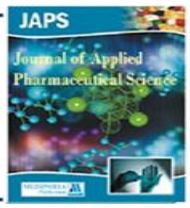
Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 02-12-2011
Revised on: 15:12:2011
Accepted on: 19-12-2011

In-vivo antimalarial and toxicological evaluation of *Chrozophora senegalensis* A. Juss (*euphorbiaceae*) extracts

Ali A. Jigam, Usman. T Abdulrazaq and Martins N. Egbuta

Ali A. Jigam, Usman. T Abdulrazaq and Martins N. Egbuta
Malaria and Trypanosomiasis Research Unit, Department of Biohemistry, Federal University of Technology, Minna, Nigeria

For Correspondence
Ali A. Jigam
Malaria and Trypanosomiasis Research Unit, Department of Biohemistry, Federal University of Technology, Minna, Nigeria.
Cell: +2348036136862

ABSTRACT

The antiplasmodial, analgesic, antiinflammatory and chronic dose effects of methanolic extract of *Chrozophora senegalenesis* A. Juss were studied in mice. *Plasmodium berghei* (NK 65 chloroquine sensitive strain) was inoculated into eighteen mice assigned to 3 groups of 6 mice each . Group I was treated with 75mg/kg bw *C. Senegalensis*, group II with 5mg/kg bw chloroquine phosphate (standard) and group III with 20ml/kg bw normal saline (Control). Anagelsia and antiinflammation were analysed by the Acetic acid induced abdominal constriction in mice and egg albumin induced paw oedema in rats respectively. Another set of 40 mice were divided into two groups of twenty each (test and control) and some serum parameters studied. The test animals were gavaged with extract while controls were given normal saline over a period of 5 weeks. *C. senegalensis* suppressed parasitemia in mice by 51.80%, had 37.05% anagelsia, and 60.92% anti-inflammatory activity. Body weights, packed cell volume and serum triacylglycerides significantly ($p<0.05$) decreased in mice given *C. senegalensis* while serum glucose, Aspartate amino transferase (AST), Alanine amino Transferase (ALT) and Alkaline phosphase (ALP) increased significantly ($p<0.05$) in the test mice over the study period. In conclusion, *C.senegalensis* is effective in the management of malaria but long term consumption can predispose to adverse physiological effects.

Keywords: *Chrozophora senegalensis*, *Plasmodium berghei*, analgesia, physiological, antiinflammation, serum.

INTRODUCTION

Malaria remains a protracted global disease problem compromising improved health care and life expectancy among the poor especially in South-east Asia and sub-sahara Africa. It is a "re-emerging disease", the most vulnerable to which are pregnant women and children under 5years of age (Jigam *et al*., 2011)[b]. It is estimated that 300-500 million acute infections and up to 3 million deaths occur annually from the disease (WHO, 2008). Most common antimalarials have been rendered ineffective because *plasmodium*, species the causative parasites, have acquired resistance to them. With no viable vaccines in sight, it has become a widely accepted and common phenomenon to source for alternative compounds mostly of plant origin in the fight against malaria (Matuscheski, 2009; Jigam *et al*., 2010). The screening of herbal drugs used for treating malaria may yet offer some hope in the search for a new generation of cures for the disease. In fact quinine isolated from *Cinchona ledgeriana* (*Rubiaceae*) has been the fulcrum of malarial treatment for over a century. Similarly, artemisinin, a sesquiterpene lactone with potent antimalarial action was recently isolated from the Chinese antipyretic herb, *Artemisia annua* (Tona *et al*., 2009; Jigam and Akanya, 2007). The wide spread use of plants as medicaments is well documented. It has been

reported that over 80% of the global populace use plants as their primary source of medication (Cordell, 2000; Rahman and Choudhary, 1999; Usman *et al*; 2007). This practice is common in northern Nigeria where a variety of plant species are used in the treatment and management of malaria often without proper scientific documentation. A common problem with the use of crude plant extracts in ethnomedicine is the lack of toxicological evaluation of such plants. This could predispose to tissue and organ damage often with disastrous consequences (Gamaniel, 2000).

