www.cenresinpub.org
ISSN 2277-0143

ANTIPLASMODIAL ACTIVITY OF THE METHANOL EXTRACT OF THE ROOTS OF ARISTOLOCHIA ALBIDA IN ALBINO SWISS MICE

[*1]M. E. Khan, [1]I. Toma, [1]D. Y. Shingu and [2]C.H. Wazis
[1] Department of Chemistry Adamawa State University, Mubi, Nigeria
[2]Department of Pharmacology and Toxicology University of Maiduguri, Nigeria
e-mail: emamulu@yahoo.com

ABSTRACT

Biological investigation of the efficacy of the methanol extract of the roots of *Aristolochia albida*, locally used in forms of decoction / concoction for the treatment of malaria, diarrhea and other related diseases in kaltungo LGA of Gombe State and Gwoza LGA of Borno State, both in North Eastern Nigeria. Here, the *in vitro* antiplasmodial activity of the methanol extract of the roots of *Aristolochia albida* was evaluated in *plasmodium berghei berghei* infected mice. Oral acute infection, prophylactic effect against residual infection and the mean survival time using chloroquine sensitive *plasmodium berghei berghei* NK65 infected mice. The Oral median lethal dose of the extract in mice was determined to be about 5000mg kg^{-1} body weight. The extract at doses of (100, 200 and 400mg kg^{-1}b.w.) used, produced significant ($P< 0.05$), dose dependent activity against the parasites in the suppressive, curative and prophylactic tests. The results suggest that the methanol fraction of *Aristolochia albida* possesses antiplasmodial activity and thus lends credence to its ethno medical, folkloric, and its tropical African indigenous usage for the cure of malaria.

Keywords: *Aristolochia albida, Plasmodium berghei berghei, in vitro,* prophylactic-test, Swiss Mice

INTRODUCTION

Human disease management in Nigerian history addresses in one accord evidence(s) of the relationship of plants and medicine [1,2,3, 4]. Plants serve as the basis of traditional medicine systems for thousands of years in Cameroon, Nigeria, India, China, Indonesia etc. [2]. They posses pure secondary metabolites which could provide the expected chemotherapy for MDR-Malaria and also provide a "lead" whose chemotherapeutic index equals or even exceeds that of most common malaria drugs, used in combination. Our focus and goal has been that this plant will significantly reduce the long therapy as well as side effects of existing drugs, combat the menace of resistance and obviously be affordable to the poor. Knowing that, Malaria has made life miserable for millions and constrained socio-economic development across Africa. Everyone is waiting eagerly for a vaccine to stem the tide of deaths and the long wait might soon be over, given the significant progress in clinical trials. Africa needs to get ready now. Though poor funding and planning, inadequate infrastructure and logistical problems plague Africa's public health systems and constrains the effectiveness of malaria and other disease-control programmes. Indeed, waiting for a malaria vaccine from the North is symptomatic of a more serious African malaise — being comfortable to be co-pilots, rather than drivers, of development in our own countries. This

hands-off, conservative attitude must change. African scientists running R&D projects must make herculean efforts to mentor a new generation of Africans to tackle malaria/tuberculosis vaccine(s) head-on. This also means lobbying their governments to invest in research. We can easily understand why [5], declared that **"malaria has been responsible for the death of about half of all the people who ever lived"** and quoting from the words of the UN millennium Economist, **"There is a silent Tsunami underway all the time in rural Africa. Every month, as many children die of malaria in Africa as did in the tsunami; about 150,000 children die every month"** The situation is further complicated by the worldwide emergence and rapid spread of resistance to several existing anti-malarial by *Plasmodium falciparum* that threatens to increase the above annual death toll [6, 7,8, 9,10, 11]. Taking cognizance of this malady and problems associated with anti-drug resistance and prevalence of fake drugs in general circulations in the African markets, new drugs /drugs with fine-tuned modes of action or new combinations are urgently required hitherto for malaria treatment. Plants have been a source of medicinal agents for thousands of years and an impressive number of modern drugs find their origin from them [12, 13]. Natural product has played and will continue to play an invaluable role in the drug discovery process [14, 15]. The future of natural product in drug development thus appears to be a tale of justifiable hope. Faithful drives are needed in more intensified fashion to explore nature as a source of novel and active agents that will serve as leads and scaffold for elaboration into urgently needed efficacious drugs for a multitude of disease indications [16]. Due to limited availability and / or affordability of pharmaceutical medicines in the tropical countries, majority of the populations depend on traditional medical remedies [17, 18].

