



Original Research Article

***In vivo* antimalarial and cytotoxicity activity of ethanolic stem bark of *Petersianthus macrocarpus* and leaf of *Astonia boonei* in experimental mice model**

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ABSTRACT

The purpose of this study was to evaluate *in vivo* antiplasmodial effect of ethanolic extracts of *Alstonia boonei* leaves and *Petersianthus macrocarpus* stem bark and compare their antiplasmodial effects with chloroquine phosphate, a standard antimalarial. Phytochemical analysis of the extracts was carried out using standard procedures. The oral acute toxicity was evaluated in mice using modified Lorke's method and the *in vivo* anti-plasmodial effect against early infection and curative effect against established infection were studied in chloroquine-sensitive *Plasmodium berghei berghei* NK65-infected mice. Results of the phytochemical tests showed relative abundance of alkaloids, glycosides, tannins, resins, steroids, flavonoids, terpenoids and saponins in the extracts. The oral median lethal dose of the extracts in mice was determined to be 3,807.9 mg/kg body weight for *P. microcarpus* and >5000 mg/kg body weight for *A. boonei*. Comparatively, chloroquine (5 mg/kg) gave the same chemo-suppressive effect as 200 mg/kg b. wt. of each of the extract and also exhibited similar curative effect as 400 mg/kg b. wt. p.o. of each of the extract, which were significantly ($p \leq 0.05$) greater than that of negative control (5 mL/kg distilled water). Overall, the antimalarial effects in the curative and suppressive tests were higher in *A. boonei* than *P. macrocarpus*. Results of this study has shown that ethanolic extracts of *A. boonei* leaves and *P. microcarpus* stem bark possess potent antimalarial effects and could therefore offer potential for safe, effective and affordable antimalarial phytomedicines. Further experiments will be required to identify the active constituent(s) responsible for the observed antimalarial effects and their mechanism(s) of action. Malaria is undoubtedly the single most destructive and dangerous infectious disease in the developing world. Attempts at eradicating the disease with insecticides and chemotherapeutic drugs failed because of the emergence of drug-resistant parasites and female *Anopheles* mosquitoes that are resistant to insecticides. Herbal medicines are widely used due to their therapeutic efficacy coupled with least side effects, which initiate the scientific research regarding the antimalarial activity. *A. boonei* and *P. macrocarpus* are medicinal plants widely used in folkloric medicine in Nigeria in the management of acute malaria and other ailments. The present study will help the industry to develop herbal medicine as alternatives to synthetic and semi-synthetic agents in the treatment of malaria with less likelihood of resistance and at a lower cost.

Keywords

Alstonei boonei;
Petersianthus microcarpus;
Antimalarial;
Mice;
Chloroquine;
Plasmodium berghei berghei.

Introduction

Malaria, a life threatening disease caused by a parasitic infection of the red blood

cells by *Plasmodium* parasites transmitted through a bite of the female anopheles

mosquitoes, is undoubtedly the single most destructive and dangerous infectious agent in the developing world, predominantly tropical and subtropical regions, including parts of the Americas, Asian and Africa (Winter *et al.*, 2006). Clinical symptoms of malaria include headache, fever, chills and vomiting which are usually mild but if not treated immediately could lead to delirium, metabolic acidosis, cerebral malaria and multi-organ system failure (Iyiola *et al.*, 2011). This vector-borne infectious disease is a classic example of one that affects the productivity of individuals, families and the whole society, since it causes more energy loss, more debility, more loss of work capacity and more economic damage than any other human parasitic diseases (Sachs and Malaney, 2002). World Health Organization (WHO) estimates that each year, more than 200 million people are infected with malaria worldwide (WHO, 2008). The severity of malaria caused by *Plasmodium falciparum*, the most widespread etiological agent for human malaria, depends on a complex interplay between the infecting parasite and the immune status and genetic background of the host (Sharma *et al.*, 2004). The increasing resistance of this species to standard anti-malarial drugs as well as misuse of chloroquine and other synthetic and semisynthetic agents has posed great obstacle towards preventing and curing malaria, and thus necessitates the need for a continuous scientific effort to search for new drugs, particularly with novel modes of action (Guadalupe *et al.*, 2007). In addition, lack of affordability of anti-malarial drugs, especially the currently approved drug combinations [artemisinin-based combination therapies (ACTs)] by the majority of the population has forced many to seek alternative sources of

treatment using traditional herbal remedies, such as plants and minerals. In other words, the increasing cost of conventional antimalarial treatment coupled with the upsurge in side effects of many synthetic and semisynthetic antimalarials in addition to multi-drug resistant *Plasmodium falciparum* have spurred scientists on the research for orthodox (e.g. plant-based) antimalarials of therapeutic potential (Njoroge, and Bussmann, 2006; , Lewis and Ausubel, 2006). The primary benefits of using plant-derived medicine include that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments (Iwu, 1993).

