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Original Research Article

Invivo antiplasmodial activity of the ethanol stem extract and fractions of *Citrus sinensis* in mice

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Abstract

Treatment of malaria in Africa is becoming increasingly difficult due to increasing resistance to antimalarial drugs. The invivo antiplasmodial activity of 70% ethanol extract of C*itrus sinensis* and its n-hexane, ethyl acetate, and aqueous fractions was investigated in this study. Healthy Albino mice were inoculated with *Plasmodium berghei* and a hydro alcoholic crude extract of *C. sinensis* and its solvent fractions were administered at doses of 300, 500 and 700 mg/kg. The antipyretic effect of the extract was determined in rats using Brewer's yeast- induced pyrexia. The phytochemical analysis and the acute toxicity of the crude extract were also evaluated. The crude extract and fractions showed varying degrees of antiplasmodial activity. The percentage suppression of parasitaemia was 33.65, 43.93 and 53.27% at 300, 500 and 700 mg/kg respectively. The ethyl acetate fraction was the most potent among the fractions exhibiting percentage chemo-suppression of 16.48, 19.81 and 26.42%, at 300, 500 and 700mg/kg respectively. There was no significant reduction in the rectal temperature at the doses tested (P>0.05). Acute toxicity test of the crude extract indicated an LD_{50} more than 5000 mg/kg. The qualitative phytochemical analysis revealed the presence of tannins, saponins, flavonoids, alkaloids, proteins and carbohydrates. These results indicate the potentials of the stem of *Citrus sinensis* in combating malaria.

Keywords: Antiplasmodial effect, Antipyrexia, *Citrus sinensis, Plasmodium berghei*

INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans of the genus *Plasmodium*. Commonly, the disease is transmitted by a bite from an infected female *Anopheles* mosquito, which introduces the organisms from its saliva into a person's circulatory system. Malaria causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death. (Biswajit, 2013).

The disease is widespread in tropical and subtropical regions around the equator, including much of Sub-Saharan Africa, Asia, and the Americas. The World Health Organization estimated that in 2010, there were 219 million documented cases of malaria. That year, the disease killed between 660,000 and 1.2 million people, many of whom were children in Africa (Nayyar *et al*, 2012)

Five species of *Plasmodium* can infect and be transmitted by humans. Severe disease is largely caused by *Plasmodium falciparum* while the disease caused by *Plasmodium vivax*, *Plasmodium ovale* (Sutherland et al, 2010) and *Plasmodium malariae* is generally milder, and rarely fatal. *Plasmodium knowlesi* is a zoonosis that causes malaria in macaques but can also infect humans (Singh et al, 2004).

Malaria transmission can be reduced by preventing mosquito bites using mosquito nets and insect repel-lents, or by mosquito-control measures such as spraying

insecticides and draining standing water. The production of a widely available malaria vaccine that provides a high level of protection for a sustained period is still to be met, although several vaccines are under development (Kilama and Ntoumi, 2009). The search for effective antimalarial agent is unrelenting

Several medicinal plants are employed traditionally for the treatment of malaria, and many of them have been screened for antimalarial activity (Iwalewa et al, 1990, 1997. Usha devi et al 2001, Isah et al 2003, Tona et al 2004, Subeki et al 2005, Okokon et al 2008)

The stem bark of *Citrus sinensis* (sweet oreange) (Rutaceae) is a popular antimalarial ingredient in many parts of Nigeria. Few scientific studies have been reported on the orange peel (Najimu *et al* 2003, Anna et al 2003, Dawit and Bekelle, 2010). The present study was undertaken to evaluate the antiplasmodial activity of the stem of *Citrus sinensis* using rodent model of Plasmodium.

Plant material

Fresh stem of C*itrus sinensis* was harvested in August, 2013, at Umujagwo Village, Awka, Anambra State, Nigeria. The plant specimen was identified and authenticated by Dr. J.K. Ebiguwai, a taxonomist in the Department of Botany, Delta State University, Abraka, Delta State, Nigeria.

The stems were cut into smaller piece and were allowed to dry at room temperature for seven days (Sofowora, 1982). The dried stem was reduced into powder using an electric mill. The crude extract was prepared by cold maceration technique (O'Neill *et al,* 1985). The milled stem was extracted by adding 1.4 kg of the powdered material in 6 litre of ethanol (70 %) at room temperature for three days. The extract was filtered using Whatman filter paper (No.3, 15cm size). The ethanol extract was concentrated in a rotary evaporator (Buchi TRE121, Switzerland).