Aristolochia albida is a Herb/shrub, rarely lianas, sub shrubs root, stems and leaves with oil cells. Leaves alternate; stipules absent; petiole present and well defined; leaf blade simple, usually pinnately. Inflorescences terminal or axillary, racemes , cymes, corymbs or flowers solitary. Flowers bisexual veined, sometimes palmately 3-5-veined, margin usually entire, rarely 3-5-lobed zygomorphic or actinomorphic. Perianth usually with 1 petaloid whorl (in *Saruma* with 2 whorls: outer one sepaloid , inner one petaloid), mostly connate into distinct tube , cylindric to campanulate or subglobose; limb rotate, urceolate , cylindric, or ligulate , 1-3-lobed; lobes valvate . Stamens 6-12 (in China), in 1 or 2 series; filaments adnate to ovary (in *Asarum*) or style column (in *Thottea*) with anthers free , or filaments and anthers fully adnate to style column to form gynostemium (in *Aristolochia*) ; anthers 2-loculed, dehiscence longitudinal . Ovary inferior to superior, 6-loculed (in *Thottea* 4-loculed) carpels connate only at base or fully fused; ovules numerous , anatropous , usually in 1 or 2 series; placentation parietal . Styles free or connate, column 3- or 6-lobed (in *Thottea* 5-20-lobed). Fruit a fleshy or dry capsule, rarely siliquiform or follicular. Seeds many; testa somewhat hard or crustaceous; endosperm copious, fleshy; embryo minute [19 , 20]. **Medicinal uses**: The flowers are used in Social: religion, superstitions, magic; sayings, and aphorisms, the leaves for the treatment of skin and mucosae diseases and the roots an antidote (venomous stings, bites etc), medicine for cutaneous and subcutaneous parasitic infections, vermifuges, and general healing and stomach troubles. The crude methanol extract of the root has

antifeedant activities and contain a good yield of aristolochic acid at 0.1% concentrations [21]. *Aristolochia albida* [*Aristolochiaceae*] *is a* twining climber of the Sahel zone of the Region. An infusion of the dried leaves, sometimes with dried root added, is used in Nigeria by Hausa and Fulanis as an anthelmintic [22]. The leaf is applied in Nigeria to certain (unspecified) painful skin-diseases, and crushed and mixed with castor-oil and applied topically on pimples [23]. To get rid of guinea-worm, the leaf may be applied, or a poultice composed of powdered root with seeds of *Lepidium sativum* Linn. (Cruciferae), garlic and native natron, and an infusion of the same mixture is drunk [23]. The root is bitter. It is sold in markets in light-coloured pieces 8–10 cm long for taking as a stomachic and tonic for which an infusion is made by pouring water repeatedly on to it through a strainer. The root mixed with lime-juice is given in cases of snake-bite, scorpion-stings, etc., against which the flowers are sometimes worn as a juju or charm [22]. English name is dutchman's pipe, *HAUSA, dúmán duútseè* = gourd of the rocks (auctt.) fiyaka (JMD; ZOG) gaďahuka, gaďakuka, gaďaukuku from Fulani (JMD) kadacin kasa (ZOG) mádaàcín κάsà = medicine of the earth (auctt.) This research reports the *in vitro* antiplasmodial activity of the methanol extract of the roots of *Aristolochia albida* evaluated in *plasmodium berghei berghei* infected mice, at dose dependent activity against the parasites in the suppressive, curative and prophylactic tests.