Recently, a lot of evidence has been collected to show immense potential of medicinal plants used in various traditional systems (Vasanth *et al.*, 1990; Iwu *et al.*, 1999). Plants have invariably been a rich source for new drugs and some anti-malarial drugs in use today (e.g. quinine and artemisinin) were either obtained from plants or developed using their chemical structures as templates (Wright *et al.*, 1993; Odugbemi and Akinsulire (2007). A number of studies have been undertaken to evaluate the inhibitory effects of various plant extracts on *P. falciparum* (Awe and Opeke, 1990). Similarly, the *in vivo* antimalarial properties of several plant extracts have been evaluated in mice (Bello *et al.*, 2009; Gbadamosi, *et al.*, 2011; Idowu *et al.*, 2010).

Alstonia boonei De Wild, also called 'devil tree' in tropical and sub-tropical Africa, Central America and Australia, is a large deciduous evergreen tree, usually up to 45m tall and 1.2 m in diameter, belonging to the family *Apocynaceae* consisting of about 40-60 species (Wright

et al., 1993). Also known as Ahun in Yoruba, Egbu-ora in Igbo, Ukhu in Edo and Ukpukunu in Urhobo, the plant is widely distributed in the lowlands and rain-forest areas of Nigeria (Odugbemi and Akinsulire, 2007). Parts of the plant are employed for the treatment of a variety of ailments in Africa and the stem bark has been listed in the African Pharmacopoeia as an agent useful for treatment of malaria, intestinal helminthes, muscular pain, insomnia, hypertension, fever, painful micturition, chronic diarrhea, rheumatic pains, as anti-venom for snake bites and in the treatment of arrow poisoning (Faparusi SI, Bassir, 1972; Oliver-Bever, 1986; Asuzu IU, Anaga, 1991; Kweifo-Okai *et al.*, 1995). However, potential nephrotoxicological effect, especially at high dose, was reported in Guinea pigs by Oze *et al.* (Oze *et al.*, 2007). The stem bark of the plant has been reported to possess potent neuroleptic and anxiolytic properties in mice (Elisabetsky and Costa-Campos, 2006) and also to contain important minerals like calcium, phosphorous, iron, sodium, potassium and magnesium in addition to alkaloids, tannins, saponins, flavonoids, cardiac glycoside and vitamin C. More so, Elijah *et al.*, (2010) reported possible and promising beneficial effect of *A. boonei* for preserving palm wine. Oigiangbe *et al.* (2010) reported insecticidal properties of an alkaloid from *Alstonia boonei* De Wild, Olajide *et al.* (2000) established anti-inflammatory, antipyretic and analgesic properties of *A. boonei* stem bark extract, while Taiwo *et al.*, (1998) investigated the activity of stem bark of *A. boonei* on human complement and polymorph nuclear leucocytes. It has been reported to contain phytochemicals such as saponin, alkaloids, tannins, triterpenoids and steroids (Faparusi and Bassir, 1972). Over 90% of the isolated chemical constituents

are alkaloids many of which are the indole types (Faparusi and Bassir, 1972; Ojewole, 1984). The major alkaloids are echitamine and echitamidine. *In vitro* antiplasmodial activity of the alkaloids against both drug sensitive and resistant strains of *P. falciparum* and *in vivo* activity against *P. berghei* in mice have been reported (Vasanth *et al.*, 1992; Awe SO, Opeke, 1990; Olajide *et al.*, 2000). Anti-malarial activity of various fractions of the stem bark extract of *Alstonia boonei* was reported by Bello *et al.*, (2009) and Iyiola, *et al.*, (2011) while Odugbemi and Akinsulire (2007), Idowu *et al.* (2010) and Gbadamosi *et al.*, (2011) confirmed indigenous medicinal usefulness of *Alstonia boonei* for malaria therapeutic usage in Southwestern part of Nigeria.