A portion of the extract was fractionated with n-hexane, ethyl acetate and water to yield n-hexane, ethyl acetate and aqueous fractions respectively. The resulting fractions were concentrated in rotary evaporator

Animal

Adult Wistar rats (150-250 g) and albino mice (18-24 g) of either sex were used in the study. All the animals were procured from the Animal House of the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Nigeria. The animals were housed under standard Laboratory conditions and fed with growers mash. They were acclimatized for two weeks with free access to food and water *ad libitum*. Animal experiments were conducted in compliance with NIH Guide for Care and Use of Laboratory Animals (pub. No. 85-23 Revised 1985), and approved by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals.

Parasite innoculation

A strain of *Plasmodium berghei* that was chloroquine sensitive was obtained from the University of Nigeria, Nsukka, Enugu, Nigeria.

Three animals were used as infected donors and as parasite reservoir. The donor mice were monitored for signs of infection which included lethargy, anorexia, ruffled appearance, shivering and heat-seeking behaviour. The inoculums consisted of *Plasmodium berghei* parasitized erythrocytes. This was prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and diluting them with normal saline in proportion indicated by both parameters. Each mouse except for the negative control groups was infected intraperitonally with 0.2 ml of infected blood containing about $1x10^7$ of *P. berghei* – parasitized erythrocyte per mL. This was prepared by determining both the percentage parasitemia and the erythrocyte count of the donor mouse and diluting the blood with isotonic 0.88% saline in proportions indicated by both determinations (Odetola and Basir, 1980).

Phytochemical Screening

Standard phytochemical screening tests were carried out for various plant constituents. The ethanol extract was screened for the presence of glucose, protein, carbohydrate, alkaloids, flavanoids, saponins and tannins using standard procedures (Trease and Evans, 1996)

Acute Toxicity Studies

The extract was evaluated for its acute toxicity in mice and rat using the modified Locke's (1983) method. The test was carried out in two phases. In the phase one of the study, nine mice and nine rat was randomized into three separate groups of three (3) mice and rat each and were given oral dose of 100, 500 and 1000 mg/kg of the extract

The animals were observed for changes in physical appearance, gross behavioural change and death for 24 hours. Based on the result obtained from phase 1, phase 2 study was carried out using another fresh set of 9 mice and 9 rat randomized into separate three groups of 3 mice and 3 rat each and was given 2000, 3500 and 5000 mg/kg body weight of the extract orally, They were observed for signs of toxicity and mortality for 24 hours.

The LD_{50} was calculated as the geometric mean of the product of the lowest lethal dose and highest non-lethal dose

Antipyretic Activity

The albino rats (36) were randomly distributed in control and test groups of six animals each. They were fed with standard laboratory diet and allowed free access to drinking water .The animals were kept in 12/12 hours dark-light cycle. Pyrexia was induced by subcutaneous injection of 20% w/v brewer's yeast suspension (10 ml/kg) into the animal's dorsum region. Eighteen hours after the injection, the rectal temperature of each rat was measured using a thermometer. Only rats that showed an increase in temperature of at least 0.7 °C were employed for the experiments. Paracetamol (150 mg/kg) was used as the positive control, normal saline (0.2ml) as the negative control and the crude ethanol stem extract (300 mg/kg, 500 mg/kg and 700 mg/kg) as the test substance. The basal rectal temperature of the animals was taken prior to brewer's yeast administration, and the animals were left for eighteen hours before the extracts and paracetamol were administered, The rectal temperature was taken at 0, 1, 2 , 3 and 4 hour after drug administration (Sini *et al*, 2011)

Evaluation of Blood Schizontocidal Activity(Curative Test)