MATERIALS AND METHODS

Collection of Plant Materials:

Fresh sample of the roots of *Aritolochia albida* were collected in Gwoza, Borno State and were identified in the Biological Sciences Department of Gombe State University. The FHI number is 044 and a specimen of the plant was deposited in the herbarium. The sample (1.5kg) was air dried in the laboratory before pounding to a fine powder using pestle and mortar to about 70 mesh sizes and then stored in a dry container.

Extraction

250g of the powdered roots was accurately weighed and percolated with 2.5L of distilled methanol for 72hrs. After which there was decantation, filtration, and concentration using rotary evaporator (Model RE100) at 45^0C to obtain methanol soluble fractions, (F_E01), labeled, F_M. [43.7g]. This fraction was heated over a water bath at reduced temperature to remove the remaining solvent and then stored in a refrigerator at 40^O C for the tests.

Antimicrobial activity: Antimicrobial tests for the extracts were carried out in International Institute for Pharmaceutical Research and Development (NIPRD) Idu Abuja, Nigeria.

Animals: The animals used in the work were four (4) weeks old-albino mice weighing 18 – 22g, obtained from the National Veterinary Institute Vom Jos Plateau State, Nigeria. Carried in cages to IPRD where they were housed in plastic cages with saw dust as beddings and given food and water *ad libitum*. They were used in accordance with NIH guide for the care and use of laboratory animals NIH Publication (No. 83 -23) revised (1985).

Acute toxicity test (LD_{50}): The oral acute toxicity of *Aristolochia albida* was carried out in mice using modified Lorke (1983). The study was carried out in two phases. In phase one, nine mice were randomized into three groups of three mice each and were given 10, 100 and 1000 mg kg^{-1} body weight (b. wt) of the extracts orally. The mice were observed for paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first four (4) hrs and subsequently daily for seven (7) days. In phase two, another fresh set of nine mice were randomized in to three groups of three mice each and were given 1600, 2900 and 5000mg kg^{-1} b. wt of the extracts orally, on the result of the first phase. They were observed for signs of toxicity and mortality for the first four critical hrs and thereafter, daily for 7 days. The LD_{50} was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose. I .E the geometric mean of the consecutive doses for which 0 % and 100% survival rates were recorded in the second phase, the oral median lethal dose was calculated using the formula:

LD_{50} =√ Minimum toxic dose X maximum toxic dose or

Oral median lethal (LD_{50}) dose = $\sqrt{2900x5000}$ = 3800mg kg^{-1}

Rodent parasite (*Plasmodium berghei berghei*): The rodent parasite *Plasmodium berghei berghei* NK 65 was obtained from the National Institute for Medical Research (NIMR) Lagos, Nigeria. The parasites were kept alive by continuous intraperitoneal passage in mice [24] every 24 days. These infected mice were used for the study. Prior to the beginning of the study, one of the infected mice was kept and observed to reproduce disease symptoms similar to human infection [25].

Anti-Plasmodial Studies

Suppressive test: The Peter`s 4days suppressive test against chloroquine sensitive *plasmodium berghei berghei* NK 65 infection in mice was employed [26]. Adult Swiss albino mice weighing 18 -22 g were incubated by intraperitoneal (IP) injection with standard inoculums of the *plasmodium berghei berghei* with 1 x 10^7 infected erythrocytes. The mice were randomly divided into five (5) groups of six (6) mice per group and treated for 4 consecutive days with 100, 200 and 400 mg extract kg^{-1} b. wt. orally daily. Two control groups were used: Positive control was treated daily with 5 mg chloroquine kg^{-1} b. wt while the negative control was given 5 mL kg^{-1} normal saline. On day 5 of the experiment, blood was collected from the tail of each mouse and smear on to a microscope slide to make a film [27]. The blood films were fixed with methanol, stained with 10 % Giemsa at pH 7.2 for 10min and parasitaemia examined microscopically. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected control with those of treated mice. I.E

Average % suppression = $\frac{A-B}{A}$ Where A = Average percentage parasitaemia in negative control group, and B = Average percentage parasitaemia in test group.