Petersianthus macrocarpus (fam. *Lecythidaceae*), common name Essia, synonymous with *Combretodendron macrocarpum*, is widely used in the folkloric medicine of the South Eastern part of Nigeria (where it is called *owewe*) for the relief of many ailments including pains, and “re-current” fever associated with malaria and as an anti-cancer agent (Mengome Line-Edwige *et al.*, 2009). The plant is also used as traditional folk medicine in Cameroon for the treatment of different infections and disorders like gastrointestinal disorders (e.g. dysentery) (Laure Brigitte Kouitcheu Mabeku *et al.*, 2011). Two new triterpenoid saponins, petersaponins III and IV (1 and 2), were isolated from an *n*-butanol extract of the bark of *Petersianthus macrocarpus* (Tiwakade A. Olugbade *et al.*, 2000). Aqueous extracts from stem bark of *Petersianthus macrocarpus* contain substances (triterpenic saponins) exhibiting both estrogenic and antiestrogenic potency (Asmahan El Izzi *et al.*, 1992).

Although a lot of research has been carried out on the anti-malarial effect of different extracts of *Alstonia boonei* (Iyiola *et al.*, 2011; Vasanth *et al.*, 1990; Odugbemi and Akinsulire, 2007; 2, 10, 13 – Idowu *et al.*, 2010; Olajide *et al.*, 2000) and *Petersianthus macrocarpus* (Mengome Line-Edwige *et al.*, 2009), to the best of our knowledge, there is no reported work on the anti-malarial property of ethanolic extracts of *Alstonia boonei* leaves and *Petersianthus macrocarpus* stem bark, which the present study was set out to investigate. Consequently, the objective of this study was to evaluate the anti-malarial activity of ethanolic extracts of *Alstonia boonei* leaves and *Petersianthus macrocarpus* stem bark against *Plasmodium berghei* parasites in mice.

Materials and Methods

Alstonia boonei leaves and *Petersianthus macrocarpus* stem bark were obtained from the Bioresources Development and Conservation Programme Centre (BDCCP), Nsukka, Enugu State, Nigeria. The two plants were authenticated by Mr. A. O. Ozioko of the Centre and the voucher specimens were deposited there for future reference.

The leaves of *Alstonia boonei* were carefully removed, washed and dried under room temperature and powdered with hand blender. Similarly, the stem bark of *Petersianthus macrocarpus* was washed, sun dried, powdered using pestle and mortar and subsequently with hand blender. A weighed quantity (1 kg) each of the air-dried powdered drug was weighed and extracted with ethanol (70 %), shaken with a GFL Shaker (No. 3017 MBH, Germany) for 72hrs, concentrated to dryness on rotatory evaporator and stored in a refrigerator at 4°C.

Experimental animals

Swiss albino mice (18-25 g) of either sex were used for the study. The animals were procured from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, and were allowed to acclimatize to the new environment for a period of two weeks prior to the study. The mice, maintained on standard rodent feed and water *ad libitum*, were housed in polypropylene cages at room temperature throughout the study and were maintained under standard conditions of humidity, room temperature and 12 h light/12h darkness cycle. This study was conducted in accordance with Ethical Guidelines of Animal Care and Use Committee (Research Ethics Committee) of Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Acute toxicity studies

The safety of the extracts to the mice was evaluated by determining the oral acute toxicity (LD₅₀) using a modified Lorke's method (34). The treatment and control animals were weighed and marked appropriately. The acute toxicity test of the plant extracts was tested on mice using six doses (10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight) administered orally for each plant extract. The study was carried out in two phases. In the phase one of the study, nine mice were randomized into three groups of three mice each and were given 10, 100 and 1000 mg/kg b. wt. of each of the extract orally. The mice were observed for paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first 4 h and subsequently daily for 7 days. In the second phase of the study, another fresh set of nine mice were

randomized into three groups of three mice each and were given 1600, 2900 and 5000 mg/kg b. wt. of each of the extract orally based on the result of the first phase. These were observed for signs of toxicity and mortality for the first critical 4 h and thereafter daily for 7 days. The LD₅₀ was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose, i.e. the geometric mean of the consecutive doses for which 0 and 100 % survival rates were recorded in the second phase. The oral median lethal dose was calculated using the formula:

$$LD_{50} = \sqrt{\text{Minimum toxic dose} \times \text{Maximum tolerated dose}} \dots\dots\dots (1)$$

Rodent parasite strain

The rodent parasite *Plasmodium berghei berghei* NK 65 used in this study was obtained from National Institute for Medical Research (NIMR) Lagos, Nigeria and kept at Animal Utility House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria. The strain of parasite was maintained by continuous intraperitoneal passaging of the parasite into uninfected mice for two weeks. The infected mice were acclimatized to the environment and used for the study. Prior to the commencement of the study, one of the infected mice was kept and observed to reproduce disease symptoms similar to human infection.

