochemistry Research Laboratory 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 the blood diluted with phosphate buffered saline (pH 7.2), such that each 0.2 ml blood had approximately 10 X 10^{7} infested cells (parasite/kg body weight). The mice (both the infected and co-infected) have free access to standard laboratory mice foodstuff (vital starter) and water; the animals being kept under standard laboratory conditions with frequent checks. Five different cages were made to contain five animals in each, making up the 5 groups based on their similar weights and project design.

In vivo treatment of the infested albino mice

Tests were performed using a 4 day curative standard test (David et al.,2004), employing the rodent malaria parasite P. berghei. Five mice were used for each group labeled from A to E., regarded as test/treatment groups. Forty-eight hours after infection with the malaria species, the plant extracts were administered to the experimental groups (Groups 1-3) at a dose of 10 mg/kg/bdwt/day for 4 days. The drugs were administered to the animals based on their average body weight. Chloroquine was administerd the Chloroquine standard control group at the standard dosage of 10 mg/kg/bdwt/day for 4 days. The negative control group animals were not treated. 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%/v/v Tween 80. Treatments were performed daily for 4 days starting 48 hours after infection; each animal receiving a total of 4 intraperitoneal doses of the standard drugs (David et al.,2004; WHO,2008).

Smears were made, fixed with methanol, stained with Geimsa at pH 7.2 and examined under microscope using X 100 (under oil immersion) to assess the level of parasitemia. The percentage parasitemia was calculated according to the technique outlined as follows:

Percetage parasitemia = No of parasites in treated X 100/No. of parasites in control x 1

% Parasitemia= No of parasites in treated/ No of parasites in control

This is normally assumed to be,

% Parasitemia= No of parasites in treated x100 /500

Preparation of samples for mineral determination.

Two grams (2g) of each of the dried powdered samples were weighed into separate beakers using micro-weighing balance treated with 20 ml of concentrated HNO_3, heated to reflux on an electric hot plate at 80^0C-100^0C until digestion is complete. 20 ml of conc. HNO_3 was also added to an empty beaker which served as control .The content of the beaker is allowed to cool, filtered through Whatman filter paper No. 42 into a volumetric flask and made up to 100 ml with deionized water. The flasks were then covered.

Determination of minerals.

The mineral content of the sample was analyzed by the methods of the Association of Official Analytical chemists (AOAC, 1990),using atomic absorption spectrophotometer (AAS 969 Unicam Solar 32); but the Na and K were determined by flame photometry (JENWAY PF7). All reagents and chemicals used were of analytical grade. Acid washed glasswares as well as de-ionized water were used throughout the analyses. The

digested samples were made up 100 ml and stored in polypropylene containers. Four working standards (1000 ppm) 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 mgg-1. All values are means of three replicate determinations. The standard error of the mean of the mineral content determined, computed and recorded.

Staining techniques.

The staining technique is aimed at identifying chloroquine resistant malaria parasite, P. berghei . The procedure is as follows:

(a)Using sterile scissors, blood was collected from the tail vein of infested albino mice unto 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.

Statistical analysis.

Each analysis of the parasitemic level was carried out in triplicate. Data obtained were analyzed using student's t-test and ANOVA. Values for p≤0.01 were taken to be significant.

RESULTS

Results are presented in tables 1-5 and figures 1, 2 and 3 respectively.

Table1. Results of qualitative phytochemical screening .

Sample	Alkaloid	Flavonoid	Anthraquinone	Glycoside	Pblobatamin	Saponins	Tannins	Sterols	Terpenoids
Phyllantus amarus	++	++	+	-	+	+++	+++	+++	+++

Table 2 . Yields of crude aqueous extract (CAE) and ethanol extract of *Phyllantus amarus*

Sample	Fraction	Vol. of extract (ml)	Sample weight(g)	Weight of extract(g)
Phyllantus amarus	CAE	350.0	50.0	11.0
Phyllantus amarus	EtOH	300.0	50.0	10.0

Table3. Percentage (%) body weight of parasitized animals

Sample	No of animals	Dosage of drug (mg/kgbw)	Initial BW of animals(g)	Final BW of animals(g)	% increase in BW
EtOH extract	5.0	10.00	21.00±0.0	22.00±0.0	4.76[a]
CAE extract	5.0	10.00	21.00±0.0	21.50±0.1	2.38[a]
Artemeter	5.0	10.00	21.00±0.0	23.00±0.0	9.52[a]
STD(control)		10.00			
Chloroquine STD (control)	5.0	10.00	21.00±0.0	19.00±0.0	-5.26[b]
Untreated	5.0	10.00	21.00±0.0	18.50±0.0	-7.50[b]

Values with the same superscript are significantly the same(p≤0 05) and different from others along the rows and columns.