Evaluation of Schizontocidal activity of *Aristolochia albida on* established infection (Curative or Rane test): Evaluation of the potential of the methanol fraction of roots of *Aristolochia albida* extracts were carried out according to the method described by [28].The mice were infected intraperitoneally with standard inoculums of $1x10^7$ *plasmodium berghei berghei* NK 65 infected erythrocytes on the first day (day 0). Seventy-two hours (72hs) later, the mice were divided into 5 groups of six mice each. The groups were orally treated with roots of *Aristolochia albida* extracts at (100, 200 and 400 mg $kg^{-1}day^{-1)}$, chloroquine (5 mg kg^{-1} day^{-1}) was given to the positive control and an equal volume of distilled water was given to the negative control group. The treatment was carried out once daily for 5 days and blood smears were collected and examined microscopically to monitor the parasitaemia level.

Evaluation of the prophylactic activity of *Aristolochia albida* **(repository test):** Evaluation of the prophylactic potential of extracts of *Aristolochia albida* roots were carried out according to the method of Peters [26]. Adult mice were randomized into 5 groups of six mice each. Group 1 was given 10 mL distilled water kg^{-1} b. wt. orally. Group 2, 3 and 4 were given 100, 200, and 400 mg extract kg^{-1} b. wt. Group 5 was however given 5 mg chloroquine kg^{-1} b. wt intraperitoneally. Treatments were initiated on day 0 and continued till day 4 when, the mice were all infected with the parasite. Blood smears were then made from each mouse 72 h after treatment [29] and increase or decrease in parasiteamia determined as above.

Statistical analysis: The one way ANOVA test was used to analyze and compare the results at a 95% confident level, values of $P \geq 0.05$ were considered significant, results were expressed as Mean ± SE of mean.

RESULTS AND DISCUSSION

Anti-plasmodium investigations: The anti-plasmodium activity of *A. albida* fraction against *Plasmodium berghei berghei* are shown in **Tables (1[a, & b] 2, 3, 4). Table 5** investigates the mean survival time

Table (1a): Acute toxicity test of the methanol extract of the roots *of A. albida*.**Phase I**

10mg / kg (1mg /mL)	**Vol. (mL)**	**Signs of toxicity**	**Survival**
18g	0.18	x	√
20g	0.20	x	√
22g	0.22	x	√ 3/3
100mg /kg (10mg /mL)			
20g	0.20	x	√
21g	0.21	x	√

20g	0.20	x	√ 3/3
1000mg /Kg (100mg /mL)			
22g	0.22	x	√
19g	0.19	x	√
20g	0.20	x	√ 3/3

Table (1b): Methanol extract of the roots *of A. albida*. **Phase II (Concentrations based on phase I)**

1600mg /kg	**Vol., (mL).**	**Signs of toxicity**	**Survival**
20g	0.20	Paw licking	√
18g	0.18	Paw licking	√
20g	0.20	Stretching /writing	√ 3/3
2900mg /kg			
21g	0.21	Salivation	√
18g	0.18	Paw licking	√
19g	0.19	Salivation	√ 3/3
5000mg / kg			
19g	0.19	Sleep	death
21g	0.21	Comatose	death
20g	0.20	Weakness	Death 0

Key: x =absent √ = present j,

Thus, Oral median Lethal (LD_{50}) dose = √ Minimum toxic dose X maximum toxic dose or Oral median lethal (LD_{50}) dose = √ 2900 x5000 = 3800mg kg^{-1}

Anti-malaria Activity:

Strain of parasite =*Plasmodium berghei berghei* NK 65Specie of animal =Swiss albino mice Model =Suppressive, Parameter evaluated =Body wt., survival time, parasite count and body temp

Table (2): Suppressive Effect of *Aristolochia albida* methanol roots extract and chloroquineagainst *P. berghei berghei* infection in Swiss Albino Mice

Treatment	Parasite count	%.tage Chemo-suppression
Normal saline 5 mL kg^{-1}(control -ve)	8.72 ± 1.21	-
Extract 100mg Kg^{-1}	5.52 ± 1.32*	45.04
Extract 200mg Kg^{-1}	4.38 ± 0.93*	51.66
Extract 400mg Kg^{-1}	3.26 ± 0.88**	68.21
CQ 5 mg kg^{-1} (Control + ve)	0.42 ± 0.27**	94.00

*Significant different from control at P≤ 0.05 and **at P≤ 0.01
Strain of parasite =*Plasmodium berghei berghei* NK 65
Specie of animal =Swiss albino mice
Model =Protective
Parameter evaluated =Body wt., survival time, parasite count and body temp.

