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## Evaluation of the Antimalarial Potential of *Vernonia ambigua* Kotschy and Peyr (Asteraceae)

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**Abstract:** Some traditional medicine practitioners in Nigeria have claimed the use of the decoction of the whole plant of *Vernonia ambigua* for the management of malaria. The aim of this study is to authenticate the antimalarial potential of this plant by evaluating its antiplasmodial activities. The freeze-dried decoctions of the whole plant of *V. ambigua* were used for the study. The phytochemical components and antioxidant activity using 2, 2-Diphenyl-1-picryl-hydrazyl radical (DPPH) were determined. The oral median lethal dose (LD<sub>50</sub>) and *in vivo* antiplasmodial activity were determined in Swiss albino mice. Different doses of the extract (50, 100, 200, 300, 400, 500 and 600 mg kg<sup>-1</sup> PO) were administered to the mice infected with 1×10<sup>7</sup> *Plasmodium berghei berghei*. Four days suppressive and curative effects against established infections as well as prophylactic activities were evaluated. The *in vitro* antiplasmodial activity was carried out on *Plasmodium falciparum* using different concentrations of the decoction. The decoction showed the presence of alkaloids, flavonoids, tannins, saponins, sterols, phenols and reducing sugars and a moderate antioxidant activity. The LD<sub>50</sub> was estimated to be greater than 5000 mg kg<sup>-1</sup>. Effective dose dependent inhibitions of parasitaemia were observed in the suppressive, curative and prophylactic tests. The *in vitro* screening also showed a moderate antiplasmodial activity (31.62 µg mL<sup>-1</sup><IC<sub>50</sub><50 µg mL<sup>-1</sup>). The effective antiplasmodial activities of *V. ambigua* could be attributed to its content of certain phytochemicals and may partly explain its use for the treatment of malaria.

**Key words:** *Vernonia ambigua*, antiplasmodial activity, phytochemical components, antioxidant property, oral median lethal dose

### INTRODUCTION

Malaria is an infectious disease which is caused by the protozoan *Plasmodium* parasite and is responsible for about 515 million disease cases, killing between one and three million people each year. Majority of these disease cases and deaths occur in sub-Saharan Africa where the disease is endemic (Greenwood and Mutabingwa, 2002; De Ridder *et al.*, 2008). In some communities in Africa excessive mortality due to the disease has been reduced by the ability of the local traditional medical practitioners to manage the disease (Okigbo and Mmeka, 2006).

Plants from different botanical sources have been used by various Traditional Medical Practitioners (TMPs) for the treatment and cure of malaria (Asase *et al.*, 2005;

Jullian *et al.*, 2006). Numerous claims by the TMPs on the potency and use of various plants species for the treatment of malaria abound. Only few of these claims have been authenticated by scientific investigations (Elujoba *et al.*, 2005).

A mini botanical survey of ten selected local government areas in North central Nigeria was conducted with the aim of collecting plants or recipes used for treating malaria. Seven out of the twenty TMPs interviewed claimed the use of the decoction of the whole plant of *Vernonia ambigua* for the management of malaria. The claim states that a prescribed quantity of the decoction of the whole plant when administered twice daily for five to ten days achieves radical cure for a suspected malaria infection and when similar quantities

are administered twice weekly protect individuals from the infection (Builders *et al.*, 2007).

*V. ambigua* known as “Orungo” in “Yoruba” and “Tab-tabá or Tattaba” in “Hausa” is an annual shrub belonging to the family Asteraceae/Compositae. The shrub is erect, coarse and bushy in nature, growing up to 600 mm high. The stem is erect, woody and ribbed as well as hairy and leafy especially at flowering time. The leaves are simple and alternate oblanceolate with a dimension of 20-60 mm long and 6-10 mm wide. The upper leaves have serrated margins and are coarsely hairy. The floescence consists of cluster of flower heads 10-15 mm in diameter with pail bluish or mauve floret usually at the end of leafy branches. The plant is easily propagated through seeds and stem cutting is also grown in other parts of Africa where they also have medicinal uses (Akobundu and Agyakwa, 1998). In Tanaganyika the roots are chewed raw or taken as a decoction for the treatment of cough and fever (Burkill, 1995); the leaves are taken orally for treating male or female infertility (Focho *et al.*, 2009).