The curative potential of the extract and fractions against *P. berghei* infection was evaluated using the method of Ryley and Peter (1970). Each mouse was inoculated on the first day intraperitoneally, with 0.2ml of infected blood containing about $1x10^7$ *P.berghei* parasitized erythrocytes. The animals were divided into five groups (n = 5) of mice and each group received 300, 500 or 700 mg/kg /day doses of the extract, chloroquine 5 mg/kg and 0.2 ml of normal saline (negative control) (Osunkwo *et al*, 2010). Treatment lasted four days, (day 1 to day 4). On the 5th day (day 5), thin blood films were made from the tail of each mouse (Saidu *et al*., 2000). The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 min and parasitaemia examined microscopically and the parasitaemia level determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage chemosuppression was calculated as: $100[(A–B)/A]$, where A is the average percentage parasitaemia in the negative control group and B is the average parasitaemia in the test/standard group (Odeghe *et al*., 2012)

Evaluation of Prophylactic Activity (Repository Test)

The method of Peters (1967) was adopted. The mice were randomly divided into five groups with 6 mice in each group. Negative control Group was given 0.2 ml of normal saline orally (Osunkwo *et al*, 2010). Group 1 was given chloroquine (5 mg/kg) intraperitoneally, while Group 3-5 were administered with extract at 300, 500 and 700 mg/kg/day respectively. Treatments were initiated on day 0 and continued until day 4, after which all the mice were infected with the parasite. Blood smears were made from each mouse 72 hours after treatment (Abatan and Makinde, 1986) and parasitaemia determined.

Statistical Analyses

Results were presented as mean ± Standard error of mean (SEM) (n=5). Data were subjected to statistical analyses using Graph pad Prism (version 5.0) and one way analyses of variance (ANOVA), followed by Dunnet Post hoc test. Difference between means were accepted to be significant at $P<0.05$

RESULTS

Acute toxicity

Gross physical and behavioural observation of the experimental mice and rat revealed no visible signs of acute toxicity like hair erection, weakness and reduction in motor activities. There was no mortality at all the doses tested, indicating that the LD_{50} is greater than 5g/kg

Phytochemistry

The results of the phytochemical analysis using standard screening tests are shown in Table 1

Anti-pyretic activity

The administration of brewer's yeast elevated the rectal temperature above the basal temperature of the animals after 18 hours of subcutaneous administration, Paracetamol (150 mg/kg) decreased the rectal temperature (Table 2). .The extract (300, 500 and 700 mg/kg) did not affect the rectal temperatures significantly ($P>0.05$)

Antiplasmodial studies

The ethanol stem extract of *Citrus sinensis* exhibited a dose dependent chemosuppressive effect at the different doses employed. Doses of 300, 500 and 700 mg/kg

Table 1. Qualitative phytochemical screening of citrus cinensis stem extract

Phytochemical constituents	composition
Saponin	+
Tannin	+++
Flavonoid	+
Alkaloid	+
Glucose	+
Protein	++
Carbohydrate	+

(+) present in small concentration, (++) present in moderately high concentration, (+++) present in high concentration

Table 2. Antipyretic effect of the extract

Groups	Dose/Treatment	Basal temperature(^{0}C)	Temperature after 18 hour(^{0}C)	Rectal temperature after 18 hours of yeast injection(^{0}C)				
				0 hour	1 hour	2 hour	3 hour	4 hour
1	Normal saline 0.2ml	38.64±0.16	38.70±0.15	38.70±0.16	38.52±0.22	38.56±0.20	38.56±0.20	38.52±0.18
2	Brewers yeast 10ml/kg	38.7±0.12	40.68±0.30	40.68±0.30	40.58±0.21	40.36±0.12	40.4±0.10	40.5±0.13
3	Paracetamol 150mg/kg	38.64±0.26	40.46±0.29	40.72±0.37	39.1±0.25*	38.98±0.25*	38.84±0.27*	38.78±0.29*
4	Extract 300mg/kg	38.28±0.19	40.20±0.14	40.00±0.10	40.18±0.08	40.16±0.05	40.22±0.05	40.34±0.05
5	Extract 500mg/kg	39.8±0.07	40.36±0.21	40.20±0.19	40.4±0.14	40.36±0.13	40.4±0.12	40.46±0.12
6	Extract 700mg/kg	39.86±0.21	40.18±0.17	40.36±0.14	40.34±0.16	40.3±0.14	40.34±0.10	40.32±0.08

