Ifeoma C.Ezenyi *et al.* / *International Journal of Phytopharmacology*. 7(1), 2016, 1-9.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

STACHYTARPHETA CAYENNENSIS METHANOL LEAF EXTRACT AND SOLVENT FRACTIONS DISPLAY ANTIPLASMODIAL ACTIVITY AGAINST *PLASMODIUM BERGHEI BERGHEI IN VIVO*

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ABSTRACT

Stachytarpheta cayennensis L.C. Rich (Verbenaceae) is used traditionally in Nigeria for the treatment of febrile illnesses, including malaria. The present study was aimed at investigating the antiplasmodial effects of *S. cayennensis* leaf extracts. A methanol extract (ME) and successive hexane (HF), dichloromethane (DCMF) methanol (MF) and aqueous (AF) fractions of *S. cayennensis* leaves were prepared. Elemental, phytochemical and total phenolic analyses of the methanol extract were carried out. Oral acute toxicity test of the methanol extract was performed in mice. The antiplasmodial activity of the extracts was determined against *Plasmodium berghei berghei* infection in mice. The antioxidant effect of the methanol extract was evaluated using phenylhydrazine-induced haematotoxicity and against DPPH radical. The oral LD_{50} of the extract in mice was 3535 mg/kg. All the elements in the plant material were within normal limits and there was no trace of lead (Pb). Glycosides, flavonoids and other phenolic compounds were detected in the extract. The methanol extract significantly ($p<0.05$) suppressed parasitaemia in early, repository and established infection while successive solvent fractions (100 mg/kg each) significantly ($p<0.05$) suppressed parasitaemia by 87.84 (HF), 84.32 (DCME), 97.57 (MF) and 94.59% (AE). The methanol extract also inhibited phenylhydrazine-induced haematotoxicity and scavenged DPPH in a concentration dependent manner (EC_{50} = 37 µg/ml). These findings indicate that *S. cayennensis* leaf extracts possesses potent antiplasmodial activity which may be mediated in part through antioxidant effects.

Key words: *Stachytarpheta cayennensis*, Extracts, malaria, Anti-oxidant, Phytochemistry.

INTRODUCTION

Despite efforts to curb malaria,its incidence still persists in many tropical and sub-tropical regions of the world. The economic burden of the disease is prevalent in sub-Saharan Africa, where many children suffer at least one episode of malaria infection before they attain the age of five. Recent estimates show that in 2012 alone, approximately 482,000 young children died from malaria in sub-Saharan Africa (WHO, 2013). Although it is evident that some reduction in malaria – related mortalities has been recorded (O'Meara, *et al*., 2010), there is a continuous threat of antimalarial drug resistance and its spread as there are recent reports of parasite resistance to currently used antimalarials in some malaria endemic areas (Van Hong *et al.*, 2014; Talundzic *et al.*, 2015; Tun *et al.*, 2015). The malaria parasite, *Plasmodium falciparum* is the most deadly of all the malaria causing *Plasmodium* species and causes deadly forms of malaria, especially in individuals with low immunity to the disease (Hochman and Kim 2012, Crompton *et al.*, 2014). Untreated malaria in such individuals after the onset of fever rapidly develops into cerebral malaria, neurological deficit and death. Malaria in pregnancy still remains a major cause of maternal death, abortion, stillbirth, premature delivery and low birth weight (Guyatt and Snow 2004). *Plasmodium*