Apart from the recent investigations on the antimicrobial activities of *V. ambigua* (Kunle and Egharevba, 2009), there is no scientific document authenticating or collaborating the antiplasmodial potential of *V. ambigua*. However, similar species such as *V. amygdalina*, *V. cinerea* and *V. colorata* have been investigated and found to possess various degrees of antiplasmodial activities (Njan *et al.*, 2008; Kraft *et al.*, 2000). Also various ethno-botanical surveys of herbal medicines used for the treatment of malaria fever in communities in Southwest Nigeria contained *V. amygdalina* in all the reports (Odugbemi *et al.*, 2007; Idowu *et al.*, 2010). The aim of this study therefore is to authenticate the antimalarial potential of the aqueous decoctions of the whole plant extract of *V. ambigua* by evaluating its antiplasmodial activities.

## MATERIALS AND METHODS

**Plant materials:** The whole plant (leaves, stems and barks) of *V. ambigua* was collected in the month of February, 2009 in Chaza village in Niger state, Nigeria. The identification and authentication were done by (Ethno botanist) Mallam Muazam Wudil, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, (NIPRD), Abuja, Nigeria where a voucher specimen (NIPRD /H/6302) was deposited at the herbarium for reference.

**Extraction of plant materials:** The plant material was air dried under shade and samples were pulverized to a

coarse powder. A 100 g quantity of the coarse powder was boiled with 1 L of distilled water for 30 min. The decoction was decanted, centrifuged at 4500 rpm (Erweka, Germany) for 30 min and freeze-dried. The total yield of dark green extract was 11.54% w/w of crude starting material. The freeze-dried powder was stored in an airtight container and used for the study.

**Animals:** A total of one hundred and eighty Swiss albino mice (20-25 g body weight) were used, eighteen for acute toxicity study while fifty-four were used for each of the *in vivo* antiplasmodial test. These were obtained from the animal facility centre of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and used for the study. The animals were fed *ad libitum* with standard feed (Ladokun feeds, Ibadan, Nigeria) and had free access to water. They were also maintained under standard conditions of humidity, temperature and 12 h light/darkness cycle. The animals were acclimatized for two weeks before the commencement of the study. A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulations (ENV/MC/CHEM (98) 17, 1998). The principle of laboratory animal care (NIH Publication No. 85-23, 1985) was also followed in this study.

**Malaria parasites:** The chloroquine sensitive *Plasmodium. berghei berghei* (NK-65) obtained from the National Institute for Medical Research (NIMR), Lagos, Nigeria and kept at the Department of Pharmacology and Toxicology, NIPRD, Idu, Abuja, Nigeria. The parasites were kept alive by continuous re-infestation (I.P) in mice (Calvalho *et al.*, 1991) every four days.

**Inoculums:** Parasitized erythrocytes were obtained from a donor- infected mouse by cardiac puncture in heparin and made up to 20 mL with normal saline. Animals were inoculated intraperitoneally with infected blood suspension (0.2 mL) containing  $1 \times 10^7$  parasitized erythrocytes on day zero. Infected mice with parasitaemia of 5-7% were allocated to nine groups of six mice each (Hilou *et al.*, 2006).

**Chemicals:** All chemicals were purchased from Sigma-Aldrich, USA.

**Phytochemical tests:** The phytochemical screening of *V. ambigua* whole plant aqueous extract was carried out to determine the presence of the following compounds; alkaloid, flavonoids, tannins, anthraquinones, saponins, glycosides, sterols, resins, volatile oil, terpenes and phenols using standard procedures.

The presence of alkaloid was detected by boiling and filtering 10 % w/v solution of the extract in acid alcohol. Five milliliter quantity of the filtrate was added 2 mL of dilute ammonia and 5 mL of chloroform were added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids (Trease and Evans, 1996).

Isoquinoline alkaloids were determined by adding to a few drops of Froede's reagent and a crystal of sodium chloride to 2 mL of acidic extract. Indole alkaloids were determined by adding 1 mL of concentrated sulfuric acid and a crystal of potassium dichromate were added to 2 mL of acidic extract (Mukherjee, 2006).