Chrozophora senegalensis A. Juss *(Euphorbiaceae),* known variously among the Hausa tribe as "*Walkin maciji, Bauren kiyashi, damagi or Damangi*" was selected on the basis of literature and folklore reports (Dalziel, 1955; Etkin, 1997; Usman et al; 2007). It is a herb with deep red flowers and violet tingle capsules commonly found in dried up inundated flats or sandy river beds (Sofowora 1993). Chrozophora has extra floral nectarines. Its medicinal applications are varied including treatment of intestinal pains, conjunctivitis, diarrhea, syphilis, boils, and fever (Tignokpa *et al*; 1986; Etkin, 1997 , Yusha'u, 2011). These reports necessitated the invivo evaluation of its antiplasmodial, analgesic and antiiflammatory potentials including the effects of chronic consumption of *C. senegalensis* in herbal medicine.

MATERIALS AND METHODS

Plant Materials

Whole *Chrozophora senegalensis* were collected between April and June in Minna, Northern Nigeria and authenticated at the Department of Biological Sciences, Federal University of Technology, Minna.

Preparation of Crude Extracts

50g of air dried plant materials were micronized and extracted exhaustively (48 h) in the cold with 2L of methanol, (Sigma-Aldrich Europe). The marc was filtered with muslin cloth and solvent removed under reduced pressure in a rotary evaporator. Green coloured paste was freeze dried and weighed prior to analysis.

Animals

Healthy swiss albino mice of either sex of about 6 weeks old weighing between 20 – 30 g each and wister rats of about 180 – 200 g weights obtained from National Institute of Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria were used for the experiments. The rodents were conveniently housed under standard environmental conditions. (Temperature 27 ± 2°C; 70% relative humidity; 12hrs daylight/night cycle) and had free access to commercial feed pellets and water. Experiments were conducted in strict compliance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (CCAC, 1997).

Parasites

P. berghei NK65 chloroquine sensitive strain was obtained from NIPRD Abuja, Nigeria and maintained in our laboratory by serial passage in mice.

Safe dose and acute toxicity (LD_{50})

Five groups (A,B,C,D and E) of four mice each were used. The animals were given extracts intraperitoneally (i.p) at doses of 50, 75, 100 and 200mg/kg body weight (bw) in A,B,C,D and E respectively. Extracts were dissolved in dimethylsulphoxide (DMSO) (Sigma chemicals; St. Louis, M. O. USA).

A control group was given normal saline (0.9% w/v NaCl) at 20 ml/kg bw. Mice were observed over 72h. Clinical signs and mortality were recorded. LD_{50} was obtained graphically as the intercept of % mortality (y-axis) and dosages (x-axis).

Antiplasmodial screening

Mice were pre-screened by microscopy of thin and thick tail tip blood smears. This was necessary to exclude the possibility of test animals harboring rodent *Plasmodium species*.

The method by Fidock *et al.*, (2004) was used. It involved the commencement of treatment on the third day post inoculation of mice with parasite. Eighteen male and female mice were divided into three groups of six each. A mouse infected with *P. berghei* (parasitaemia of about 20 - 30%) was anaesthetized with chloroform and its blood collected by cardiac puncture with a sterile syringe and needle earlier flushed with heparin. The blood was diluted with normal saline such that 0.2 ml contained about 1 x 10^7 infected cells. Each of the eighteen clean mice were inoculated (i.p.) with 0.2 ml diluted blood. The extract at a dose level of 75mg/kg body weight was administered subcutaneously once daily for four days (D3, D4, D5 and D6). A parallel test with chloroquine (5 mg/kg bw) in the second group served as reference. The third group was given normal saline and served as control. Thick and thin films were made from tail blood from D3 – D6, fixed with methanol and stained with 4% Giemsa (pH7.2) for 45 min before being examined under a microscope. Five fields were examined on each slide and the number of infected and uninfected red blood cells (RBC) counted and means taken. Percentage suppression of parasitaemia was calculated using values from controls related to those of treated animals. Standard drug equivalent was also determined from the ratio of chloroquine (standard) dose to dose of test drug giving identical average percentage suppression.