Experimental design

The anti-malarial activity of the extracts was tested using *in vivo* anti-plasmodial effect against early infection and curative effect against established infection models in chloroquine-sensitive *Plasmodium berghei berghei* NK65-infected mice

(Awe and Opeke, 1990). Forty mice (weighing 22-35 g) were divided randomly into eight groups of five mice each according to the following treatment protocol:

Group 1: Positive control (5 mg/kg of chloroquine phosphate solution).

Group 2: Negative control (5 ml/kg of distilled water).

Group 3: 100 mg/kg ethanolic stem bark extract of *P. macrocarpus*.

Group 4: 200 mg/kg ethanolic stem bark extract of *P. macrocarpus*.

Group 5: 400 mg/kg ethanolic stem bark extract of *P. macrocarpus*.

Group 6: 100 mg/kg ethanolic leaf extract of *Alstonia boonei*.

Group 7: 200 mg/kg ethanolic leaf extract of *Alstonia boonei*.

Group 8: 400 mg/kg ethanolic leaf extract of *Alstonia boonei*.

Three treatment groups (Groups 3-8) were used for each plant extract for the suppressive and curative models respectively.

Assessment of anti-plasmodial activity

Chemosuppressive test:

The Peter's 4 day suppressive test against chloroquine sensitive *Plasmodium berghei berghei* NK 65 infection in mice was employed (Iwu *et al.*, 1999; Peters *et al.*, 1975). Parasitized erythrocytes were obtained from a donor infected mouse by cardiac puncture with a sterile needle and syringe (Bello *et al.*, 2009). Forty mice were selected and inoculated intraperitoneally with infected blood suspension (0.2ml) containing 1×10^7 infected erythrocytes. The mice were randomly divided into five groups, with groups 3 and 6, groups 4 and 7, groups 5 and 8 receiving correspondingly daily doses (100, 200, 400mg/kg b. wt., p.o.) of

P. macrocarpus ethanolic stem bark and *A. boonei* ethanolic leaf extracts respectively, group 2 (negative control) received equal volume of distilled water (5ml/kg), while group 1 (positive control) was treated with chloroquine at a total dose of 5mg/ kg, all on the first day. Treatment continued daily until the fourth day. On the fifth day (day 4 post-treatment), blood was collected from the tail of each mouse and smeared on to a microscope slide to make a film (Idowu *et al.*, 2010). The blood films were fixed with methanol, stained with Giemsa at pH 7.2 for 10 min and parasitaemia examined microscopically. The percentage suppression of the parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice.

Curative (Rane) test:

The curative potential of each of the extract was carried out according to a method already described (Iyiola *et al.*, 2011). The mice were injected intraperitoneally with standard inoculum of 1×10^7 *Plasmodium berghei berghei* NK 65 infected erythrocytes on the first day (day 0). Seventy two hours later, the mice were divided into 8 groups of four mice each. The groups were orally treated with each of the extract (100, 200 and 400 mg/kg/day), chloroquine (5mg/kg/day) was given to the positive control and an equal volume of distilled water (5ml/kg/day) was given to the negative control group. The treatment was carried out once daily for 5 days and blood smears were collected and examined microscopically at a Magnification of X100 to monitor the parasitaemia level. Twenty-four hours after last drug administration, thin blood smears were prepared using blood collected from the

tail vein of each mouse. Each smear was air-dried, fixed in methanol, air-dried again and stained with Geimsa for 10 minutes and then examined under the microscope. The slides were observed under oil immersion. Each slide was observed at three different fields and the parasitized red blood cells (RBCs) and total number of RBCs for each field was recorded. Percentage parasitaemia was counted using the formulae below.

$$\% \text{ parasitaemia} = \frac{\text{No of parasitized RBCs}}{\text{Total RBCs}} \times 100 \text{ (Elisabetsky and Costa-Campos, 2006)} \quad \dots\dots\dots (2)$$

Phytochemical tests of the extracts

Various phytochemical tests were carried out on the extracts to determine the presence of saponins, flavonoids, glycosides, steroids, triterpenoids, alkaloids, carbohydrates, acidic compounds, proteins, reducing sugars, oils, resins and tannins following standard procedures (Evans, 2002). Each test was qualitatively expressed as negative (-) or positive (+); the intensity of the characteristic colour was expressed as (++) or (+++).

Test for saponins

A 5 ml of extract was vigorously shaken with 10 ml of water in a test tube. Frothing which persisted was taken as an evidence for the presence of saponins.