The presence of flavonoids was detected by two methods. First, dilute ammonia (5 mL) was added to a portion of an aqueous filtrate of the extract. Concentrated sulfuric acid (1 mL) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids. Second, 10 mL the extract was heated with 5 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids (Sofowora, 1993; Igwe, 2004).

Tanins were detected by boiling and filtering a 5% w/v aqueous solution of the extract. A few drops of 0.1% ferric chloride were added to 10 mL of the filtered solution. A brownish green or a blue-black colouration indicated the presence of tannins (Trease and Evans, 1996; Oloyede, 2005).

Anthraquinones were detected using the Borntrager's test. One gram of the powdered extract was shaken with 10.0 mL of benzene. This was filtered and 5.0 mL of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a violet colour in the ammoniacal (lower) phase indicated the presence of free hydroxy anthraquinones (Trease and Evans, 1996; Oloyede, 2005).

Saponins were detected by vigorously shaking 0.5 g of extract in 5 mL of distilled water in a test tube to observe a stable froth persistent on warming (Oloyede, 2005).

The presence of cardiac glycosides was detected using the Keller-Killiani test. To 5 mL of a 10% w/v solution of the extract in distilled water was added to 2 mL of glacial acetic acid containing one drop of ferric chloride solution and 1 mL of concentrated sulfuric acid. A brown

ring was formed at the interface. A violet ring also appeared below the brown ring while in the acetic acid layer a greenish ring was also formed just above the brown ring and gradually spread throughout this layer (Oloyede, 2005).

Terpenoids were detected using the Salkowski test. 2 mL of chloroform was added to 10 mL of a 10% w/v solution of the extract and shaken. Three milliliter of concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration was formed at the chloroform water interface (Trease and Evans, 1996).

Sterols were detected using the Liebermann-Burchard test for sterols. To 0.2 g of the powdered extract was added to 10 mL of chloroform and shaken to ensure dissolution. Two milliliter of acetic acid was added to the solution and cooled well in ice followed by the addition of concentrated H<sub>2</sub>SO<sub>4</sub> carefully. Blue, green ring indicate the presence of sterols (Sofowora, 1993).

Phenols in the in the extract was detected by dissolving 0.2 g of the powdered extract in 5 mL of ethanol. Two drops of 1M ferric chloride was added. The appearance of intense color indicated the presence of phenolic groups (Sumitra *et al.*, 2006).

Reducing sugars were detected by dissolving 0.5 g of the powdered extract in 5 mL of water and added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction (Sofowora, 1993).

The presence of volatile oil was detected by boiling 5 g of the air-dried powder of the plant was boiled with 100 mL of distilled water for 3 h. Appearance of yellow colour indicates the presence of volatile oil (Mukherjee, 2006).

Resin was detected by dispersing 0.5 g of the powdered extract in 10 mL of 95% ethanol heated to boiling on a water bath for 20 min. The formation of a precipitate on addition of 5 mL distilled water indicates the presence of resins (Mukherjee, 2006).

**Antioxidant potential:** Ascorbic acid was used as the antioxidant standard and the methanol soluble portion of the freeze-dried aqueous extract of *V. ambigua* was extracted and concentrated and used for the antioxidant assessment. *V. ambigua* and ascorbic acid at equal concentrations of 0.0875, 0.175, 0.25, 0.5 and 1 mg mL<sup>-1</sup> in methanol were prepared. The radical scavenging activities of the extract against 2, 2-Diphenyl-1-picryl hydrazyl radical (DPPH) were determined by UV spectrophotometric methods (Brand-Williams *et al.*, 1995; Ayoola *et al.*, 2006). One milliliter quantities of the extract and ascorbic acid were placed in a test tube and 3 mL of methanol added followed by 0.5 mL of 1 mM DPPH in methanol. These were allowed to stand for 30 min before

the absorbances were taken at 517 nm. The blank solution consists of the same amount of methanol and DPPH. The radical scavenging activity presented as percentage inhibition was calculated using Eq. 1:

$$\% \text{ Inhibition} = [(Ab-Aa)/Ab] \times 100 \quad (1)$$

where, Ab is the absorbance of the blank sample and Aa is the absorbance of the drug (*V. ambigua* or ascorbic acid).