Table (3): Curative Effect of *A. albida* methanol roots extract and chloroquine against *P. berghei berghei* infection in Swiss Albino Mice

Treatment	Parasite count	%.tage Chemo-suppression
Normal saline 5 mLkg^{-1}(control)	43.6 ± 2.22	-
Extract 100mg Kg^{-1}	18.5 ± 2.02*	40.54
Extract 200mg Kg^{-1}	12.2.00 ± 0.61*	48.73
Extract 400mg Kg^{-1}	9.360 ± 0.11**	62.17
CQ 5 mg kg^{-1}	0.21 ± 0.13**	99.59

*Significant different from control at P≤ 0.05 and **at P≤ 0.01

Table (4): Prophylactic Effect of *A. albida* methanol roots extract and chloroquine against *P. berghei berghei* infection in Swiss Albino Mice

Treatment	Parasite count	%.tage Chemo-suppression
Normal saline 5 mLkg^{-1}(control)	7.89 ± 1.41	-
Extract 100mg Kg^{-1}	5.42 ± 1.32*	27.01
Extract 200mg Kg^{-1}	3.74 ± 0.18*	32.20
Extract 400mg Kg^{-1}	1.91 ± 0.76**	39.53
CQ 5 mg kg^{-1}	0.62 ± 0.32**	89.41

*Significant different from control at P≤ 0.05 and **at P≤ 0.01

Table (5): Mean survival period of Swiss Albino Mice treated with methanol roots extract of *A. albida* and chloroquine in established malaria infection.

Dose of extract (mg / kg / day)	Survival time (days)
Norman saline 5 $mLkg^{-1}$(control)	09
Extract 100mg Kg^{-1}	14
Extract 200mg Kg^{-1}	15
Extract 400mg Kg^{-1}	18
CQ 5 mg kg^{-1}	30

Acute Toxicity: The mice were treated intraperitoneally with single dose each of 10 – 5000 mg kg^{-1} b. wt. of *A. albida* roots extracts after being starved for 24h. The route was chosen because of its sensitivity and rapid results. The extract at 10 – 1000 mg kg^{-1} (phase 1) produced no physical signs of toxicity in the mice 24h after administration. But from 1600 to 5000 mg kg^{-1} (phase 2) there were some physical signs: salivation, paw licking, stretching / writing, calmness etc, within the first minutes of administration. There was however no mortality at all dose levels used. The median lethal doseLD_{50} was estimated to be ≥ 5000mg kg^{-1} b. wt. However, the observed reduced activity of the treated mice showed that the extract possess central depressant effect. The absence of death following oral administration of the extract, at below 5000mg extract kg^{-1} b. wt. observed in mice suggested that the extracts were practically non-toxic acutely [30].This high safety profile of anti-malaria efficacy in human than in rodent models, the later have also been validated through the identification of several conventional anti-malaria, drugs such as chloroquine, halofantrine, mefloquine, maldox and more recently artemisinin derivatives [28]

Suppressive Test: *A. albida* roots extract exerted dose dependent chemo-suppressive effect against *Plasmodium berghei berghei* NK 65 malaria parasite. The extract caused a significant ($P<0.05$) chemo-suppression of 45.04, 51.66, 68.20 & 94.0 %, when compared to the control. The standard drug chloroquine caused chemo-suppressions of 94.0% (Table2) which was higher than the groups treated with the plant extract. The observed higher efficacy of chloroquine may in part be due to non selectivity of the extract or slow absorption and poor bioavailability of the crude extract. This is common with medicinal plants extracts[24]. The significant chemo-suppression produced by the extracts on day 4 is consistent with the traditional use of the plant as a herbal medicament against malaria in Northern Nigeria.