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falciparum developed resistance to relatively cheap antimalarials such as chloroquine, sulphadoxine, pyrimethamine and it became necessary to adopt the artemisinin-based derivatives and their combinations with other antimalarial drug classes for malaria therapy. Although this has recorded significant success in the fight against malaria, there are increasing reports of parasite resistance to artemisinin based combinations in some areas of high transmission and drug burden (Vijaykadga *et al*., 2006; Rogers *et al.,* 2009; Carrara *et al.,* 2009). In the face of this challenge and with limited drug options, it is important to search for effective alternatives. Prospecting higher plants with history of use against febrile illnesses for new antimalarial compounds afforded the discovery of quinine and artemisinin (Klyaman 1985; Bruce-Chwatt 1998). This favors the search for new antimalarial leads from plant sources and many studies have reported the antiplasmodial effects of different plant extracts (Benoit *et al.,* 1996; Abiodun *et al.,* 2011; Venkatesalu *et al*., 2012). *Stachytarpheta cayennensis* L.C. Rich (Verbenaceae) is used traditionally as fever medicine and for stomach ache (Burkill, 2000). In Trinidad, the leaf-sap is an eye wash for opthalmia, drunk for dysentry, worm infestation, chest colds and heart failure, while the leaf infusion is taken as a tea for heat and fever (Wong, 1976). Preparations of the leaves are used for the treatment of acute symptoms of malaria in south eastern Nigeria. Previous studies on extracts of this plant revealed its antispasmodic, antiinflammatory, antimicrobial, antidiabetic and antiulcer properties (Vela *et al*., 1997; Schapoval *et al*., 1998; Adebajo *et al*., 2008; Okoye *et al*., 2010; Souza *et al*., 2010). A number of phytochemical compounds responsible for some of these pharmacological activities have been isolated. These include ipolamide, verbascoside, betulinic acid, martinoside, hydroxyipolamide, ipolamide, isoverbascoside, leucosceptoside A and Jionoside D (Schapoval *et al*., 1998; Adebajo *et al*., 2008; Froelich *et al*., 2008; Souza *et al*., 2011).

Although an earlier report has documented a preliminary antiplasmodial activity (Okokon *et al*., 2008) of an extract of this plant, the present study evaluated different extracts of the plant to identify the most active fraction for further investigation (Rasoanaivo *et al*., 2004). There is also a documented evidence for the role of adjunctive antioxidant therapy in malaria which prevents the oxidative stress caused by the malaria toxin responsible for the development of complicated disease (Reis *et al*., 2010). Based on these, the present study was designed to evaluate the antiplasmodial profile of leaf extracts of *S. cayennensis* and its antioxidant action, which may influence its antimalarial potency.

MATERIALS AND METHODS

Drugs

Chloroquine sulphate, Folin Denis reagent, gallic acid, 1, 1-diphenyl-2-picrylhydrazyl-radical (DPPH), ascorbic acid, phenylhydrazine (Sigma Aldrich, Germany) were sourced through a regional representative. All solvents and other reagents used were of analytical grade.

Parasite strain

Chloroquine-sensitive *Plasmodium berghei berghei* (NK 65 strain) was sourced from the National Institute for Medical Research (NIMR), Lagos, Nigeria. The parasite was maintained in the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD) by continuous reinfection through intraperitoneal passage in mice.

Animals

Adult male and female Swiss mice (18 – 35 g) of both sexes obtained from the Animal Facility Center of the National Institute for Pharmaceutical Research and Development (NIPRD) were used. The mice and rats were housed separately in plastic cages at room temperature in a well-ventilated room, fed with standard rodent chow and allowed free access to potable water. All experiments were carried out in accordance with NIH Guide for the Care and Use of Laboratory Animals (NIH, 2011) and NIPRD's standard operating procedures. Prior to each study, the mice were acclimatized for two weeks to laboratory conditions in the Animal Facility Centre.

Collection and identification of plant material

Fresh leaves of *S. cayennensis* were collected in July from Orba, Enugu State, Nigeria (coordinates: 6^{o} 51' 24" North, 7^{o} 23' 45' East).The leaves were identified at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka. A voucher specimen of the plant material was prepared and deposited in InterCEDD (InterCEDD/52). The leaves were air-dried under shade for two weeks and milled mechanically to coarse power.

Elemental analysis

The elemental analysis of the extract was done according to a method described previously (Samali *et al.,* 2012). An atomic absorption spectrometer was employed under standard conditions using a gas mixture of air-acetylene. Concentrated nitric acid and deionized water were used for the standard and sample preparation. Reference standard solutions of calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), lead (Pb), zinc (Zn) and aqueous plant extract (1 g/L) were prepared in deionized water and used for the analysis. Working standards of the metals of interest were prepared by dilution of the analytical grades of the stock standards appropriately. The equipment was calibrated with the working standard for

the investigation of the element of interest prior to the sample analysis. Concentrations of metals were calculated as:
Actual Concentration = Conc. Read (µg/ml) x V x M;
Where V = volume of the sample taken and M = further dilution ratio where necessary.