**Acute toxicity test:** The acute toxicity of the extract was determined by evaluating its median lethal dose (LD<sub>50</sub>) using the Lorkes method (Lorke, 1983). The test was carried out in two phases. Phase 1: Nine mice were divided into three groups (A, B and C). The three groups were administered orally with graded concentrations (10, 100 and 1000 mg kg<sup>-1</sup>, respectively) of the extract. Phase 2: Another nine mice were divided into three groups (D, E and F) each group consisting of three mice each, these also received graded concentration of 1600, 2900 and 5000 mg kg<sup>-1</sup> of the extract, respectively.

#### **In vivo antiplasmodial test**

**Suppressive test:** A total of fifty-four mice were used for this study. Each mouse was given standard intraperitoneal inoculums of  $1.0 \times 10^7$  *P. berghei berghei* parasites with the aid of a 1 mL disposable syringe. The animals were divided into nine groups of six mice each. Different doses of the extract (50, 100, 200, 300, 400, 500 and 600 mg/kg/day) were administered orally to these groups. Chloroquine diphosphate 25 mg/kg/day was given as positive control and 0.2 mL of normal saline as negative control for four consecutive days (D<sub>0</sub> to D<sub>3</sub>). On the fifth day (D<sub>4</sub>), thick blood smears were prepared and blood films were fixed with methanol. The blood films were stained with Giemsa and then microscopically examined with 100-x magnification. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice (Knight and Peters, 1980).

**Curative test:** On the first day (D<sub>0</sub>), standard inoculums of  $1 \times 10^7$  *P. berghei berghei* infected erythrocytes were injected intraperitoneally. Seventy-two hours later, the mice were divided into nine groups of six mice each. Different doses of the extract (50, 100, 200, 300, 400, 500 and 600 mg/kg/day) were administered orally to these groups. Chloroquine diphosphate (25 mg/kg/day) was given to the positive control group and 0.2 mL of normal saline to the negative control group. The extract was given once daily for 5 days. Thick blood smears were

prepared from tail of each mouse for 5 days to monitor the parasitaemia level. The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post-inoculation) in each group over a period of 28 days (D<sub>0</sub>-D<sub>27</sub>) (Ryley and Peters, 1970; Chandel and Bagai, 2010).

**Prophylactic test:** Nine groups of six mice per group were used for the study. Seven groups were administered with 50, 100, 200, 300, 400, 500 and 600 mg/kg/day doses of the extract orally. The other two groups were administered with 25 mg/kg/day of chloroquine diphosphate and 0.2 mL/mouse/day of normal saline orally. The animals were dosed for four consecutive days (D<sub>0</sub>-D<sub>3</sub>). On the fifth day (D<sub>4</sub>), the mice were inoculated with *P. berghei berghei* infected red blood cells. Seventy-two hours later, the parasitaemia level was assessed by studying Giemsa stained blood smears (Peters, 1965; Okokon *et al.*, 2005).

#### **Antiplasmodial assay**

**Patients' selection:** Three fresh blood specimens were collected from three patients suffering from fever and other malaria symptoms with confirmed infection by *P. falciparum*. Already prepared dried -in-acridine orange-stained thin smears were examined for *Plasmodium* species identification. The parasite density was determined by counting the number of infected erythrocytes among 20,000 erythrocytes from each patient, 4 mL of venous blood was collected in a tube coated with EDTA. Samples with mono-infection due to *Plasmodium falciparum* and a parasite density between 1 and 2% were used for the in vitro antimalarial tests (WHO, 2001).