Curative Effect: *Aristolochia albida* roots extract produced significant ($P<0.05$) dose dependent reduction in parasitaemia levels in the extract treated groups of *Plasmodium berghei berghei* NK 65 malaria parasite with a similar reduction in the chloroquine treated group (positive control). The average percentage parasitaemia reduction of the extract

treated groups on day 7 were 40.54, 48.73, 62.17 % for the 100, 200 and 400mg /kg /day. Chloroquine 5mg /kg b. wt exerted 99.59% reduction of the parasite (Table 3).While there was a daily increase in the parasitaemia in the negative control group, the average percentage prasitaemia decreases in the extract and the positive control. This is in consonance with the earlier reports ([31, 32, 32, 34,and 35] using the plant *Alstonia boonei*. This is consistent with natural products of plant origin due to the crude nature of the extract.

Prophylactic Effect: The methanolic extract leaves of *Aristolochia albida* produced significant (P< 0.05) dose dependent reduction in parasitaemia levels in the extract treated groups of *Plasmodium berghei berghei* NK 65 malaria of 27.01, 32.20 & 39.53% while 5mg chloroquinekg-1 b. wt. caused 89.40%reduction in parasite count (Table 4).The result indicated that the roots extract of *Aristolochia albida* possesses blood schizontcidal activity as evident from the chemo-suppression obtained during the four day early infection test and the 30 days curative / established infection which is comparable to the standard drug chloroquine, 5 mg / kg / day.

Survival Period: From (Table 5), the extract appears to be highly effective against the species of *Plasmodium berghei berghei (*NK 65).The mean survival period of the Swiss albino mice treated with the extracts in established infection during a period of one month showed that as the dose increases, the survival time increases. Mice treated with chloroquine 5mg / kg b. wt. per day survived for 30 days. Those treated with the extract at 100mg, 200mg and 400mg / kg b. wt. per day survived for 14, 15, & 18 days respectively. The animals in the negative control group, which were treated with distilled water /normal saline, were found to have a mean survival period of 9 days. *Plasmodium berghei berghei* parasite is used in predicting the treatment outcomes of any suspected anti-malaria agent due to its high sensitivity to chloroquine making it the most appropriate parasite for this research [36] .
Currently, no single drug is effective for the treatment of multidrug resistant malaria and combination therapy includes artemisinin derivatives such as artesunate [37] or mixtures with older drugs such as atovaquone [38], proguaml [40] combination malarone/ maldox [41 and 42]. Unfortunately, first report on drug resistance to arteminin-derivatives [43] and to drug combination therapies [44] have already appeared. So, in the absence of a functional, safe and widely available malaria vaccine, efforts to develop new anti-malaria drugs most continue.

CONCLUSION AND RECOMMENDATION

There is a consensus among the scientific community that natural products have been playing a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases [15]. Indeed, the vast majority of the existing anti-malaria chemotherapeutic agents are based on natural products and this fact anticipates that new anti-malaria may constantly emerge from our tropical plants sources if well harnessed, [45]. The results of the present investigation, suggests that the extracts of the indigenous plants

A. albida are safe and possesses potent anti-malaria activity which justifies their continuous folkloric usage as anti-malaria remedies.
Further research to isolate, identify, elucidate and characterize the active ingredients is ongoing in the laboratory.

ACKNOWLEDGEMENTS
The authors wish to thank Dr. Mrs. Salawu and Staff of the Department of Pharmacology and Toxicology, International Institute for Pharmaceutical Research and Development (NIPRD), PMB 21 Garki Abuja, Nigeria, for the technical support they provided for the success of the work.

REFERENCES

Bannerman,P .C; Mirsky, R; Jessen, K. R., Timpl, R; and Duance,V.C; (1986),Light microscopic mmunolocalization of laminin, type IV collagen, nidogen, heparin sulfate proteoglycan and fibronectin in the enteric nervous system of rat and guinea pig. *Journal of Neurocytology*, 15; 432-443.

Hammer, K; (1999): Antimicrobial activity of essential oils and other plant extracts *Journal of applied Microbiology* **86:**985-990.