Test for flavonoids

A 3 ml of the extracts plus small 0.5 ml of magnesium chips plus two drops of concentrated hydrochloric acid down the side of test tube. Reddish coloration was taken as evidence for the presence of flavonoids.

Test for alkaloids

A 2 ml of the extracts plus picric acid were added. An orange colouration was taken as evidence for the presence of alkaloids.

Test for tannins

Extract plus 4 ml of water and drops of ferric chloride were added. Immediate green precipitate was taken as evidence for the presence of tannins.

Test for steroids

Extract plus 9 ml of ethanol followed by refluxing and filtration. The filtrate was concentrated and about 5 ml of hot water were added, allowed to stand for 1 hour, filtered, and the filtrate extracted with 2.5 ml of chloroform. To 0.5 ml of the chloroform extract in a test tube was carefully added 1 ml of concentrated sulphuric acid to form a lower layer. A reddish brown interface shows the presence of steroids.

Test for terpenoids

Another 0.5 ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicates the presence of terpenoids.

Test for glycosides

Extract plus 5 ml dilute sulphuric acid and drops of 20 % potassium hydroxide solution plus 10 ml of a mixture of equal parts of Fehlings solutions I and II plus boiled for 5 minutes. A more dense brick red precipitate indicates the presence of glycosides.

Test for acidic compounds

About 0.1 g of the extract was placed in a clear dry test tube and sufficient water added. This was warmed in a hot water bath and cooled. A piece of water-wetted litmus paper was dipped into the filtrate and colour change on the litmus paper observed.

Test for proteins

Extract plus two drops of Million reagents in a test tube. A white precipitate indicates the presence of protein.

Test for reducing sugars

About 0.1 g of the extract was shaken vigorously with 5 ml of distilled water and filtered. To 1 ml portion of the filtrate was added 2 ml of Benedict's reagent. The mixture was shaken, heated on a water bath for 5 minutes. A rusty brown precipitate indicates the presence of reducing sugar.

Test for carbohydrates

About 0.1 g of the extract was boiled with 2 ml of water and filtered. To the filtrate, few drops of naphthol solution in ethanol (Molisch's reagents) were added. Concentrated sulphuric acid was then gently poured down the side of the test tube to form a lower layer. Purple interfacial rings indicate the presence of carbohydrate.

Test for oils

About 0.1 g of the extract was passed between filter paper and the paper observed. Translucency of the filter paper indicates the presence of oils.

Test for resins

Extract plus 15 ml of 96 % ethanol plus poured into 20 ml of distilled water in a beaker. A precipitate occurring indicates the presence of resins.

Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA followed by, Dunnet's test (SPSS-15). Differences between groups were considered significant at $p < 0.05$ levels. All values were expressed as Mean \pm SEM.

Result and Discussion

Acute toxicity studies

The LD₅₀ of ethanolic stem bark extract of *Petersianthus macrocarpus* was 5000 mg/kg. Behavioural signs of toxicity observed in mice given 5000 mg/kg and above include drowsiness, weakness, reduced activity in treated mice and paw licking. However, no mortality was observed in mice after oral administration of the *Alstonia boonei* ethanolic leaf extract, even at doses as high as 5000 mg/kg signifying that the oral LD₅₀ was greater than 5000 mg/kg. There was mild drowsiness and mild weakness, little reduced activity in treated mice; *Alstonia boonei* leaf extract did not produce any major clinical signs of toxicity in mice during a 4-day observation period. Generally, there was reduced activity among the mice.

Antiplasmodial activity

Suppressive effect

The results of this study (shown in Tables

and 2) indicated that the ethanolic leaf extract of *Alstonia boonei* and stem bark of *Petersianthus macrocarpus* displayed some activity against *P. berghei* malaria parasite.

The plant extracts exhibited potent dose-dependent activity at the various doses employed (100, 200 and 400mg/kg). *P. macrocarpus* gave 8.80 ± 1.53 %, 4.00 ± 0.45 % and 3.80 ± 0.58 chemo-suppression respectively and *A. boonei* gave 6.00 ± 1.14 %, 4.20 ± 0.80 % and 2.80 ± 0.86 % chemo-suppression respectively. The standard drug (chloroquine 5 mg/kg) exhibited 4.2 ± 0.97 % chemo-suppression and this value was approximately equal to the chemo-suppression produced by the 200 mg/kg dose of both plant extracts. The chemo-suppression effect of both extracts at all doses employed in this study were significantly ($p < 0.05$) greater than the value obtained from the negative control group (distilled water, 5 ml/kg).