**In vitro test:** The assay was performed in duplicate in a 96-well microtiter plate, according to WHO method *in vitro* micro test (Mark III) (WHO, 2001). RPMI 1640 (Sigma Company, USA) was the culture medium used for cultivation of *P. falciparum* (Flores *et al.*, 1997; Alshawsh *et al.*, 2007). Dilutions were prepared from the crude plant extract and the final concentrations prepared by dilution were (100, 50, 25, 12.50, 6.25 and 3.125 µg mL<sup>-1</sup>). Negative controls were treated with solvent and positive controls (Chloroquine phosphate) were added to each set of experiments. Fifty microliters from blood mixture media was added to each well in plate and incubated in a candle jar (with gas environment of about 3% oxygen, 6% carbon dioxide and 91% nitrogen) (Dicko *et al.*, 2003; Ridzuan *et al.*, 2005) at 37.0°C for 24-30 h. After incubation, contents of the wells were harvested and stained for 5 min in an already prepared dried -in-acridine orange reagent. The parasites were

counted in five fields of vision (>200 total cells) using a fluorescence microscope (Partec cyscope fluorescence microscope, Germany) at a magnification of 40.

**Data analysis:** Data were expressed as the Mean±Standard Error of Mean (SEM). The IC<sub>50</sub> values were determined graphically on a log dose-response curve (log concentration versus percent inhibition curves) by interpolation while those values for the antioxidant activities were calculated from the linear regression of plots of concentration of the test compounds (mg mL<sup>-1</sup>) against the percentage of inhibition of DPPH.

## RESULTS

**Phytochemical tests:** The result of the phytochemical screening of the freeze dried aqueous whole plant extract of *V. ambigua* is presented in Table 1. The analysis revealed the presence of saponins, tannins, terpenes, flavonoids, isoquinoline and indole alkaloids, phenols, reducing sugars and sterols, anthraquinone, resins and volatile oil were however, absent.

**Antioxidant potential:** Figure 1 shows the comparative antioxidant effect of the crude extract of *V. ambigua* and the ascorbic acid. The antioxidant activities of *V. ambigua* and ascorbic acid increased with increase in concentration. The sensitivity of the antioxidant activity of ascorbic acid after 0.0875 mg mL<sup>-1</sup> was reduced as a near total mopping up of the scavenging free radicals was obtained at a concentration of 0.0175 mg mL<sup>-1</sup> such that at higher concentration only small changes in antioxidant activity occurred.

**Acute toxicity tests:** The toxic signs observed after the administration of the extract were rubbing of the nose and mouth on the floor of the cage and restlessness. These signs however disappeared 24 h after the extract's administration. No death occurred in any of the doses administered to the different groups of mice. The absence of death at any of the doses up to 5000 mg extract/kg show that the LD<sub>50</sub> of the aqueous extract of the whole plant of *V. ambigua* is greater than 5000 mg kg<sup>-1</sup> P.O.

**In vivo antiplasmodial activity:** The aqueous whole plant extract of *V. ambigua* exhibited a dose dependent inhibition of parasitaemia at the different doses employed. The highest activities: 56.85, 62.98 and

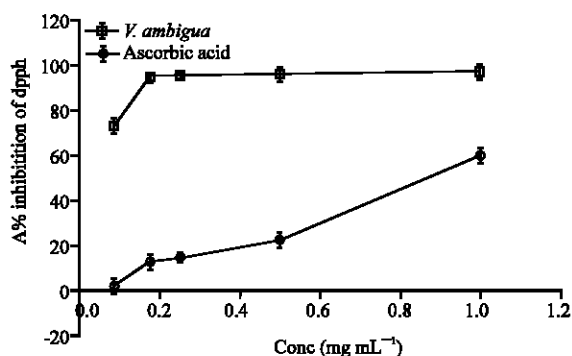


Fig. 1: Antioxidant activity of *V. ambigua* and ascorbic acid

Table 1: Phytochemical composition of the decoction of the whole plant of *V. ambigua*

Phyto-compounds	Remarks
Saponins	+
Glycosides	+
Tannins	+
Flavonoids	+
Sterols	+
Terpenes	+
Resins	-
Anthraquinones	+
Reducing sugars	+
Phenols	+
Volatileoil	-
Alkaloids	+

+ = Present; - = Absent

57.73% were obtained for suppressive, prophylactic and curative, respectively at a dose of 600 mg kg<sup>-1</sup> PO (Fig. 2).

**In vivo antiplasmodial activity of aqueous extract of whole plant of *V. ambigua* versus chloroquine:** The aqueous extract of *V. ambigua* showed dose dependent chemosuppressive activities similar to chloroquine (Fig. 3).

