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Evaluation of the antimalarial activity of aqueous leaf extracts of *Casuarina equistifolia* and *Mangifera indica* against *Plasmodium berghei* in mice

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Abstract

The evaluation of anti-malarial properties of crude leaf extract of *Mangifera indica* and *Casuarina equistifolia* were carried out using standard procedures in laboratory mice. The results showed that water extract of *M. indica* displayed a very good activity against *Plasmodium berghei* malaria parasites in a dose dependent fashion with a statistically significant difference ($p< 0.05$) while *C. equistifolia* extract showed no significant difference ($p> 0.05$) at all the dose levels chosen in the suppressive test. Furthermore, in the curative, all the test substances were able to reduce the parasitaemia level with a clear significant difference between the treated groups and the control ($p< 0.05$) although parasites were not totally cleared after termination of treatment. The repository effect of the test substances produced a significant difference ($p< 0.05$) suppression of parasitaemia, but *C. equistifolia* failed to prove any repository effect as the parasitaemia level increased to 12.40± 1.91 than the control 11.67±1.56. The test extracts did not prevent body weight loss associated with malaria infection as only the 5mg/kg chloroquine phosphate (CQ) group prevented weight loss. Prevention of toxic effect resulting from treatment was determined based on the LD_{50} of the extracts to be 891.25mg/kg *M. indica* and 2884.03mg/kg for *C. equistifolia* inoculated intraperionneally in mice. The phytochemical screening of the two extracts revealed the presence of tannins, flavonoids, cardiac glycosides, steroids/ terpenoids and carbohydrates. In addition to these, *M indica* extract contained alkaloids, saponins and anthraquinones.

Keywords: Malaria, *Plasmodium berghei*, *Mangifera indica*, *Casuarina equistifolia*, aqueous extract.

INTRODUCTION

Malaria, a parasitic haemoprotozoan disease, is caused by *Plasmodium* species. It is a huge public health problem in Africa and other tropical and subtropical regions of the world (Nyarango *et al*., 2006; Dikasso *et al*., 2006). Globally about 300-500 million estimated clinical cases were reported with 1.4-2.6 million deaths which occur yearly and 80 – 90% of these being in tropical regions of Africa (Snow *et al*., 1999; Salako, 2006). The disease was known of and treated even in ancient times, but it was centuries before the true cause of the disease was well-understood. Malaria is one of the most important human diseases included among the World Health Organization's (WHO) control priority of infectious agents (Anonymous, 1990). Although, the epidemiology and prevention of this disease has been improved in recent years, human treatment is still necessary to control this global pandemic (Chinchilla *et*

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al., 1998).

There are four species of *Plasmodium* that are of economic importance to man and of these, *Plasmodium falciparum* is one of the most widespread and lethal. The disease, if untreated, can lead to total cerebral dysfunction which is a leading cause of morbidity and mortality especially in children and immune-compromised individuals with an increasing emergence or re-emergence of chloroquine resistant *P. falciparum* (Ekanem *et al*., 1990; Jiya *et al*., 2006). The other three common species of malaria (*P. vivax, P. malariae* and *P. ovale*) are generally less serious and are usually not life-threatening. *P. falciparum* accounts for > 90% of all human malaria infection while *P. malariae* infections are frequent during the dry season and mostly of lower density. *P. malariae* and *P. ovale* together are responsible for < 5% of malaria infections in Nigeria (Molta *et al*., 1993). However, another relatively new species, *P. knowlesi*, is also a dangerous species that is typically found only in long-tailed and pigtail macaque monkeys. Like *P. falciparum, P. knowlesi* can lead to human malaria the exact mode of transmission remains unclear but deadly to anyone infected (Desai *et al*., 2007).