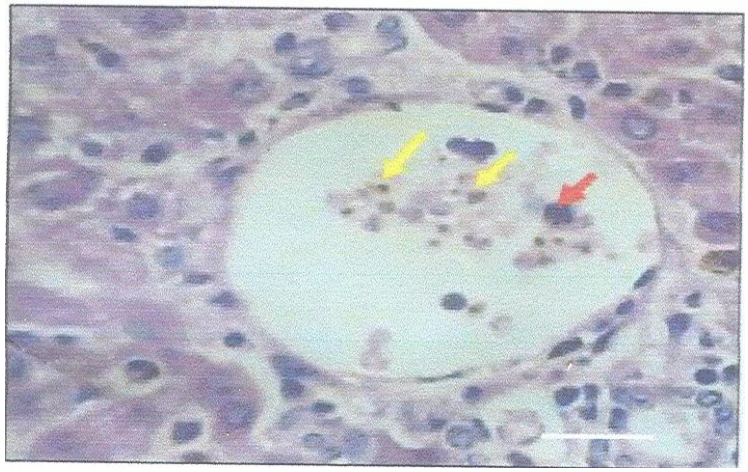

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Fig. 2 Thin blood film smear showing red cells of P.berghei infected and treated with ethanol extract of Phyllantus amarus treated at day 4. Red arrows represent parasitized zones; yellow arrows show cleared zones

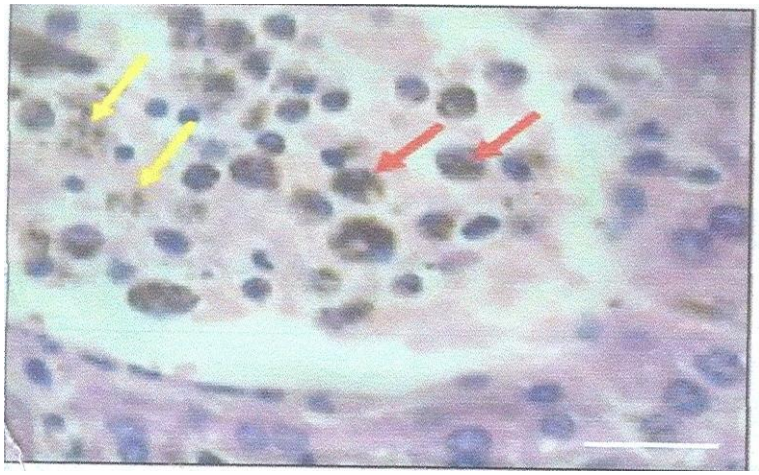

Fig3. Thin blood film showing red cells of *Plasmodium berghei* from infected mice not treated at day 4. Red arrows show parasitized zones. There are no cleared zones.

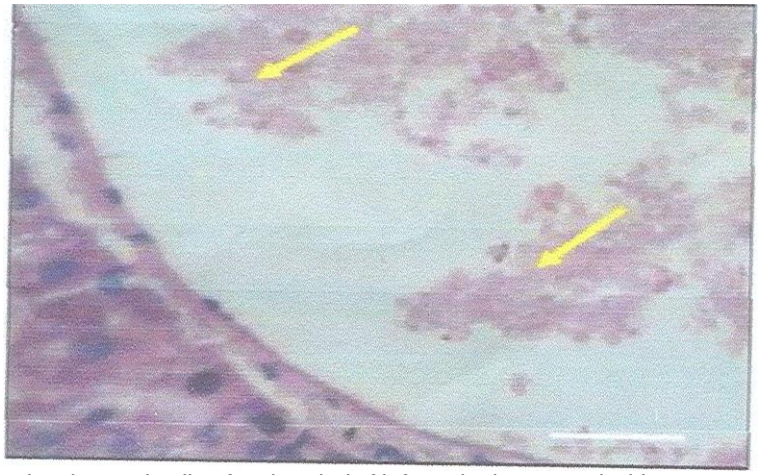

Fig4.Thin blood film smear showing, red cells of *P. berghei* of infested mice treated with Artemeter at day 4. Red arrows are virtually absent , while yellow arrows show cleared zones.

Table 4.Antimalaria effects of ethanol and crude aqueous extracts of *Phyllantus amarus*, using malaria infested albjno mice

Sample	No of mice	Dosage(mg)	Day 0	Day 1	Day 2	Day 3	Day 4	Day5	Day 6	Day 7	Day 8	Day 9	Day10	Day 11
CAE	5.0	100.0o	1.53±0.2	1.60±0.0	1.46±0.1	1.30±0.1	0.86±0.1	0.73±±0.1	0.53±0.2	0.60±0.1	1.06±0.2	1.13±0.1	1.13±0.1	1.00±0.0
EtOH	5.0	100.0	1.60±0.1	1.30±0.1	1.20±0.1	1.13±0.2	0.53±0.2	0.46±0.2	0.40±0.2	0.40±0.0	0.67±0.1	0.60±0.2	0.45±0.2	0.40±0.2
ArtemeterSTD control	5.0	100.0	1.60±0.1	1.20±0.1	1.20±0.1	0.80±0.2	0.53±0.2	0.33±0.1	0.20±0.1	0.40±0.2	0.40±0.01	0.26±0.2	0.06±0.2	0.40±0.1
Chloroquinestd Control	5.0	100.0	1.46±0.10	1.66±0.1	1.73±0.1	2.06±0.2	1.93±0.1	2.13±0.2	2.08±0.2	2.13±0.2	2.08±0.2	3.30±0.1	3.50±0.0	4.00±0.0
Untreated	5.0	====	1.53±0.1	1.66±0.1	1.86±0.2	2.66±0.2	2.60±0.2	2.60±0.0	2.80±0.2	3.10±0.1	3.40±0.0	4.00±0.0	4.05±0.2	4.00±0.0

Values in the table are the Mean ± SD from triplicate determinations. Values having the same superscript are significantly the same at p≤0.05 and different from others.