**Mean survival time:** The mean survival time for the extract treated mice was dose dependent and ranged from 12.5±1.5-20.2±1.0 days only mice treated with chloroquine diphosphate survived the total duration of the study (Fig. 4).

**In vitro antiplasmodial activity:** The photomicrographs of the *in vitro* antiplasmodial activity of the aqueous extract of *V. ambigua* are presented in Fig. 5. The aqueous extract of *V. ambigua* whole plant showed a concentration dependent growth inhibition of *P. falciparum* (Fig. 6). Maximum plasmodia inhibition of 60±1.8 % at a concentration of 100 µg mL<sup>-1</sup> for the aqueous extract of *V. ambigua* and 98.4±1.0% for chloroquine phosphate at a concentration of 0.2 µg mL<sup>-1</sup> (Fig. 7).

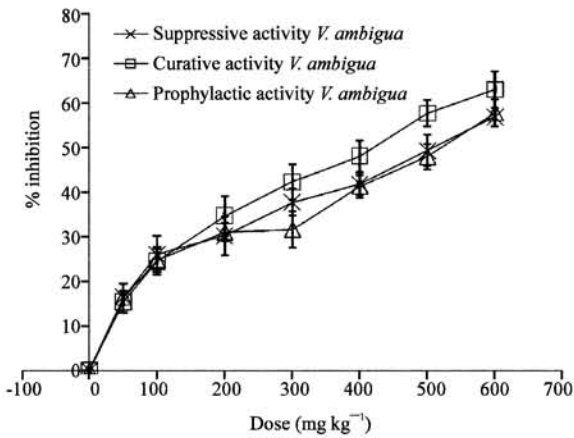


Fig. 2: Suppressive, curative and prophylactic activities of aqueous extract of the whole plant *V. ambigua*

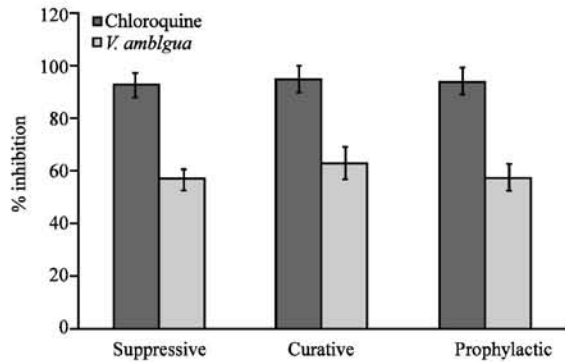


Fig. 3: Antiplasmodial activity of aqueous extract of whole plant *V. ambigua* versus chloroquine

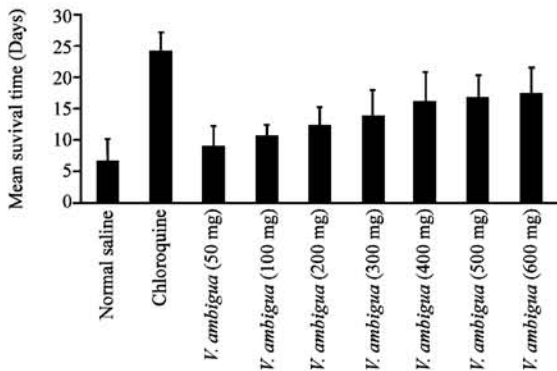


Fig. 4: Mean survival time of mice treated with aqueous plant extract of *V. ambigua*

The IC<sub>50</sub> of the aqueous extract of *V. ambigua* was determined as 31.62  $\mu\text{g mL}^{-1}$ .