Malaria parasites are transmitted from person to person as a result bite of a female Anopheline mosquito of which only sixty of the over three hundred species have been incriminated. In Nigeria, the major vectors of malaria include *Anopheles gambiae*, *A. arabiense, A. funestus* and *A. melas* (FMOH, 1991) while in Guinea, the vectors are: *A. funestus, A. gambiae s. l.* and *A. melas* (Sharp *et al*., 2007). Malaria is preventable, treatable and curable by anti-malarial drugs such as. This assertion does not hold nowadays in certain regions of the world because, the parasite have developed resistance to certain anti-malarial drugs particularly chloroquine which is the cheapest and most widely available first line drug used in routine therapy (Olliaro and Trigg, 1995; Molta *et al*., 1993, 2004 and Salako, 2006).

Regions such as Latin America, Africa and Asia, patients require treatment with other advanced drugs because of the complications arising from malaria infections as well as resistance to first line drugs of treatment. Surprisingly, some ancient malaria treatments were *Artemesia annua* a remarkable example has been used for the last 2000 years in China. The active ingredient artemisinin was only recently scientifically identified (Olliaro and Trigg, 1995). Quinine an active product extracted from *Cinchona* (Rubiaceae) was probably the first important drug used in malaria treatment before the development and use of synthetic drugs (Belding, 1942).

In recent years, natural products have gained considerable interest because drug resistance has increased. Included amongst these products are Qinghaosu and other artemisinin derivatives with an active anti-malarial power (Olliaro and Trigg, 1995). This development has necessitated the alternative choice for cheaper and easily available drugs. Furthermore, the high cost of development and production of new effective drugs that may be likely unaffordable to the poor and generally the increasing reported cases of insecticide resistant mosquitoes (Okon *et al*., 1992; Beales and Gilles, 2002). Therefore the rational search for active substances in medicinal plants is a very promising and cost effective strategy for anti-malarial discovery.

EXPERIMENTAL

Collection and preparation of plant materials. Plants used for the experiment were *Casuarina equistifolia* (Whistling pine) family Casuarinaceae and *Mangifera indica* (Mango) family Anacardiaceae. The leaves of both plants, obtained from the School of Forestry Jos, were randomly picked and put inside clean polythene bags and immediately transported to the laboratory. The leaves were spread thinly on a flat clean tray and allowed

to air dry at room temperature for seven days to prevent spoilage due to moisture condensation and then reduced to coarse powder using a wooden pestle and mortar (Sofowora, 1993).

Extraction of plant materials. The methods described by Ekpendu *et al*. (2000) were adopted. 100g each of coarse powder of *C. equistifolia* leaf and *M. indica* leaf were transferred into two separate conical flasks and macerated in 300mls each of distilled water. The mixtures were allowed to stand overnight then later shaken for 3 hours using a mechanical shaker. Filtration of the extracts was carried out through a Buckner flask using a suction or vacuum pump. The filtrates obtained were evaporated to dryness using a rotary evaporator. The weight of each extract was taken before storage in the refrigerator.

Determination of median lethal dose (LD_{50}). Eighty four (84) mice grouped into 7 (n=6) and each administered with 0.2ml normal saline, 100, 200, 400, 800, 1600, 3,200mg/kg of *M. indica* extract and 0.2ml, normal saline, 500, 750, 1000, 2,500, 5,000 and 7,500mls of *C. equistifolia* extract respectively. Prior to the LD_{50} test a preliminary study was conducted using six mice three per extract administered doses ranging from 10mgkg to 5000mg/kg. The mortality in each group was assessed 24hr following administration of test substances. The percentage mortality were converted to probit units using a standard probit table and plotted against the logarithm of the administered doses of the extract. Regression lines were fitted using a straight-line linear equation, $Y = a + bx$ and the LD_{50} value for each extract calculated (Litchfield and Wilcoxon, 1947).

Parasite species. Chloroquine sensitive NK65 (Peters *et al*., 1975a) *P. berghei* was used for evaluating the plant test extracts. The parasite was obtained from the National Institute for Pharmaceutical Research and Development (NIPRD) Idu Abuja.