Table 5. Mineral content of the leaves of *Phyllantus amarus.* Values expressed as mg/g or mgg-[1]

Mineral(mg/g)	Ca	K	Na	Mn	Mg	Fe	Cu	Zn	Cd	Pb
Conc(mg/g	157.42±1.42	11.34±0.10	14.33±0.2	7.92±0.2	59.62±0.15	34.55±0.15	3.08±0.11	3.30±0.22	-	-

Values in the table are the Mean ± STD from triplicate determinations. Cadmium and lead were not dictated in the sample.

DISCUSSION

The normal Swiss mice injected with Plasmodium berghei and not treated(Group 5) and Group 4 treated with chloroquine became weak after day 6; while the infected Group 3 treated with Artemeter (10 mg/kg bw) survived and the parasitemia cleared in day 4. However, comparing day 0 and day 11 of treatments with the ethanol extract of P. amarus , the level of parasitemia greatly reduced from (1.60±0.2 to 0.4±0.2) . The ethanol extract was more efficacious than the crude aqueous extract (CAE) in reducing the parasitemia (1.53±0.2 to 1.0±0.2) as compared to (1.53±0.1 t0 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 ethanol and crude aqueous extracts of the plant showed significant difference (p≤0.05) when compared with other treatment groups like the infested untreated group and the chloroquine treated group. There was no significant difference (p≤0.05) observed when Group 4 mice, treated with chloroquine and Group 5 (untreated animals) were compared. The difference in the efficacy of the ethanol 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 the group treated with the ethanol and aqueous extracts, there was dramatic reduction as shown in table 5. The antimalarial effects of extracts from Phyllantus amarus have been documented (Ogata et al., 1992;Bagaran et al.,2011). Apart from the treatment of malaria, the plant has equally exhibited various actions in the treatment of hepatitis B and C (Akinjogunta et al.,2010;Xia et al.,2011) and even urolithiasis (Ajala et al.,2011)

The use of ethanol and water as solvents for the extraction of the active metabolites is in consonance with folkloric procedure of the use of decoctions and alcohol extracts. The percent (%) yield of the extracts 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 crude aqueous extract had more yield suggesting more solubility of most aqueous components of the metabolites in the solvent. In the study, the ethanol extract demonstrated higher antimalarial activity than the crude aqueous extract. Ethanol is less dense than water and might possess greater diffusibility in the same medium than water. This might account for the greater efficacy exhibited by ethanol extract over the CAE. It might also be possible that the most active metabolites were more soluble in ethanol than water conferring this advantage on the ethanol extract. There was no significant (p≤0.05) percent increase in body weight of the experimental animals treated with ethanol and crude aqueous extracts. This may be that the active metabolites and other components of the plant extract possess lipid lowering effect . 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 antimalarial activity exhibited by the plant (Ene et al.,2008a;Ene et al.,2008b;Njunda et al.,2013). Also present in the plant extract are some nutrient and micro elements which may be responsible for the activity of the active principles acting as cofactors, coenzymes and catalysts. Some of the mineral elements are cofactors to many metabolic enzymes, drugs, metabolic intermediates involved in many 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 combination of drugs developed. Many theories emanating from the tropical localization of this syndrome (Cox,2002;Carter and Mendis,2002;Dobson,1994) exist, but also have not yielded the desired effects. Conventional synthetic drugs have failed to ameliorate the condition and this explains while traditional Africans resort to herbal remedies which have been found to be novel therapies for acute malarial infestations and for which malarial species have failed to gain resistance(Asfaw et

al.,1999;Alison,2009).Apart from its role as an antimalaria plant,a lot of literature have cited and indicated enormous biochemical roles such as hepatoprotective, anticancer,anti HIV 1 and 2 (Godwin,2010;Taesokikul et al.,2012;Thakur et al.,2012).Others include dyslipidemic,antimicrobial,anti-hepatitis B and C(Appiah-Opong et al.,2011;Nayak et al.,2010;Abhyankar et al.,2010). As a result of the endemicity of malaria parasite and its ubiquitousness 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 (Collins et al.,2008;Perkins and Austin,2008). We suggest that research be intensified in the discovery of phytomedicines as a panacea for the therapy of most tropical diseases such as malaria and sickle cell disease. There is no doubt that the antimalarial activity of the plant extracts(Phyllantus amarus) compared favorably with that of the most current antimalarial, Artemeter, that has overcome chloroquine resistance in chloroquine resistant Plasmodium falciparum malaria.This plant endowed with the preponderance of phytochemicals , nutrients vitamins(some antioxidants) and mineral elements, would onetheless provide radical cure for all types of benign malaria attacks and their accompanying sequale when administered at appropriate dosages..

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