Preparation of extracts

A sixty gram (60 g) quantity of *S. cayennensis* leaf powder was extracted by cold maceration with 500 ml methanol. The mixture was initially agitated using a mechanical shaker for 3 h then allowed to stand for 24 h. After 24 h, the mixture was filtered under vacuum and the filtrate concentrated by rotary evaporation under reduced pressure. The concentrate was dried on a hot water bath maintained by 50^{o}C to give 3.25 g of the methanol extract (ME).

A separate batch of the powdered plant material (2.5 kg) was subjected to solvent-guided extraction with n-hexane (10 L), dichloromethane (8 L) and methanol (22.5 L) in a soxhlet extractor to give the corresponding extracts in increasing polarity. The residual plant material was air-dried and extracted with distilled water (10 L) by cold maceration for 24 h with occasional mechanical agitation. The macerate was filtered and the filtrates were individually concentrated in a rotary evaporator to obtain the hexane (HF; 19.75 g), dichloromethane (DCMF; 15.75 g), and methanol (MF; 207.5 g) fractions. The aqueous fraction was freeze dried to obtain the aqueous extract (AE; 147.5 g). All the extracts were transferred to air-tight glass vials and stored in a refrigerator until required for experimental use.

Phytochemical analysis of methanol extract

Phytochemical screening of the methanol extract for identification of the phytoconstituents was carried out in accordance with standard procedures (Trease and Evans 1983).

Acute toxicity study of methanol extract

Acute toxicity testing of the methanol extract was estimated in mice using a modification of Lorke's method (Lorke 1983) in two phases. In the first phase, two groups of three mice each were orally given 300 and 1000 mg/ kg of the extract respectively and monitored for physical signs of toxicity such as writhing, decreased motor activity, respiratory depression and death. In the second phase, three groups of three mice each were orally administered 1250, 2500 and 5000 mg /kg respectively and monitored for physical signs of mortality. In both phases, the number of deaths that occurred in any of the groups within 24 h was recorded. The median lethal dose in mice (LD_{50}) was calculated as the geometric means of the maximum dose that produced 0% mortality and the minimum dose that produced 100% mortality (Lorke, 1983)

Determination of total phenolic content of the methanol extract

A. optimized method of George *et al*., (2005) was adopted with slight modification for phenolic estimation. All the tests were carried out in duplicates.

Preparation of gallic acid calibration plot

A 1 mg/ml stock solution of gallic acid was prepared by weighing 20 mg of gallic acid and dissolving with distilled water. Serial dilutions of the stock solution (0.03125 – 0.5 mg/ml) were prepared and treated as follows: A 2.5 ml of Folin Denis reagent (diluted 1:9 in distilled water) was added to 3 ml of gallic acid solution. The mixture was allowed to stand for two minutes at room temperature after which 2 ml of sodium carbonate solution (75 g/L) was added. The resulting mixture was maintained at 50^{o}C for 15 min in a water bath and afterwards, cooled in ice – cold water for 3 min. The final mixture was diluted with 15 ml of distilled water and absorbance was read spectrophotometrically at 760 nm.

Phenolic content assay of extract

A solution of the extract (5 mg/ml) was prepared in distilled water and tested as described for gallic acid. An extract blank was prepared by replacing Folin Denis reagent with distilled water, to correct for absorbance produced by extract alone. Concentration of phenolics was determined from the standard gallic acid plot. The result obtained was expressed as gallic acid equivalent per gram of extract.