Preparation of parasite inoculums. A donor mouse with parasitaemic level of (++) = 11-100 parasites per 100 thick film field was anaesthetized with chloroform. Blood (1ml) was extracted through cardiac puncture using 1ml needle and syringe and made up to 20ml with normal saline (Adzu *et al*., 2007).

4-Day suppressive (Peters) test. Thirty five (35) mice of either sexes were each inoculated with 0.2ml of the diluted inoculums intra–peritoneal (i.p.), then grouped into groups of A-G (n = 5) as described by Adzu *et al*., (2007). Treatment of animals commenced immediately (Peters *et al*., 1993; Saidu *et al*., 2000; Dikasso *et al*., 2006; Adzu *et al*., 2007) and then daily day one to day three (D1-D3) until day four (D4) when blood was collected from the tail of each mouse after each mice was restrained in a mouse restrainer and the tail cut with a pair of scissors and the blood was quizzed out and smeared unto a microscopic slide to make a thick film (Saidu *et al*., 2000). The weight and mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice in each group over a period of 14days (day 0-14) of post inoculation with *P. berghei.*

Curative (Rane) test. The procedure described by Adzu *et al*., (2007); Saidu *et al*., (2000) was adopted. This method is similar to the suppressive test described earlier, except that in curative test, treatments started on day three (D3) after infection was established. On the D3 a pretreatment blood smear of each mouse was collected and the mice are then treated as described above. Treatment is then continued on a daily bases day four to day six (D4-D6) until day seven (D7) when post treatment blood smear was collected and examined for parasite suppression. The mean survival time for each group was determined arithmetically by finding the average survival

time (days) of the mice in each group over a period of 30days (day 0-30) of post inoculation with *P. berghei.*

Repository test. The ability of the test extracts to exhibit prophylactic activity was tested using the residual infection procedure described by (Adzu *et al*., 2007). In this test, treatment as described in the suppressive test was initiated on day zero (D0) and continued till day four (D4) when the mice are all infected with the parasite as earlier described. Blood smears were then made from each mouse 72 hours after treatment day seven (D7) and increase or decrease in parasitaemia was determined.

Collection of blood and smear preparation. The tail of each mouse was cut with a pair of scissors. A gentle pressure was applied to the tail to squeeze out blood and a drop placed on the middle of a clean grease free microscopic slide to make thick smear.

Staining films with Giemsa stain. Dried blood smears were stained with 3% Geimsa at pH 7.2 for 30 minutes on a slide staining rack (Cheesbrough, 1998).

Microscopic examination and parasite density estimation. The estimation of parasite density involves obtaining an average of parasites counts per high power field. The average number of parasites per high power field (100% objective) was multiplied by 500 and 10-50 fields (depending on the parasitaemia level) were examined to determine the average number of trophozoites per high power field (Cheesbrough, 1998).

Phytochemical screening. Phytochemical investigation was carried out as described by Evans (1989).

Statistical analysis. Results are expressed as mean ± standard error of mean (SEM). The student t-test was used to compare means of treated groups and control for any significant difference in parasitaemia of mice treated with leaf extracts and the control group.
All data were analyzed at a 95% confidence interval ($\alpha = 0.05$).

$$\% \text{ suppression} = \frac{\text{parasitaemia in control} - \text{in treated group}}{\text{parasitaemia in control}} \times 100$$

(Mesia *et al*., 2005; Adzu *et al*., 2007).