Table 3. Percentage parasitaemia against *P.berghai* induced malaria in mice

	0.2 ml	5 mg/kg	300 mg/kg	500 mg/kg	700 mg/kg
Normal saline	10.7 ± 0.46	-	-	-	-
Chloroquin	-	3.7 ± 0.34*	-	-	-
Extract	-	-	7.1 ± 0.64*	6.0 ± 0.35*	5.0 ± 0.27*
Ethyl acetate fraction	-	-	9.1 ± 0.40*	8.5 ± 0.41*	7.8 ± 0.25*
n-hexane fraction	-	-	9.7 ± 0.12	9.0 ± 0.47*	8.6 ± 0.26*
Aqueous fraction	-	-	9.7 ± 0.20	9.6 ± 0.37	9.3 ± 0.25*

*$p < 0.05$ (n=5)

caused chemosuppression of 33.65%, 43.93% and 53.27% respectively (Figure 1). The effect of the extract was significant ($P<0.05$) when compared with the normal saline control. The standard drug, chloroquine (5mg/kg/day), caused 65.42% suppression. The ethylacetate fraction exhibited a significant ($P<0.05$) chemosuppression of 16.48%, 19.81% and 26.42% at the three dose levels respectively.

The aqueous fraction exhibited chemosuppressive effect at the three doses tested, however, only the effect of the 700 mg/kg of the aqueous fraction was significant ($P<0.05$) when compared with the normal

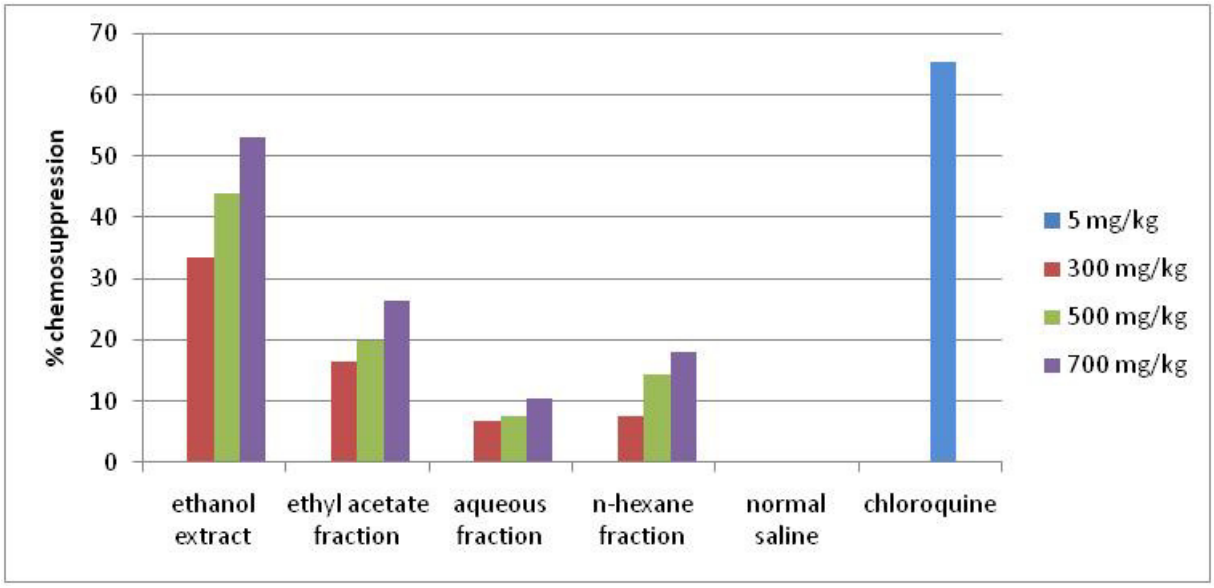

Figure 1. Percentage chemosuppression of *P.berghai* induced malaria in mice

saline.

The n-hexane fraction at the three doses of 300, 500 and 700 mg/kg caused chemosuppression of 7.62%, 14.29% and 18.10% respectively (Table 3, Figure 1). The effects of the 500 mg/kg and 700 mg/kg fractions were significant ($P<0.05$) when compared with the negative control.

The ethanol stem extract exhibited significant prophylactic antimalarial effect at doses of 300, 500 and 700 mg/kg with chemosuppression of 17%, 28% and 42% respectively. The standard drug, chloroquine (5 mg/kg/day), caused 59% suppression .

DISCUSSION

The present study was carried out to investigate the antimalarial activity of *Citrus cinensis* stem extract and fractions in experimental animals.