Antioxidant activity of the methanol extract

Effect on phenylhydrazine – induced erythrocyte haematotoxicity

The assay was carried out according to the procedure described by Cazana *et al*., (1990), with slight modifications. A healthy rats were anaesthetized with ketamine (60 mg/kg) and blood was collected from the retro orbital plexus of the rat using a capillary tube into an EDTA containing tubes. The tubes were centrifuged at 1600 rpm for 5 min and the plasma discarded. From the packed cells, a 20% erythrocyte suspension in phosphate buffered saline (PBS) was prepared. For the assay, incubation mixture comprised of 1 ml of phenylhydrazine hydrochloride (0.5 mM) prepared in Phosphate buffered sulphate, 1.9 ml of different concentrations of ME (1.25 – 20 mg/ml) prepared in PBS and 0.1 ml of 20% erythrocyte suspension made to a total volume of 3 ml with PBS solution. The mixture (RBC, extract and phenylhdrazine) was incubated at 37^{o}C for 1 h then centrifuged at 1000 g for 10 min. The amount of methaemoglobin generated was measured by reading the

absorbance of the supernatant at 540 nm using a UV-VIS spectrophotometer. Suitable blank controls were kept to nullify the effect of solvents and inherent hemolysis.

DPPH radical scavenging activity

The free radical scavenging activities of the methanol extract and ascorbic acid were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH method (Mensor *et al*., 2001). Briefly, 0.01% w/v solution of DPPH was freshly prepared in methanol and was protected from light by maintaining it in the dark (covered with aluminum foil). A 150 µl volume of this solution was added to 50 µl of varying concentrations (0.0161- 1mg/ml) of extract or ascorbic acid prepared in methanol. After 30 min of incubation in the dark, absorbance was read spectrophotometrically at 518 nm. The percentage inhibition activity was calculated using:

$$100 - [\{\frac{(As - Ab)}{Ac}\} x\ 100]$$

Where As – Ab= net absorbance of sample; Ac= absorbance of control

Methanol was used as a blank, whereas DPPH solution plus methanol was used as a negative control. For each concentration, the experiment was done in triplicate. The radical scavenging activity was also expressed as EC_{50}, or the amount of antioxidant necessary to decrease the initial DPPH absorbance by 50% and was calculated by linear regression, where the abscissa (x) represents extract concentrations and the ordinate (y) represents the average percentage of scavenging capacity.

Antiplasmodial assay

Parasite inoculation

Using the parasitaemia and red blood cell count of an infected donor mouse, tail vein blood was diluted with normal saline such that 0.2 ml contained approximately $10^5 – 10^7$ parasitized red blood cells. Each experimental mouse was injected intraperitoneally with 0.2 ml of the diluted infected blood.

Suppressive antiplasmodial screening

The method of (Peters and Robinson, 1990) was adopted. Within 4 h after inoculation of mice with standard inoculum of *P. berghei berghei* on day 0, the infected mice were randomized into groups of six mice each. The treatment groups (n=6) received oral administration of one of ME (100, 200 and 400 mg/ kg), HF (100 mg/kg), DCMF (100 mg/kg), MF (100 mg/kg) and AF (100 mg/kg). Control groups received either distilled water (0.5 ml/kg) or the reference antimalarial, chloroquine (5 mg/kg). Treatment was carried out at the same time each day on days 1, 2 and 3. On day 4, thin films of tail vein blood were prepared and stained with Giemsa stain. The films were examined microscopically and parasitaemia (%) was expressed as the mean number of parasitized erythrocytes counted in 10 fields of approximately 250 erythrocytes per field, converted to a percentage.

The level of suppression of parasitaemia (%) was calculated using the following equation:

$$\frac{Pc - Pt}{Pc} \times 100$$

Where Pc= mean parasitaemia (%) of control; Pt= mean parasitaemia (%) of treated

Curative antiplasmodial test

The schizonticidal activity of ME in established infection was evaluated using the method of Ryley and Peters (1970). Thirty mice were injected intraperitoneally with dilute infected blood and on day 3, thin blood films of the mice were prepared to determine pre-treatment parasitaemia of the mice. Treatment was done as follows: Groups 1 and 2 served as controls and received distilled water (5 ml/kg) and chloroquine (5 mg/kg) respectively, while groups 3, 4 and 5 were treated with the methanol extract at 100, 200 and 400 mg/kg respectively. Treatment was continued once daily on days 4 to 6. On day 7, thin blood films made from the tail vein blood of each mouse were stained and assessed for the level of parasitaemia. The mice were subsequently monitored for survival for 30 days and mean survival time of each group was recorded.