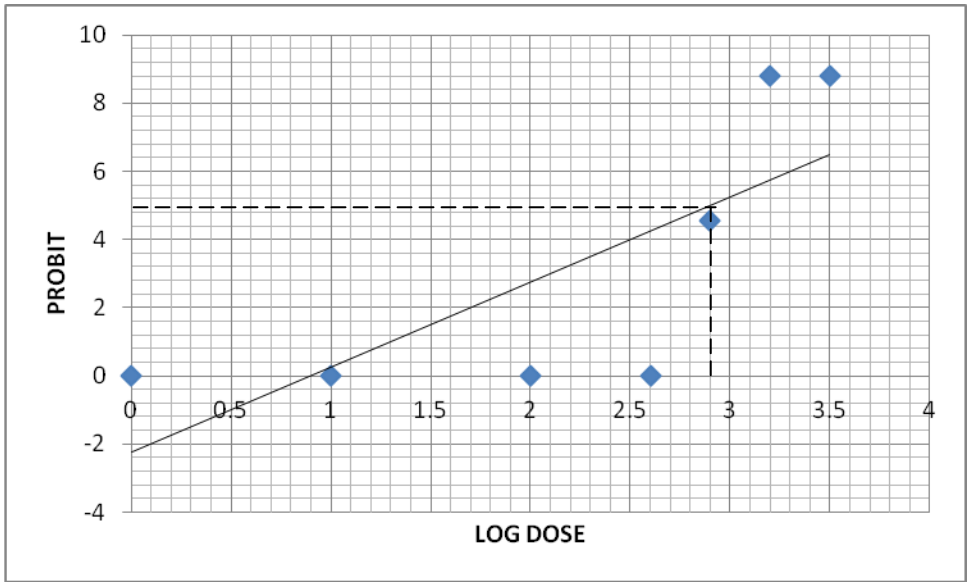

Figure 1: Line of Regression showing 24hours mortality of mice after administration of crude leaf extract of *Mangifera indica*
Antilog 2.90=794.33mg/kg

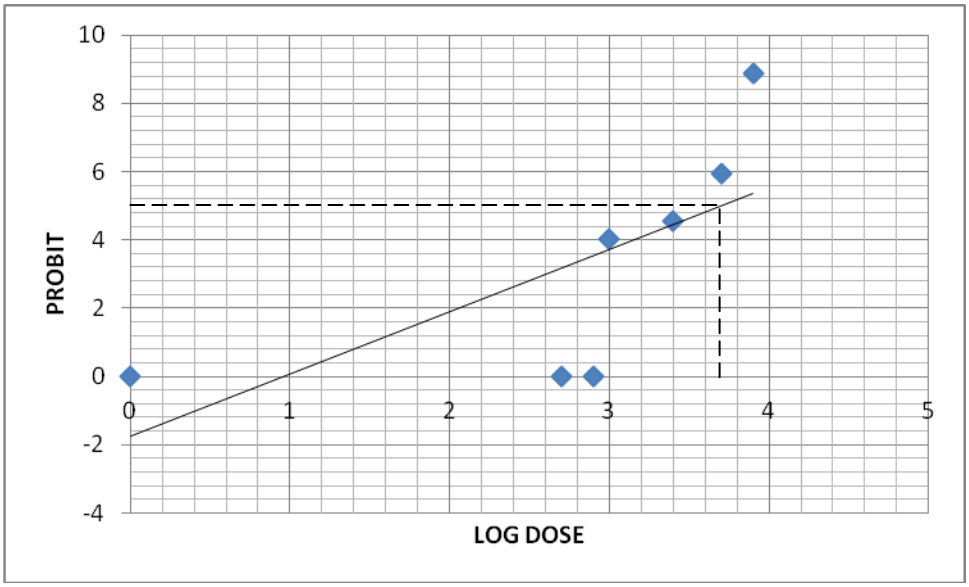

Figure 2: Line of Regression showing 24hours mortality of mice after administration of crude leaf extract of *Casuarina equistifolia*
Antilog 3.70=5012mg/kg

Table 1: Antimalarial activities of crude water extracts of *M. indica* and *C. equistifolia* against *P. berghei* in mice (suppressive test)

Test substances	Dose(mg/kg/day)	% Parasitaemia	% Reduction
M. indica	300	7.20±3.72	76.39*
	200	6.00±1.76	80.33*
	100	10.40±2.99	65.90*
C. equistifolia	400	29.80±8.73	4.26
	200	33.80±8.87	-10.82
Chloroquine phosphate	5	5.40±1.08	82.30*
Control(Normal Saline)	0.2ml	30.50±1.85	0.00

*Indicates significant difference ($p < 0.05$), each result is having a mean of 5 mice.