Phytochemical screening tests indicated that the extract contained saponins, tannins, flavonoids, alkaloids, glucose, proteins and carbohydrates in varying amounts

The extract might be considered very safe since there were no observed untoward effects during the toxicity tests. There was no mortality in mice and rat even at 5000 mg/kg. In addition, gross physical and behavioural observation also revealed no visible signs of acute toxicity

The extract was screened for antipyrexia to ascertain if its use as an antimalarial agent is symptomatic. No significant antipyretic activity was obtained indicating that its antimalarial effect may not be symptomatic.

Phytochemical analysis of the extract revealed the presence of some bioactive substances. The antimalarial activities of flavonoids have been reported. Adele and Kelvin (2008) report the antimalarial activity of dietary flavonoid luteolin against chloroquine resistant Plasmodium. Frederique *et al* (2006) also reported that the flavonol derivatives of silybin (dehydrosilybin), and the natural flavonoid kaempferide modified with hydrophobic substituents (DiMethylAllyl: DMA), expressed an antiplasmodial activity invitro on chloroquine sensitive and resistant strains and that the two flavonoid derivatives had a direct antimalarial activity on several strains of *P. falciparum*

It is believed that flavonoids act by inhibiting the fatty acid biosynthesis (FAS II) of the parasite (Freundlich *et al*, 2005). Some flavonoids have also been shown to inhibit the influx of *L*-glutamine and myoinositol into infected erythrocytes (Elford, 1986). Flavonoids are known to elevate red blood cell oxidation and inhibit the parasite's protein synthesis (Chandel and Bagai, 2010).

Alkaloids are also known to possess antiplasmodial effect. Dua *et al* (2013) reported that the steroidal alkaloid conessine isolated from the bark of *H. antidysentrica* exhibited substantial anti-malarial activity with slight cytotoxic effect. Similarly, the Naphthylisoquinoline alkaloids extracted from Dioncophyllaceae and Ancistrocladaceae species exhibited strong invitro activity against *P. falciparum* and *P. berghei* erythrocytic stages (Francois *et al*, 1996)

The antimalarial activity of plant species (especially *Alstonia scholaris, A. macrophylla* and *A. glaucescens*) were attributed to bisindole alkaloids, notably villalstonine and macro-carpamine, which possessed IC_{50} of 270 and 360nM, respectively, against a CQR strain of *P. falciparum* (Keawpradub et al., 1999)

Several 1-aminopolycyclic-carbolin alkaloids isolated

from marine sources were reported to possess high in vitro and in vivo antimalarial properties (Ang et al., 2000).

Although the mechanism of action of this plant has not been elucidated, some plants are reported to exert antiplasmodial activity either through red blood cell oxidation (Pedroni *et al*, 2006) or by inhibition of protein synthesis (English *et al*, 2006).

Alkaloids, flavonoids or a combination of them may be responsible for the observed antiplasmodial activity since they have been implicated in antiplasmodial activities of many plants such as *Anthocleista grandiflora* (Odeghe *et al*., 1012), *Gongronema latifolium* (Osunkwo *et al*., 2010), *Bombax buonopozense* (Tom *et al*., 2012).

CONCLUSION

The results of this study show that the extract of *Citrus sinensis* possesses potent antimalarial activity, hence justifying its popular local use in treating malaria.

REFERENCES

Abatan MO, MJ Makinde (1986). Screening Azadirachta indica and Pisum sativum for possible antimalarial activities. J. Ethnopharmacol. 17: 85-93.

Adele ML, Kevin JS (2008). Common dietary flavonoids inhibit the growth of the intraerythrocytic malaria parasite. *Biomed Central.* 1:26

Ang KKH, Holmes MJ, Higa T, Hamann MT, Kara UAK (2000). In vivo antimalarial activity of the beta-carboline alkaloid manzamine A. Antimicrob. Agents Chemotherapy. 44, 1645—1649

Anna PKL, Jing YC, Sobri H, Abdul RH (2008). Physiological Responses of *Citrus sinensis* to Gamma Irradiation. World Appl. Sci. J. 5 (1): 12-19

Biswajit B (2013). A short review of laboratory diagnosis of malaria. Int. J. Pharm. Res. Bio-sci. 2(5):177-181