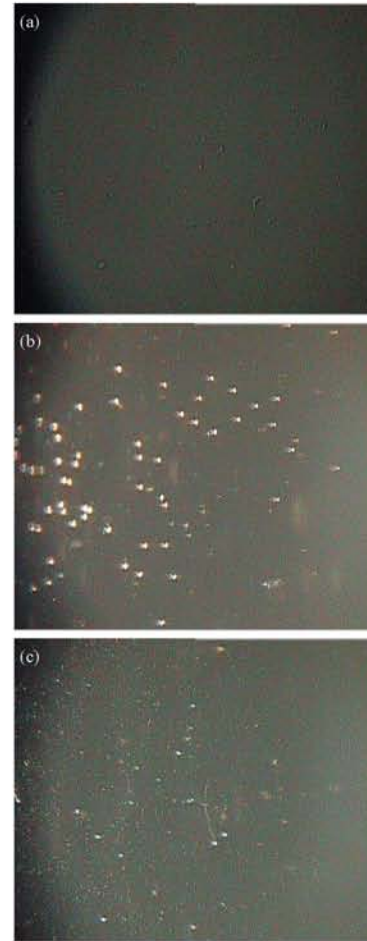


Fig. 5: Photomicrographs of *in vitro* antiplasmodial activity of the aqueous extract of *V. ambigua*, (a) complete RPMI medium (No parasites), (b) Untreated RPMI medium with *P. falciparum* and (c) RPMI medium treated with *V. ambigua* extract

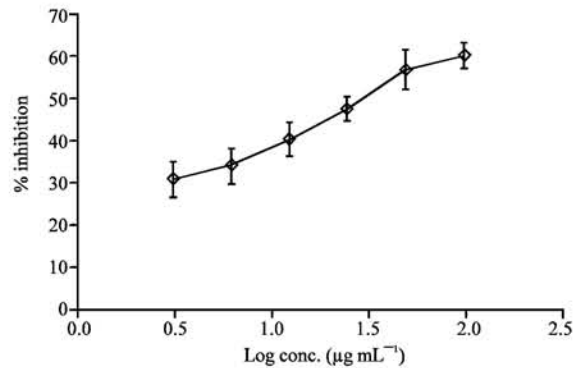


Fig. 6: *In vitro* antiplasmodial activity of aqueous extract of whole plant of *V. ambigua* on *P. falciparum*

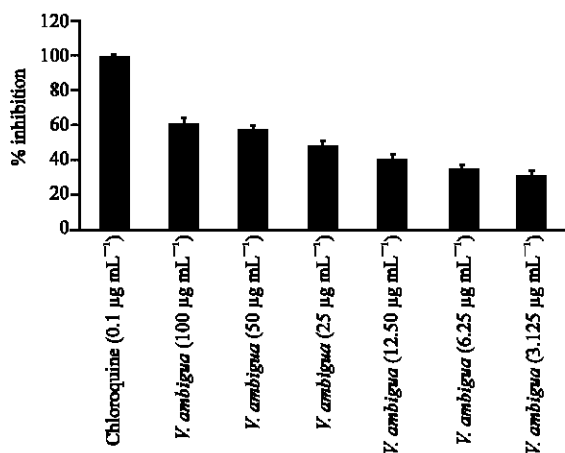


Fig. 7: Comparative inhibition effect of *V. ambigua* and chloroquine on *P. falciparum*

## DISCUSSION

Phytochemicals constitute an integral part of medicinal plants and are responsible for their numerous bioactivities. The phytochemical analysis of the freeze dried decoction of the whole plant extract of *V. ambigua* showed the presence of saponins, flavonoids, tannins, terpenes and alkaloids, phenols, reducing sugars and sterols. Similar research had also been conducted (Kunle and Egharevba, 2009), though these results were similar, but did not detect the presence of terpenes, phenols and sterols. This difference may be attributed to certain critical factors, which include time and place of plant collection and extraction technique. In present studies the technique used for extraction is that employed by the TMPs the plant was also appropriately obtained from Chaza village where the plant is commonly used for malaria treatment and the plant was harvested in February which is their optimal time of collection. The traditional use of *V. ambigua* for the treatment of malaria could be attributed to the presence of certain phytochemicals that constitute the bioactive principles in the plant. Numerous plants containing a wide variety of phytochemicals as their bioactive principle have shown antiparasitic activities (Matur *et al.*, 2009; Alshawsh *et al.*, 2007; Steele *et al.*, 1999). Although the mechanism of action of the extract has not been evaluated in the present study, some of the metabolites detected have however been implicated in antiparasitic activities by different mechanisms. The anti-plasmodial activity of *Croton zambesicus* has been tracked to the alkaloids, flavonoids and terpenes contained in the plant (Okokon *et al.*, 2005). The antioxidant flavonoids and phenolic compounds have also been shown to exert antiparasitic activity by