Curative effect

The results of the curative test (Tables 3 and 4) showed that ethanolic leaf extract of *A. boonei* and stem bark extract of *P. macrocarpus* caused a dose- dependent reduction in mean parasitemia in mice, the extent of which is similar to those of chloroquine treated groups, while the control group showed a daily increase in parasitemia.

Results showed that chloroquine (5 mg/kg) gave a percent reduction in parasitaemia of 2.00 ± 0.55 % as compared to 9.60 ± 0.68 %, 4.00 ± 0.32 % and 1.60 ± 0.24 % observed with 100, 200 and 400 mg/kg of *A. boonei* extract and 13.00 ± 0.84 %, 5.60 ± 0.51 % and 2.40 ± 0.24 of *P. macrocarpus* extract.

Table.1 Suppressive effect of *P. macrocarpus* ethanolic stem bark extract and chloroquine against *P. berghei berghei* infection in mice

Treatments	Parasitaemia (%±SEM)
Distilled water 5mLkg ⁻¹	11.00 ± 3.27
Extract 100 mgkg ⁻¹	8.80 ± 1.53*
Extract 200 mgkg ⁻¹	4.00 ± 0.45
Extract 400 mgkg ⁻¹	3.80 ± 0.58
Chloroquine 5 mgkg ⁻¹	4.20 ± 0.97

*significantly different from the control at p≤0.05.

Table.2 Suppressive effect of *A. boonei* ethanolic leaf extract and chloroquine against *P. berghei berghei* infection in mice

Treatments	Parasitaemia (%±SEM)
Distilled water 5mLkg ⁻¹	11.00 ± 3.27
Extract 100 mgkg ⁻¹	6.00 ± 1.14
Extract 200 mgkg ⁻¹	4.20 ± 0.80
Extract 400 mgkg ⁻¹	2.80 ± 0.86
Chloroquine 5 mgkg ⁻¹	4.20 ± 0.97

Table.3 Curative effect of *P. macrocarpus* ethanolic stem bark extract and chloroquine against *P. berghei berghei* infection in mice

Treatments	Parasitaemia (%±SEM)
Distilled water 5mLkg ⁻¹	13.00 ± 1.10
Extract 100 mgkg ⁻¹	13.00 ± 0.84*
Extract 200 mgkg ⁻¹	5.60 ± 0.51*
Extract 400 mgkg ⁻¹	2.40 ± 0.24
Chloroquine 5 mgkg ⁻¹	2.00 ± 0.55

*significantly different from the control at p≤0.05.

Table.4 Curative effect of *A. boonei* ethanolic leaf extract and chloroquine against *P. berghei berghei* infection in mice

Treatments	Parasitaemia (%±SEM)
Distilled water 5mLkg ⁻¹	13.00 ± 1.10
Extract 100 mgkg ⁻¹	9.60 ± 0.68*
Extract 200 mgkg ⁻¹	4.00 ± 0.32*
Extract 400 mgkg ⁻¹	1.60 ± 0.24
Chloroquine 5 mgkg ⁻¹	2.00 ± 0.55

*significantly different from the control at p≤0.05.

Figure.1 Microscopic view of non parasitized red blood

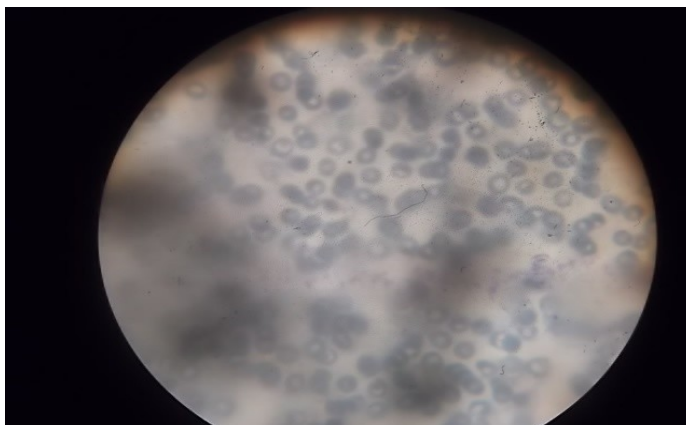


Figure.2 Microscopic view of parasitized red blood cells



Table.5 Phytochemical constituents of the extracts

Constituent	Observation	
	<i>A. boonei</i>	<i>P. microcarpus</i>
Alkaloids	+++	+++
Resin	ND	+++
Reducing sugars	ND	+++
Acidic compound	+	+
Oil	+++	ND
Steroids	++	++
Terpenoids	++	++
Glycosides	+++	+++
Flavonoids	++	+
Tannins	+++	+
Carbohydrates	+++	+++
Saponins	+++	+
Proteins	++	ND

+++ = Relatively high abundance of compound; ++ = Moderate abundance of compound; + = Relative low presence of compound; ND = Not detected

Microscopic observation of thin film slide

The microscopic images of non-parasitized and parasitized red blood cells are shown in Figures 1 and 2 respectively.