Dawit KZ, Bekelle J (2010). Evaluation of Orange Peel *Citrus Sinensis* (L) as a Source of Repellent, Toxicant and Protectant against *Zabrotes Subfasciatus* (Coleoptera: Bruchidae) Momona Ethiopian Journal of Science. 2 (1): 61-75

Dua VK, Gaurav V, Bikram S, Rajan A, Bagai U, Agarwal DD, Gupta NC, Kumar S, Rastogi A (2013). Anti-malarial property of steroidal alkaloid conessine isolated from the bark of Holarrhena antidysenterica. *Malaria J.*12:194

Ekechukwu PA (2005). *Prevalence rate of Plasmodium species among the inhabitants in Owerri Metropolis*. B.Sc. Project Report submitted to the Department of Medical Laboratory Sciences Imo State University Owerri, Nigeria.

Elford BC (1986). *L*-Glutamine influx in malaria-infected erythrocytes: a target for antimalarials? *Parasitology Today* , 2:309-312

English MC, Wuruiri C, Lightowler C. Murphy SA, Kirigha G, Marsh K (1996). Hyponatreamia and dehydration in severe malaria. Archives Disease in Childhood; 74: 201-205.

Francois G, G Timperman, J Holenz, L Ake´ Assi, T Geuder, L Maes, J Dubois, M Hanocq, G Bringmann (1996). Naphthylisoquinoline alkaloids exhibit strong growth inhibiting activities against Plasmodium falciparum and P. berghei in vitro structure-activity relationship of dioncophylline C. *Annals of tropical medicine and parasitology. Parasitol*. 90:115–123

Frederique de M, Mathias M, Christine L, Franck B, Francois P, Denis B, Stephane P (2006). In vitro antimalarial activity of flavonoid derivatives dehydrosilybin and 8-(1;1)-DMA-kaempferide. *Acta Tropica* 97 : 102–107

Freundlich JS, Anderson JW, Sarantakis D, Shieh HM, Yu M, Valderramos JC, Lucumi E, Kuo M, Jacobs WR Jr, Fidock DA, Schiehser GA, Jacobus DP, Sacchettini JC (2005). Synthesis, biological activity, and X-ray crystal structural analysis of diaryl ether inhibitors of malarial enoyl acyl carrier protein reductase: part 1: 4′-substituted triclosan derivatives. Bioorganic & Medicinal Chemistry Letters, 15:5247-5252

Isah AB, Ibrahim YKE, Iwalewa EO (2008). Evaluation of the antimalarial properties and standardization of tablets of *Azadirachta* indica (Meliaceae) in mice. Phytotherapy Research 17: 807-810.

Iwalewa EO, Lege-Oguntoye L, Rai PP, Iyaniwura TT (1997). Invivo and Invitro antimalarial activity of two crude extracts of *Cassia occidentalis leaf*. Nig. J. Pharm. Sci. 5:23-28.

Iwalewa EO, Lege-Oguntoye L, Rai PP, Iyaniwura TT, Etkin NI (1990). Invitro antimalarial activity of leaf extract of *Cassia occidentalis* and *Guiera senegalensis* on *Plasmodium yoelli nigeriensis*. West Afr. J. Pharmacol. Drug Develop. 4(Suppl): 19-21.

Iwuanyanwu TC, Akuodor GC, Essien AD, Nwinyi FC, Akpan JL, Okorafor DO, Osunkwo UA (2012). Evaluation of antimalarial potential of aqueous stem bark extract of *Bombax buonopozense P. Beauv*. (Bombacaceae). Eastern J. Med. 17: 72-77

Keawpradub N, Kirby GC, Steele JC, Houghton PJ (1999). Antiplasmodial activity of extracts and alkaloids of three Alstonia species from Thailand. *Planta Medica*. 65: 690—694

Kilama W, Ntoumi F (2009). Malaria: a research agenda for the eradication era. *Lancet* 374 (9700): 1480–1482.

Lorke D (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*., 54: 275-287.

Najimu NS, Anu SA, Syed NRJ (2013). Antibacterial activity of citrus sinensis peel against enteric pathogens. Int. J. Pharm. Res. Bio-sci. 2(5):1-13

Nayyar GML, Breman JG, Newton PN, Herrington J (2012). "Poor-quality antimalarial drugs in southeast Asia and sub-Saharan Africa".*Lancet Infectious Diseases* 12 (6): 488–96.