Table 2: Body weights of *P. berghei* infected mice after the administration of *M. indica* and *C. equistifolia* extracts in suppressive test

Test substances	Dose (mg/kg/day)	Weights		% Change
		D-0	D-4	
M. indica	300	27.52±1.90	24.58±2.02	-10.68
	200	20.19±0.32	19.39±0.87	- 3.96
	100	18.14±0.13	15.83±0.67	-12.73
C. equistifolia	400	17.11±0.16	14.93±0.49	-12.74
	200	16.41±0.09	15.24±0.17	-7.13
Chloroquine phosphate	5	15.12±0.67	14.86±0.67	-1.72
Control(Normal Saline)	0.2ml	19.12±2.95	18.26±4.00	-6.31

D-0 = day infection was initiated; D-4 = 5th day of infection; Each result is with a mean of 5 mice.

Table 3: Antimalarial activities of extracts of *M. indica* and *C. equistifolia* against *P. berghei* in mice (curative test)

Test substances	Dose (mg/kg/day)	Parasitaemia count D-3	Parasitaemia count D-7	% Reduction
M. indica	300	6.86±0.77	2.71±0.18	60.50*
	200	9.00±1.77	3.57±0.43	60.33*
	100	7.43±0.93	4.00±0.31	46.16*
C. equistifolia	400	6.43±0.53	2.86±0.26	55.52
	200	7.00±0.53	3.67±0.21	47.57
Chloroquine phosphate	5	8.67±0.26	1.86±0.26	78.55*
Control(Normal Saline)	0.2ml	8.67±1.16	12.43±1.71	55.38

* Indicates significant difference ($p< 0.05$) each result is with a mean of 7 mice.
D-3 = day 3, D-7 = day 7 after infection was initiated

Table 4: Antimalarial activities of plant extracts of *M. indica* and *C. equistifolia* against *P. berghei* infected mice (repository test).

Test substance	Dose (mg/kg/day)	Parasitaemia
M. indica	300	4.50±0.34*
	200	4.60±0.40*
	100	7.17±0.87*
C. equistifolia	400	10.17± 0.70*
	200	12.40±1.91
Chloroquine phosphate	5	2.83±0.31*
Control (NS)	0.2ml	11.67±1.56

*Indicates significant difference ($p< 0.05$), each result is a mean of 6 mice; NS = Normal Saline

Table 5: Phytochemical constituents of powdered leaves extracts of *M. indica* and *C. equistifolia*

Substances	Result	
	M. indica	*C. equistifolia*
Alkaloids	+	-
Saponins	+	-
Tannins	+	+
Flavonoids	+	+
Cardiac Glycosides	+	+
Steroids	+	+
Anthraquinones	+	-
Carbohydrates	+	-

Key: + present - absent

RESULTS AND DISCUSSION

The rodent parasite model *P. berghei* discovered by Vincke and Lips, (1948) has been used as a good model for evaluating potential anti-malarial drugs (Peters *et al.*, 1975). Plants have proved to be sources of anti-malarial agents especially with the success of quinine isolated from Peruvian *Cinchona* bark and artemisinin from *Artemisia annua* (Adzu *et al.*, 2007; Olliaro and Trigg, 1995; Belding, 1942).