Qualitative Phytochemical analysis of the extracts

The results of the qualitative phytochemical analysis (Table 5) show that the dried ground powder of *Petresianthus macrocarpus* stem bark contains alkaloids, flavonoids, glycosides, saponins, tannins, carbohydrates, steroids, terpenoids, resins, acidic compound and reducing sugars. Proteins and fat and oil were absent in the extract. The results also showed that the dried powdered ethanolic extract of *Alstonia boonei* leaves contains alkaloids, flavonoids, glycosides, saponins, tannins, carbohydrates, protein, steroids, terpenoids, fats and oil, and acidic compounds. Resins and reducing sugars were absent in the extract.

The present study was undertaken to evaluate the antiplasmodial activity of two commonly used plants in Nigerian traditional medicine for treatment of malaria (Odugbemi and Akinsulire, 2007; Mengome Line-Edwige *et al.*, 2009). Ethnopharmacological data has been one of the common useful ways for the discovery of biologically active compounds from plants (Kuria, 2001). The big advantage of the ethnopharmacological information is that the extensive literature may already allow for some rationalization with respect to the biological potential of a reputed use (Kuria, 2001). Ethnopharmacological use of plants can therefore be a basis for phytochemical and phytopharmacological investigation.

There is consensus among the scientific community that natural products have been playing a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases, including multi-drug resistant malaria (Iyiola *et al.*, 2011; Bello *et al.*, 2009; Idowu *et al.*, 2010). Most of the existing antimalarial chemotherapeutic agents are based on natural products and this fact anticipates that new leads may certainly emerge from the tropical plant sources, since biological chemodiversity continues to be an important source of molecular templates in the search for antimalarial drugs (Iyiola *et al.*, 2011).

In this study, the antimalarial activity of ethanolic stem bark of *P. macrocarpus* and leaf of *A. boonei* in mice. The result of acute toxicity test showed that ethanolic leaf of *A. boonei* has a wide range of effective doses. The acute behavioural signs of toxicity observed were mild drowsiness and mild weakness, little reduced activity in treated mice; *Alstonia boonei* leaf ethanol extract did not produce any major clinical signs of toxicity in mice during a 4-day observation period. The oral median lethal dose (LD₅₀) was estimated to be greater than 5000 mg/kg b.wt. The observed reduced activity of the treated mice showed that the extract possess central depressant effect, consistent with previous studies (Odugbemi, T.O and Akinsulire, O.R (2007; Awe SO, Opeke, 1990; Bello *et al.*, 2009; Gbadamosi *et al.*, 2011; Idowu *et al.*, 2010; Olajide *et al.*, 2000). The absence of death following oral administration of *A. boonei* leaf ethanolic extract at 5000 mg/kg b.wt. observed in the animals indicates that the extract is practically non-toxic acutely (Iyiola *et al.*, 2011, Vasanth *et al.*, 1990; Lorke, 1983). This high safety profile might have

contributed to its widespread use in different ethno-therapeutic interventions. On the other hand, the *P. macrocarpus* was found to be toxic at 3,807.9 mg/kg. This implies that doses below this can be safe while doses above it can be detrimental to the human system, in agreement with earlier studies on the plant (Mengome Line-Edwige *et al.*, 2009; Tiwakade A. Olugbade *et al.*, 2000; Asmahan El Izzi *et al.*, 1992). The acute toxicity results however guided in the choice of doses chosen for the antimalarial studies.