Prophylactic antiplasmodial assay

This was done according to the method of Peters, described by (Chandel and Bagai, 2010). Thirty mice were randomized into five groups of 6 mice each and treated once daily for three consecutive days (days 0, 1, 2). Control groups 1 and 2 received distilled water (5 ml/kg) and pyrimethamine (1.2 mg/kg) respectively, while the remaining groups (3 – 4) were treated with ME at doses of 100, 200 and 400 mg/kg respectively. On the fourth day, the mice were inoculated with *P. berhgei berghei.* After 72 h, a thin blood film of each mouse was made and parasitaemia levels were assessed.

RESULTS AND DISCUSSION

The methanol extract evoked significant ($P<0.05$) dose-related reduction in parasite count in early infection and suppressed parasitaemia by 77.27, 80.46 and 100% at doses of 100, 200 and 400 mg/kg respectively (Table 1). The suppressive effect produced by 400 mg/kg of the methanol extract was similar to that produced by chloroquine (5 mg/kg). The successive fractionss also caused significant ($P<0.01$) reduction in parasite counts in early infection (Table 2). Of the four successive fractions, the methanol successive fraction caused the highest antiplasmodial activity and suppressed parasitaemia by

97.57%. It is likely that the antiplasmodial constituents of the leaves of this plant are resident in the successive methanol fraction and its further separation may afford the isolation of these compounds. Further evaluation of the methanol extract following a 5-day treatment course showed significant ($P<0.05$) reduction in parasitaemia in established infection (Table 3). The methanol extract (400 mg/kg) significantly ($P<0.05$) reduced parasiteamia and offered protection against the parasites post-infection in a non-dose related manner, a similar effect was produced by pyrimethamine (Table 4). The activity produced by constituents of the extract which may act alone or synergistically could be elicited through different parasite-dependent mechanisms like inhibition of the biomineralization of free heme in parasitic food vacuole, or host-dependent mechanisms such as the inhibition of pro-inflammatory mediators generated by the malaria toxin. It is noteworthy that the anti-inflammatory properties of *S. cayennensis* extract have been reported in a separate study (Schapoval *et al*., 1998). The malaria toxin and the malaria pigment (haemozoin) are pro-inflammatory and they generate a cascade of inflammatory response in malaria. Thus, the extract may act by modulating host response to this cascade in malaria infection.

The observation that the extract did not significantly prolong survival in infected mice after discontinuation of treatment in established infection can result from parasite recrudescence. It is likely that the antiplasmodial principles in the extract have a short biological half–life, which may have enabled the multiplication of recrudescent parasites in the absence of treatment. A similar pattern has been reported for some effective plant-based antimalarials with short half-lives (Mueller *et al*., 2004, Jiang *et al*., 2005).

The inhibition of erythrocyte haemolysis was measured as a reduction in the absorbance generated due to methemoglobin, a product of oxidized haemoglobin. Table 5 shows that the extract (2.5 – 20 mg/ml) significantly ($P<0.01$, $P<0.001$) inhibited methemoglobin formation in a concentration –dependent manner. The concentration required to produce 50% of this effect was estimated to be 6.5 mg/ml. The concentration-dependent radical scavenging effect of the extract is shown in Figure 1. Its effect was concentration-dependent up to the maximum concentration used. A 50 µg/ml concentration of the extract exhibited 68.33% radical scavenging activity, compared to 90.89% effect produced by ascorbic acid. The estimated antioxidant EC_{50} of the extract and ascorbic acid were 37 and 3.9 µg/ml respectively. The extract prevented phenylhydrazine (PHZ)-induced RBC toxicity and exhibited pronounced free radical scavenging activity, possibly mediated by its antioxidant phytoconstituents such as flavonoids. Phenylhydrazine is a potent methaemoglobin generator from erythrocytes. Its haematotoxicity results from its ability to react with oxygenated haemoglobin to form reactive oxygen radicals and methaemoglobin. The radicals in turn cause lipid peroxidation and subsequently, membrane disruption. Recent evidence suggests that PHZ haematotoxicity occurs mainly as a result of ROS-induced oxidative damage of erythrocyte proteins and their binding to membrane cytoskeleton (McMillan *et al*., 2005). The methanol extract inhibited the generation of methaemoglobin in erythrocytes exposed to PHZ and this effect is likely related to its ability to prevent oxidative damage of haemoglobin. This indicates the antioxidant role of the methanol extract which is also evident in its DPPH radical scavenging activity and is supported by earlier findings (Souza *et al*., 2010). Oxidative stress plays a crucial role in the development of systemic complications caused by malaria. Thus, the antimalarial effect of the methanol extract may be mediated in part by its ability to ameliorate oxidative stress and augment the activity of endogenous enzymatic and non-enzymatic antioxidant defense in infection. It has been proposed that parasiticidal activity of some extracts was through their ability to modulate parasitic cellular signaling pathway and not by a directly lethal effect (Percário *et al*., 2012). Their use as supplements was shown to also minimize or reverse oxidative damage induced by disease and improve treatment outcomes (Ghashghaeinia *et al.*, 2010; Reis *et al*., 2010).