M. indica and *C. equistifolia* have both been reported to posses antimicrobial properties (Onwuliri and Umeruzumba, 2003), treatment of chills, fever, diarrhoea, skin rashes and sores (Coe and Anderson, 1996; Longue fosse and Nossin, 1996), malaria in West Africa (Adesegun and Coker, 2001). We employed these plants to evaluate their anti-malarial activity to justify their usefulness in traditional medicine. The result of the acute toxicity (LD_{50}) indicated that *M. indica* was more toxic 794.33 mg/kg than 5012 mg/kg *C equistifolia* in mice (fig.1 and 2). The LD_{50} of *M. indica* is close to that of > 1000mg/kg earlier reported by Satyavati *et al*, (1987). The toxicity of the extract could be attributed to the presence of terperiods which

are reported to have toxic effects on mice (Phillipson and Wright, 1996; Mesia *et al.*, 2005).

The 4-day suppressive test is a standard test commonly used for anti-malarial screening (Peters *et al.*, 1975a, Mukherjee, 2002) and the determination of percent inhibition of parasitaemia is the most reliable parameter (Dikasso *et al.*, 2006). The study revealed that the water extract of the leaf of *M. indica* showed that the percentage parasitaemia measured changed significantly (P> 0.05) at all doses chosen compared to the control (Table, 1) in agreement with the earlier reports of Dikasso *et al.* (2006) and Adzu *et al.* (2007). The observed anti-malarial activity is consistent with the traditional use of the plant as an herbal remedy against malaria (Adesegun and Coker, 2001). Also, the activity of this extract may be attributed to the traces of some chemical substances of importance such as flavonoids, terpenes and alkaloids which had been detected in the phytochemical screening and previous anti-malarial studies (Tona *et al.*, 2001; Mesia *et al.*, 2005, and Adzu *et al.*, 2007).

It is evident based on the findings that the water extracts of *C. equistifolia* leaf produced no significant difference (P> 0.05) chemo-suppression when compared to the control at all the dose levels considered. The lower dose of 200mg/kg produced a negative percentage suppression (-10.82%) compared to the control (0.00%). This result is similar to -23.00% chemo-suppression discovered by Mesia *et al*, (2005) when they used 80% methanolic extract of *Pyrenacantha staudtii* against *P. berghei* in mice their extract was also discovered to indicate only a few traces of flavonoids and a complete absence of alkaloids.

The results of curative effect gave a significant difference at all the dose levels employed (P> 0.05). Furthermore, the significant difference was dose-dependent where higher doses produced higher percentage reduction in parasitaemia compared to the control (Table, 3). The findings is in agreement with Adzu *et al.* (2007) and Saidu *et al*. (2001) whose results showed a significant difference only with higher doses of ZS-2A fraction of *Zizyphus spina-christi* root bark and *Erythrina senegalensis* bark respectively. However, the water extracts of *M. indica* exhibited a significant repository effect in all the treatment groups (Table, 4) and contrast with the result of Adzu *et al.* (2007) where only the higher doses of ZS-2A fraction of *Z. spina-christi* produced repository effect. Similarly, *C. equistifolia* water extract at 400mg/kg produced a slightly significant difference compared to the control (P>0.05) while the lower dose of 200mg/kg failed to produce any repository effect as was the case in the 4-day suppressive test. This result could be attributed to the short duration of action of the extract probably limited by rapid metabolism (Adzu *et al.*, 2007).

The extracts failed to protect the animals from loosing weights in contrast to findings of Dikasso *et al.* (2006) in which the animals gain weight with increasing level of parasitaemia (Table 2).

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REFERENCES

Adesegun, S.A, and Coker, H.A.B (2001) Plants used in traditional Medicine against malaria. *The Nigeria Journal of Pharmacy* 32:50-62.

Adzu, B., Haruna, A., Salawu, O.A., Katsayal, U.M. and Njan, A. (2007) In vivo antiplasmodial activity of Zs-2A: a fraction from chloroform extract of *Zizyphus spina-christi* root bark against *Plasmodium berghei berghei* in mice. *International*

Journal of Biological and Chemical sciences 1 (3):281-286.

Anonymous (1990): World report on tropical diseases. *World Health Organization Features*, 139: 1-12.