Plasmodium berghei berghei parasite is used in predicting treatment outcomes of any suspected antimalarial agent due to its high sensitivity to chloroquine making it the appropriate parasite for this study (Iwu *et al.*, 1999). The anti-malarial experiment results show that ethanol extract of *Alstonia boonei* and *P. macrocarpus* possess intrinsic anti-plasmodial activity that is evident from the chemo-suppression it produced during the 4- day suppressive test. This method of 4-day suppressive testing for anti-malarial activity has become popular during scientific evaluation of potential phyto-medicines for treatment of experimental malaria (Bello *et al.*, 2009; Peters *et al.*, 1975). Our investigation of the scientific reasons behind the folkloric use of *A. boonei* and *P. macrocarpus* in the treatment of malaria attack in a traditional African setting can be partially satisfied with this result. Again, this result of the chemo-suppression and curative studies implies that the extract can suppress parasite growth to non-detectable levels in erythrocytes. The significant chemo-suppression produced by the extract on day 4 and the percentage reduction in parasitaemia in the curative test is consistent with the traditional use of the

two plants as herbal medications against malaria in Southern part of Nigeria (Odugbemi and Akinsulire, 2007; Mengome Line-Edwige *et al.*, 2009).

The phytochemical analyses of the two plant extracts showed relative abundance of alkaloids, glycosides, tannins, resins, steroids, flavonoids, terpenoids and saponins. Many alkaloids are known to have effect on the central nervous system (such as morphine, a pain killer) and some act as anti-parasitic (for example, quinine was widely used against *Plasmodium falciparum*) (Elisabetsky and Costa-Campos, 2006). Previous reports indicates that alkaloids could be highly responsible for the antimalarial activity of these plants (Oigiangbe *et al.*, 2010; Oigiangbe, 1984). From Table 5 it could be seen that *Alstonia boonei* has higher quantities of tannins, saponins, terpenoids, steroids, glycosides, proteins, fats and oil, and acidic compounds (which are the core anti-plasmodial agents) than *Petersianthus macrocarpus*; these could be responsible for its greater antimalarial efficacy than *Petersianthus macrocarpus*. Steroidal compounds are known to behave like hormones, owing to their structural semblance. From phytochemical screening it is found that both plants contain steroids which has played vital role in the treatment of cerebral malaria, thus these steroids might have contributed to the anti-plasmodial activity seen in these plants (David *et al.*, 2004). Tannins have also been found to be potentially anti-viral, anti-bacterial and anti-parasitic agents (Ene *et al.*, 2008). Having found to be higher in *Alstonia* than *Petersianthus* as shown in Table 5 as well as considering its antimalarial property, it could be inferred therefore that this would have contributed to the higher efficacy of *A. boonei* when compared with *P.*

macrocarpus. Artemisinin such as glycosides, terpenoids etc. act more rapidly than other types of anti-malarial, both in killing parasites and in inhibiting their major metabolic processes, such as glycolysis, nucleic acid and protein synthesis (Vasanth *et al.*, 1990; Odugbemi and Akinsulire, 2007). Artemisinins also attack the broadest age-range of parasites, from the tiniest rings that have recently invaded erythrocytes to more mature stages of parasites such as developing trophozoites and schizonts (Awe and Opeke, 1990). Their relatively broad stage-specificity of action extends to an ability to impede the development of gametocytes (Olajide *et al.*, 2000). High concentration of glycosides and terpenoids in the plants particularly *Alstonia* might have contributed to their high anti-plasmodial activity and higher anti-plasmodial activity seen in *Alstonia boonei*. Other metabolites present in these plant extracts as presented in Table 5 are known to elicit a number of pharmacological responses and justifies why these plants are used traditionally in combating malaria. Overall, the anti-plasmodial properties observed in these plant extracts may be attributed to the presence of flavonoids, tannins, terpenes and alkaloids or even a combined action of more than one metabolite (Gbadamosi *et al.*, 2011).

The results presented herein suggest that the ethanolic extracts of *A. boonei* leaves and *P. macrocarpus* stem bark are safe and possess potent antimalarial activity which justified their continuous folkloric use as antimalarial remedies. The oral median lethal dose of the extracts in mice was determined to be 3,807.9 mg/kg b. wt. for *P. microcarpus* and >5000 mg/kg b. wt. for *A. boonei*. Comparatively, the antimalarial efficacy of ethanol extract of

A. boonei leaf is greater than that of *P. macrocarpus* stem bark but the antimalarial effects of both plants were significantly greater ($p < 0.05$) than that of the standard antimalarial drug (chloroquine, 5 mg/kg) at certain dose levels. The significantly higher antimalarial potency of *A. boonei* than *P. macrocarpus*. could be attributed to the high level concentration of the core anti-plasmodial agents such as alkaloids, tannins, terpenoids, and glycosides. However, further phytopharmacological investigations is ongoing in our laboratory to isolate, identify and characterize the active molecule(s) responsible for the observed effect of antimalarial effect from these plants.

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