In the acute toxicity test, the median lethal dose was calculated to be 3535.53 mg/kg. The estimated median lethal dose of the extract implies that the extract may be slightly toxic when administered acutely. This implies that acute administration of high doses of the extract may predispose to systemic toxicity and even death. This observation is supported by a previous investigation of the acute toxicity and median lethal dose of the extract in mice (Okokon *et al*., 2008). Significant amounts of copper, iron, manganese and zinc were found in the plant material (Table 6). Only trace quantities of calcium and magnesium were found in the sample whereas lead was undetected. Macro, micro and trace elements have important biological functions in plant and human metabolism, as co-factors in reactions (Roberts *et al*., 2000). Their deficiency or excess can affect health adversely. Their analysis is also important for quality control of plant-derived products (Welna *et al.*, 2001). The elements that were detected in *S. cayennensis* were within standard limits and at these levels may enhance metabolic physiology. For example, Iron is required by erythroid progenitor cells to proliferate and differentiate during erythropoiesis (Muñoz Gómez *et al*., 2005). The absence of lead in *S. cayennensis* signifies that it would not present a source of lead toxicity which can pose a serious health hazard. Thus, the levels of elements present

in the plant material are non – toxic and may be of benefit when and/or if consumed as food or medicine.

Phytochemical analysis revealed the presence of carbohydrates, free reducing sugars, combined reducing sugars, free anthraquinone glycosides, saponins, combined anthraquinone glycosides and oleo-resins. Flavonoids and tannins were also present but terpenes, sterols and alkaloids were absent. Phenolic content of the methanol extract was determined to be 83.36 gallic acid equivalents per gram of methanol extract. Plant phenolics comprise simple phenols, coumarins, lignins, lignans, condensed and hydrolysable tannins, phenolic acids and flavonoids (Soto-Vaca *et al*., 2012).Some secondary metabolites in higher plants have been reported to display anti-infective properties (Harvey, 2008). Anthraquinone glycosides, flavonoids and tannins found present in the extract have shown anti-infective properties and may account for the antiplasmodial activity observed (Rigano *et al*., 2007; Buzzini *et al*., 2008; Li *et al*., 2009).

Table 1. Effect of *S. cayennensis* methanol extract on early *P. berghei* infection in mice

Treatment	Dose (mg/kg)	Mean parasitaemia	Suppression (%)
Distilled water	-	11.00 ± 1.21	-
Methanol extract	100	2.50 ± 1.45*	77.27
	200	2.15 1.95*	80.46
	400	0.0 ± 0.0*	100
Chloroquine	5	0.0 ± 0.0*	100

*P<0.05; (Data analysis tests and post hoc test)

Table 2. Antiplasmodial activity of *S. cayennensis* solvent fractions on early *P. berghei*infection in mice

Treatment	Dose (mg/kg)	Mean parasitaemia	Suppression (%)
Distilled water	-	3.7 ± 0.41	-
HE	100	0.45 ± 0.24***	87.84
DCME	100	0.58 ± 0.01**	84.32
ME	100	0.09 ± 0.04***	97.57
AE	100	0.20 ± 0.13***	94.59
Chloroquine	5	0 ± 0***	100