Raghavendra MP, Satish S, Raveesha K A *(2006:)* Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant Agriculture Microbiology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, 570 006, India. (mySCIENCE 1(1), 2006, 72–78 ℃ University of Mysore http://myscience.uni-mysore.ac.in

Ayandele, A. A; and Adebiyi, A. O; (2007) The phytochemical analysis and antimicrobial screening of extracts of *Olax subscorpioidea African Journal of Biotechnology* 6 (7): 868-870.

Riscoe M, Kelly J. X and Winter R (2005); Xanthones as anti-malaria: discovery, mode of action and optimization. *Current Med Chem*. **12,** 2539 -2549

Mitsuyama J, Hiruma R, Yamaguchi A, Sawai T (1987), Antimicrob.Agents: *Chemothe.* 31: 379–384.

Gutmann, L., Billot-Klein, D., Williamson, R., Goldstein, F., Mounier, J., Acar, F., Collatz, E., (1988) Antimicrob. Agents: *Chemothe.* 32: 195–201.

Mathias AJ, Somashekar RK, Sumithra S, Subramanya S (2000). Ant-microbe. Agents: *Indian J. Microbio.* 40: 183–190.

Gangly, R., Mishra, P., Sharma, A., (2001) Microbes and infection *Indian J. Microbio* 41: 211–213.

Martino PD, Gagniere H, Berry H, Bret L (2002). Anti-microbial Agents.*Microbes and infection.* 4: 613–620.

Meshnick S. R (2002): Arteemisinin: mechanisms of action, resistance and toxicity. Int. J. Parasitol. **32**:1655 -1660.

Cragg G. M. and Newman D. J (2001): Medicinal for the millennia: the historical record. *Ann N.Y Acad. Sci*. **953**: 3-10.

Brahmachari G (2006**):** Prospects of natural products research in the 21st century: a sketch. In Chemistry of Natural products; Recent trends and developments (ed, G. Brahmachari), Research Signpost , Kerala, India pp. 1 -22.

Harvey A (2000): Strategies for discovery drugs from previously unexplored natural products. Drug Discovery Today **5**, 294.

Newman D. J, Cragg G. M and Snader K. M (2003): Natural products as sources of new drugs over the period 1981 -2002. *J. Nature Prod* **66**, 1022.

WHO, (2002): Severe falciparum malaria. Trans. R. Trop Med. Hyg., 94: 36 -37.

Zirihi G.N; Mambu L. Guede-Guina. B Bodo and Grellier P (2005): In vitro antiplasmodial activity and cytotoxicity of 33 west African plants and used for treatment of malaria. J. Ethnopharmacol., 98: 281 -285

Turrill, W. B., (1952): – eds.Flora of tropical East Africa. (F TE Afr)

Keay, R. W and Hepper, F .N., (1953–1972): Flora of west tropical Africa, ed. 2. (F WT Afr)

Lee M. L, and Schnieder G. J (2001): Scaffold Architecture and Pharmacophoric Properties of Natural Products and Trade Drugs. Application in the Design of Natural Product – Based Combinatorial Libraries. *Comb. Chem*. **3**: 284-288

Iwu, M., (1993) Handbook of African medicinal plants CRC Press, Boca Raton, FL

Johnston, B., (1997): One-third of nation's adults use herbal remedies. HerbalGram 40:49-53.

Adzu B and Haruna A (2007): Studies on the use of *Zizyphus spina-chriti* against pain in rats and mice. *Afr. J. Biotechnology*. 6: 1317 – 1324.

English M (1996): Life threatening severe malarial anaemia. *Trans. R. Soc. Trop. Med. Hyg*. 94: 585 -588

Peters W (1967): Rational methods in the search for anti-malaria drugs. *Trans. R. Soc. Trop. Med.*Hyg., 61: 400 – 410.

Saidu K, Onah J, Orisadipe A, Olusola A, Wambebe C and Gamaliel K (2000): Anti-plasmodial analgesic and anti-inflammatory activities of aqueous extract of *Erythrina senegalensis.J. Ethnopharmacol*., 71:275 – 280.