Beales, P.F and Gilles, H.M. (2002) Rationale and technique of malaria control. In Warrel, D.A. and Gilles, H.M. (eds.) *Essential Malariology* 4th edition. Book Power pp. 107- 190.

Belding, D.L. (1942) *Clinical Parasitology*. D. Appleton New York, 888pp

Cheesbrough, M. (1998) *District Laboratory Practice in Tropical Countries* Part 1. Cambridge University Press, UK, 453pp

Chinchilla, M., Guerrero, O.M., Abaraca, G., Barrios, M. and Castro, O. (1998): An *in vivo* model to study the anti-malaric capacity of plant extracts. *Revista de Biologia Tropica*, 46(1): 1-6

Coe, F.G. and Anderson, G.J. (1996) Screening of Medical Plants used by the Garifuna of Eastern Nicaragua for bioactive compounds. *Journal of Ethnopharmacology* 53:29-50.

Desai, F.O., Francis, N., Rose, M., Kwame, A., Bernard, B. and Robert, D. (2007): Epidemiology and burden of malaria in pregnancy. *Lancet Infectious Disease*, 7: 93-104.

Dikasso, D., Makonnen, E., Dabella, A., Abebe, D., Urga, K., Makonnen, W., Melaku, D., Assefa, A. and Makonnen, Y. (2006) In vivo antimalarial activity of hydroalcoholic extracts from *Asparagus africanus* Lam. in mice infected with *Plasmodium berghei*. *Ethiopian Journal of Health and Development* 20 (2): 112 – 118.

Ekanem, O.J., Weisfeld, J.S., Salako, L.A., Nahlen, B. L., Ezedinachi, E.N.U., Walker, O., Breman, J.G., Laoye, O.J. and Hedberg, K. (1990) Sensitivity of *Plasmodium falciparum* to cholroquine and sulfadoxine/pyrimethamine in Nigerian children. *Bulletin of the world Health Organization* 68 (1):45-52.

Ekpendu, T.O., Ibrahim, K., Orisadipe, A.T., Obande, O.D., Anyogo, P.U., Enwerem, N.M., Ekwuno, P., Okogun, J.I. and Wambebe, C. (2000) Studies of Nigeria *Tephrosia* species part two. *Journal of Phytomedicine and Therapeutics* 5: 33-37

Evans, W.C. (1989) *'Trease and Evans Pharmacognosy'*, 13th Edition. English Language Book Society, Bailliere Tindall, pp. 185-186,247-248

Federal ministry of Health (1991) Malaria in Nigeria, epidemiology and control. *Nigerian Bulletin of Epidemiology* 1 (3):1-19.

Jiya, N.M., Airede, K.I. and Ahmed, H. (2006) Cerebral malaria: Presentation and outcome in children in Sokoto. *Nigerian Medical Practitioner* 50 (3-4): 55-61

Litchfield, J.T. and Wilcoxon, F.A. (1947): A simplified method of evaluating dose-effect experiments. *Journal of Pharmacy and Experimental Therapeutics*, 95: 99-113.

Longuefosse, J-L. and Nossin, E. (1996) Medical ethno-botany survey in Martinique. *Journal of Ethnopharmacology* 53 : 117 – 142.

Mesia, G. K., Tona, G. L., Penge, O., Lusakibanza, M., Nanga, T. M., Cimanga, R. K., Apers, S., Van Miert, S., Totte, J., Pieters, L. and Vlietinck. A. J. (2005) Anti – malarial activities and toxicities of three plants used as traditional remedies for malaria in the Democratic Republic of Congo: *Croton mubango*, *Nauclea pobeguinii* and *Pyrenacantha staudtii*. *Annals of Tropical Medicine and Parasitology* 99 (4): 345 – 357

Molta, N. B., Daniels, H.I., Oguche, S.O., Watila, I.M. and Otu, T.I. (1993) Malaria treatment failures in north eastern Nigeria. *Nigerian Journal of Parasitology* 14: 21 – 26