elevating the red blood cell oxidation and inhibiting the parasite's protein synthesis (Phillipson and Wright, 1990; Chandel and Bagai, 2010). This activity counteracts the oxidative damage induced by the malaria parasite (Ayoola *et al.*, 2008; Hilou *et al.*, 2006; Arokiyaraj *et al.*, 2008). Thus, the antiparasitic activities of *V. ambigua* can similarly be related to the presence of these same phytochemicals.

The acute toxicity of *V. ambigua* has been investigated to determine any adverse effect that may arise as a result of a single contact or multiple exposures in a short time within 24 h period. Though *V. ambigua* has been used by TMPs without any mortality due to toxicity, this claim has been authenticated by the lack of death at oral treatment of over 5000 mg kg<sup>-1</sup> body weight of the extract. The results thus suggest that the freeze dried decoction of the whole plant of *V. ambigua* is acutely non toxic (Corbett *et al.*, 1984).

Chloroquine in this study was used as the standard antiparasitic (Ajaiyeoba *et al.*, 2006). Chloroquine has been used for curative, suppressive and prophylactic antiparasitic activities. In early and established infection chloroquine interrupts with the heme polymerization by forming a FP- chloroquine complex. This complex is responsible for the disruption of the parasite's cell membrane function and ultimately leads to auto digestion. Though the chloroquine exerted a higher suppressive, prophylactic and curative antiparasitic activities by the extent of inhibition of parasitemia, the *V. ambigua* extract also indicated similar antiparasitic activities however to a lower potency. The extract also exhibited a dose dependent chemosuppressive and curative activities and also enhanced the mean survival time period of the treated mice particularly the group administered with the 600 mg/kg/day of the extract.

In this study the *in vitro* methods has been used for the assessment of the antiparasitic properties of *V. ambigua*. Apart from offering a high sensitivity to the bioactive agents been assessed it also permits the direct testing of the herbal extract on *P. falciparum* the causative agent of human malaria (NIAID Science Education, 2007).

The Cyscope fluorescence microscope is based on the use of Plasmodium nucleic acid-specific fluorescent dyes to facilitate detection of the parasites. Some of its attributes includes detection of parasites at low levels of parasitaemia due to its high contrast with the background, rapidity, sensitivity, reproducibility and ease of result interpretation (Hassan *et al.*, 2010).

According to the norm of plants antiparasitic activity of Rasoanaivo *et al.* (1992) *V. ambigua* (31.62 µg mL<sup>-1</sup>) has a moderate antiparasitic activity as its IC<sub>50</sub> is less



than 50  $\mu\text{g mL}^{-1}$ . The moderate antiplasmodial activity of *V. ambigua* (31.62  $\mu\text{g mL}^{-1}$ ) when compared with that of the standard, chloroquine phosphate (0.042  $\mu\text{g mL}^{-1}$ ), this result is similar to those obtained for other medicinal plants material with high antiplasmodial potential. The relative higher values of  $\text{IC}_{50}$  obtained for the extract is due to crude nature of the bioactive materials. The decrease in parasitaemia with increasing concentration of the extract also reflects an inhibitory activity on parasite replication. This may be indicative of a significant potential for isolating purer compound (Palamiswamy *et al.*, 2008).

### CONCLUSION

The phytochemical assessments of the decoction of the whole plant of *V. ambigua* showed the presence of flavonoids, tannins, terpenes, alkaloids, phenols, saponins, sterols and reducing sugars some of which are implicated with antioxidant and antimalarial activities. The high value of the  $\text{LD}_{50}$ , effective *in vivo* and *in vitro* antiplasmodial activities could partly explain its safety, effectiveness and traditional use for the treatment of malaria. Though the *in vivo* and *in vitro* antiplasmodial assessment of the decoction showed lower activities when compared to chloroquine phosphate, fractionation of this crude extract may yield purer compounds with more potent antiplasmodial activities and could be a source of potential leads to new antimalarial molecules.

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