**P<0.01; ***P<0.001(Data analysis tests and post hoc test)

HE= hexane, DCME= dichloromethane, ME= successive methanol, AE= aqueous extracts

Table 3. Effect of *S. cayennensis* methanol extract on established *P. berghei*infection in mice

Treatment	Dose (mg/kg)	Mean parasitaemia	Survival (Days)
Distilled water	-	2.88 ± 0.39	18.33 ± 0.56
Methanol extract	100	0.66 ± 0.36*	17.80 ± 0.86
	200	1.38 ± 0.89	17.80 ± 1.59
	400	0.40 ± 0.04**	18.80 ± 1.56
Chloroquine	5	0.01 ± 0.008**	20.75 ± 1.60

*P<0.05; **P<0.01(Data analysis tests and post hoc test)

Table 4. Prophylactic antiplasmodial activity of *S. cayennensis* methanol extract

Treatment	Dose (mg/kg)	Mean parasitaemia	Suppression (%)
Distilled water	-	3.00 ± 0.67	-
Methanol extract	100	2.24 ± 0.27	25.33
	200	2.74 ± 0.92	8.67
	400	0.21 ± 0.197*	93
Pyrimethamine	1.2	0.0 ± 0.0**	100

*P<0.05; **P<0.01(Data analysis tests and post hoc test)

Table 5. Anti-haematotoxic activity of *S. cayennensis* methanol extract

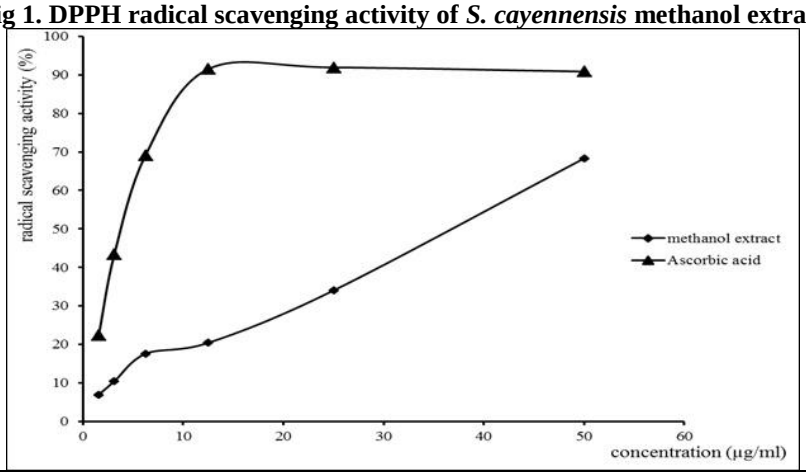
Treatment	Concentration (mg ml^{-1})	Mean absorbance[a] Due to methaemoglogin generation	Activity (%) *
Distilled water	-	0.643 ± 0.02	-
Methanol extract	1.25	0.379 ± 0.002***	41
	2.5	0.441 ± 0.05**	31.41
	5	0.375 ± 0.03***	41.73
	10	0.206 ± 0.04***	67.91
	20	0.026 ± 0.03***	97.11

* Activity was quantified as percentage (%) reduction of absorbance generated due to methemoglobin formation. [a] n = 3, mean ± SEM, **P<0.01; ***P<0.001 (Data analysis tests and post hoc test)

Table 6. Elemental composition of *S. cayennensis* leaves

S/No.	Element	Concentration(µg/g)	WHO limit(mg)
1	Calcium (Ca)	0.0125	-
2	Copper (Cu)	6.45	25
3	Iron (Fe)	33.50	100
4	Lead (Pb)	0.000	10
5	Magnesium (Mg)	0.025	-
6	Manganese (Mn)	48.15	-
7	Zinc (Zn)	18.05	25

Fig 1. DPPH radical scavenging activity of *S. cayennensis* methanol extract



CONCLUSION

These findings show the antiplasmodial potential of *S. cayennensis* leaf extract which was revealed to be enriched in the methanol successive extract. The potent antioxidant effect of the extract may likely contribute to its aniplasmodial property

ACKNOWLEDGEMENT

This study was supported by the L'Oreal – UNESCO regional fellowship for women in science, 2011, awarded to Ezenyi I.C.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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