Molta, N. B., Watila, I. M. and Oguche, S. (2004) Responses of *Plasmodium falciparum* infections to antimalarial drugs in north eastern Nigeria – part 1 : 1988-1995. *Journal of Pharmacy and Bioresources* 1 (1) : 51 – 60

Mukherjee, P. K. (2002) *Quality control of herbal drugs*: An approach to evaluation of botanicals. Business Horizons, India, pp. 61 – 616

Nyarango, P. M., Gebremeskel, T., Mebrahtu, G., Mufunda, J., Abdulmumini, U., Ogbamariam, A., Kosia, A., Gebremichael, A., Gunawardena, D., Gbebrat, Y. and Okbaldet, Y. (2006) A steep decline of malaria morbidity and mortality trends in Eritrea between 2000 and 2004 : the effect of combination of control methods. *Malaria Journal* 5: 33

Okon, O.E., Braide, E.I., Ekong, R.G. and Itam, I. H. (1992) Transplacental transmission of malaria in Cross River State. *The Nigerian Journal of Parasitology* 13 : 9 – 12.

Olliaro, P.L. and Trigg, P.I. (1995) Status of antimalarial drugs under development. *World Health Organization Bulletin* OMS 73 (5): 565–571

Onwuliri, F.C. and Umezurumba, I. C. (2003) Investigation of the claimed antimicrobial potency of selected plant parts on *Salmonella typhi*. *Journal of Medicine in the Tropics* 5 (1): 30 -35

Peters, W., Portus, J.H. and Robinson, B.L. (1975a) The chemotherapy of rodent malaria, xxii. The value of drug-resistant strains of *Plasmodium berghei* in screening for blood schizontocidale activity. *Annals of Tropical Medicine and Parasitology* 69 (2): 155-171

Peters, W., Robinson, B. L., Rossier, J. C. and Jefford, C.W. (1993): The chemotherapy of rodent malaria XLVIII. Ther activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine- resistant parasites. Part 1: Studies leading to the development of novel cis-fused cyclopenteno derivatives. *Annals of Tropicsal Medicine and parasitology* 87 (1): 1 – 7

Phillipson , J.D. and Wright, C.W. (1996) Plant with anti-protozoal activity. In: *Trease and Evans Pharmacognosy* 14th edition. Edited by Evans, W.C. W.B. Saunders Company Limited, pp, 426-433

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

Salako, L. (2006) Reflections on malaria control and research in Nigeria. *Nigerian Journal of Clinical and Biomedical Research* 1 (1): 21 - 25

Satyavati, G.V., Gupta, A. K., Tandon, S.N. and Seth, S.D. (eds) (1987) *Medicinal Plants of India*. Indian Council of Medical Research, New Delhi (India), pp. 209 – 214

Sharp, L. B., Ridl, C.F., Govender, D., Kuklinski, J. and Kleinschmidt, I. (2007) Malaria vector control by indoor residual insecticide spraying on the tropical island of Bioko, Equatorial Guinea. *Malaria Journal* 6:52

Snow, R.W., Craig, M. and Marsh, k. (1999) Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bulletin of World Health Organization* 77 (8): 624-640

Sofowora, A. (1993) *Medicinal plants and traditional medicine in Africa*. John Wiley and Sons Limited, New York pp. 143-145

Tona, L., Mesia, K., Ngimbi, N.P., Chrimwami, B., Okond, A., Cimanga, K., Bruyne, T., Apers, S., Hermans, N., Totte, J., Pieters, L. and Vlietinck, A.J. (2001) In-vivo antimarial activity of *Cassia occidentalis*, *Morinda Morindoides* and *Phyllanthus niruri*. *Annals of Tropical Medicine and Parasitology* 95 (1): 47-57

Vincke, I.H. and Lips, M. (1948): Un noveau *Plasmodium* rengeur sauvage de Congo. *Plasmodium berghei de la Societe Belge de Medicine Tropical*, 28: 97-104