O'Neill MJ, Bray DH, Boardman P, Phillipson JD (1985). Plants as source of antimalarial drugs. Part 1. In vitro test method for the evaluation of crude extracts from plants. *Planta Medica*. 61:394-398

Odeghe OB, Uwakwe A, A Monago CC (2012). Antiplasmodial activity of methanolic stem bark extract of *Anthocleista grandiflora* in mice. Int. J. Appl. Sci. Technol. 2 (4):144.

Odetola A, Bassir O (1980). Evaluation of Antimalarial properties of some Nigerian Medicinal Plants. In: Sofowora, A. editor, Proceeding of African Bioscience Network, Fed. Min. of Science and Tech., Nigeria Soc. Of Pharmacognosy and Drug Res. And Production Unit, University of Ife

Okokon JE, Ettebong E (2008). Anita BS. Invivo antimalarial activity of ethanolic leaf extract of *Stachytarpheta cayennensis.* Indian J. Pharmacol. 40(3): 111-113

Osunkwo UC, Akuodor GC, Idris-Usman M, Theresa CU, Akpan JL, Ghasi SI (2010). In vivo schizonticidal activity of ethanolic leaf extract of *Gongronema latifolium* on Plasmodium berghei in Mice. Ibnosina J. Med. Biomed. Sci. 2(3):118-124

Parle M, Chaturvedi D (2012). "Orange: Range of benefits" Int. Res. J. Pharm. 3 (7): 59-63

Pedroni HC, Betton CC, Splalding SM, Coaster TD (2006). Plasmodium: Development of Irreversible experimental malaria model in Wister rats. Experimental Parasitology; 113: 193-196.

Peters W (1967). Rational methods in the search for antimalarial drugs. *Transaction of the Royal. Society of Tropical Medicine Hygeia* ., 61: 400-410.

Ryley JF, Peters W (1970). The antimalarial activity of some quinolone esters. *Annuals of Tropical Medicine and Parasitology*, 84: 209-222

Saidu K, Onah J, Orisadipe A, Olusola A, Wambebe C, Gamaniel K (2000). Antiplasmodial, analgesic and anti-inflammatory activities of the aqueous extract of the stem bark of *Erythrina senegalensis*. J. Ethnopharmacol. 71: 275-280.

Singh B, KimSung L, Matusop A (2004). A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings *Lancet* 363 (9414): 1017–1024.

Sini KR, Sinha BN, Karpakavalli M, Sangeetha PT (2011). Analgesic and antipyretic activity of *Cassia occidentalis* Linn. Annals of Biological Research, 2 (1) :195-200

Sofowora A (1982). *Medicinal plants and Traditional medicine in west Africa*,John wiley and sons, pp. 256, New York.

Subeki MH, Takahashi K, Yamasaki M, Yamato O, Maede Y, Katakura K, Kobayashi S, Chairul T (2005). Antibacterial and antiplasmodial compounds from *Phyllanthus niruri*. J. Nat. Prod. 68(4): 537-539.

Sutherland CJ, Tanomsing N, Nolder D, Oguike M, Jennison C, Pukrittayakamee S, Dolecek C, Hien TT (2010). Two Nonrecombining Sympatric Forms of the Human Malaria Parasite Plasmodium ovale Occur Globally. The J. Infectious Dis. 201 (10): 1544–1550

Tom CI,Akuodor GC, Essien AD, Nwinyi FC, Akpan JL, Okorafor DC, Osunkwo UA (2012. Evaluation of antimalarial potential of aqueous stem bark extract of Bombax buonopozense P. Beauv.(Bombacaceae).Eastern Journal of Medicine 17: 72-77

Tonna L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers S, Hernans N, VanMiert S, Pieters L, Totte J, Vlietnick AJ (2004). In vitro antiplasmodial activity of extract and fractions from seven medicinal plants used in Democratic Republic of Congo. J. Ethnopharmacol. 93: 27-32.

Trease GE, Evans WC (1996). Pharmacognosy. 14th Ed. London: ELBS, Bailliere Tindal; p. 565-6.

Usha devi C, Nana valedra PK, Pillai CR (2001). Antiplasmodial effect of three medicinal plants. A preliminary study. Current Study 80: 917-919.