Ryley J. F and Peters W (1970): The anti-malaria activity of some quinolone esters. *Am. Trop. Med. Parasitol*., 84; 209 -222.

Abatan M. O and makinde M. J (1986): Screening *Azadirachta indica* and *pisum sativum* for possible anti- malaria activities. *J. Ethnopharmacol*. 17: 85 -93

Salawu O. A, Chindu B. A,Titanji A. Y, Obidike I. C and Akingbasote J. A (2009): Acute and sub-acute toxicological evaluation of the methanolic sterm bark extract of *Crossopteryx febrifuga* in rats. *Afr. J. pharmacy Pharmacol.*, 3: 621 -626.

Adjanohoun J. E, Aboubakar N, Dramane K, Ebot M. E, and Ekpere J. A (1996): Traditional Medicine and pharmacopoeia: Contribution to Ethno Botanical and Floristic studies in Cameroon. Organization of African Unity, Addis Ababa

Odeku O.A, Adegoke O. A and Majekodunmi S. O (2008): Formulation of the extract of the stem bark of *Alstonia boonei* as tablet dosage form *Trop, J. Pharma. Res*., 7: 987 – 994

Titanji, V.P.K. and Ngemenya, M. N. (2008): The anti-malaria potential of medicinal plants used in the treatment of malaria in Cameroonian folk medicine. Afri*. J Trad. Complimentary Alternative Med.*,5 : 302 -321.

Idowu O.a, Soniran O.T, Ajana O, and Aworinde D. O (2010): Ethno botanical survey of anti-malaria plants used in Ogun State Southwest Nigeria. *Afr. J. Pharmacy Pharmacol*., 4: 055 – 060

Tantchou P. K, Titanji V, Aldivo and Jensen (1986): Studies on Cameroon ian medicinal plants; Anti-malaria activity of the extracts of *Alastonia bonei* and *Guibourtessmanii* on

the Vietnam smith strain of *Plasmodium falciparum. Revue Science et Technique*, **3**: 69 – 77.

Peter L. T, and Anatoli V. K (1998a): The Current Global malaria Situation. Vol. 22. Malaria Parasite Biology, Pathogenesis and Protection. ASM Press Washington DC

David A. F, Philip J. R, Simon R. C, and Solomon N (2004): Anti-malaria drug discovery: efficacy models for compounds screening. *Nature Rev.*, 3: 509 -520.

Deprez-Poulain R, and Melnyk P (2005): 1,4-Bis (3-aminopropyl0 piperazine libraries: From the discovery of classical chloroquine –like anti-malaria to the identification of new targets. Comb. Chem. High Throughput Screen. 8: 39 -48.

Portet B, Fabre N, Roumy V, Gornitzka H and Bourdy *et al* (2007): Activity guided isolation of anti-plasmodial dihydrochalcones and flavones from *Piper hostmannianum* var.berbicense. *Phytochemistry*, 68: 1312 – 1320.

Jones M. K and Good m. F (2006): Malaria parasites up close *Nat. Med*. 12: 170 -171

Winter R. W, Kelly J. X, Smiikstein M. J, Dodean R, and Bagby G. C *et al* (2006): Evaluation and lead optimization of anti-malaria acridones. *Exp. Parasitol.*, 114: 47 -56.

Taylor W. Rand White N. J (2008): Anti-malaria drug toxicity: A review. *Drug Saf.,* 27: 25 - 61.

Jambou R, Legrand E, Niang M, Khim N and Lim p *et a*l (2005): Resistance of *Plasmodiumfalciparum* field isolates to *in vitro* artemether and point mutation of the SERCA type Pf ATPase. *Lancet*, 366: 1960 – 1963.

Lamidi, M., E. Ollivier, R. Faure, L. Debrauwer, L. Nze-Ekekang, and G. Balansard. (1995): Quinovic acid glycosides from *Nauclea diderichii*. Planta Med. 61:280–281.

Lorke D (1983): A new approach to acute toxicity testing. *Arch. Toxicol.*,54; 275 -287