Analgesic activity

Analgesia was assessed by the method of Koster *et al.*, (1959). Fifteen mice were divided into three groups. The extract (75 mg/kg bw) was administered mice in groups A, an hour before they were challenged with acetic acid (0.75% v/v). Animals in group B were however pretreated with Acetyl Salicylic acid (150 mg/kg bw) as reference drug, while group C which were given normal saline (20 ml/kg bw) served as controls. Five minutes elapsed before the numbers of abdominal constrictions induced by acetic acid were counted. Observations were made over ten minutes and mean value for each group calculated. Percentage inhibition of abdominal constriction by the plant extracts and ASA

were determined in relation to the control. ASA equivalent was also calculated.

Anti-inflammatory activity

The anti-inflammatory activity of the extract was tested using egg albumin induced paw oedema in rats (Winter *et al.*, 1962). Eighteen Adult rats were divided six per each treatment group and used for the analysis. Inflammation was induced by the injection of 0.01 ml egg albumin into the sub-planter surface on the right hind paw 30 min after administering the extracts (75 mg/kg bw i.p). The increase in volume (cm^3) of the hind paw was measured with a LETICA digital Plethysmometer (LE 7500) before and at 20 min interval after the injection of egg albumin for a period of 2 hr. Control rats received an equivalent amount of normal saline while ASA (150 mg/kg bw) served as reference. The percentage inhibition of oedema was calculated for each dose.

Evaluation of the effect long term dosage of crude extract in mice

Forty mice were kept in two groups (A and B) of twenty each. Group A was used as test and gavaged with 75mg/kg bw extract daily while B was control and given 20ml/kg bw normal saline daily. All animals were monitored for different biochemical parameters at weekly intervals for five weeks.

Weights of mice were taken with an Avery Balance (W and T) Avery Ltd, Birmingham, UK Packed Cell Volume (PCV) was determined using the microhaematocrit method (Green, 1976). Serum glucose was assayed with Randox glucose diagnostic kit (Cat/Kat NR GL 2623) based on the glucose oxidase reaction. Total proteins were evaluated with the Randox Protein Diagnostic Kit (TP 245) based on the interaction of cupric ions in alkaline media with protein peptide bonds. The AGAPPE, Triglyceride Kit (Cat. 1121500, Kerala, India) was employed for serum triglycerides. This involves the reactions by lipases, glycerol kinase, glycerol – phosphate oxidase and peroxidase which produced a red quinone dye read at 630nm (Annoni, *et al.;* 1982). Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were analyzed using the DIALAB IVB standard diagnostic kits based on the method of Wolf, (1980). Alkaline phosphatase (ALP) was however determined on the basis of the conversion of P – nitrophenol to its intensely yellow coloured derivative, 4 – nitrophenoxide (Tietz, 1983). The diagnostic reagent (DIALAB Cat D95560) kit was used.

Statistical Analysis

Results are expressed as mean ± standard error of the mean. While student's t-test was used to test for differences between groups using Statistical package for social sciences (SPSS) version 16. A value of $P<0.05$ was accepted as significant and the data compared using Analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The crude extract yield of *C. senegalenses* in methanol was 2.35g (4.7%), the safe dose was 75mg/kg body weight of mice and LD_{50} was 175mg/kg body weight.

Antiplasmodial activity

The effect of crude *C senegalensis* against *P. b erghei* in mice are given in Table 1 which indicates 51.80% suppression of parasitemia. The result of analgesic effects of *C. senegalensis* are in Table 2 and it exhibits a minimal 37.05% (activity). Antiinflammation (Table 3) by the plant extract was high (60.9%).

Table 1: *P. berghei* suppression in mice using methanolic extract of *C. senegalensis.*

Treatments	*Dose(mg/kg b.w)*	*Parasitaemia (X + SEM)*	*Decrease (%)*
C. senegalensis	75	38.00± 2.15	51.80
Chloroquine	5	23.50± 2.48	70.20
phosphate	20ml	78.85±3.13	-
Normal saline			

n=6

Table 2: Effects of *C. senegalensis* extract on acetic acid induced abdominal constriction in mice (analgesia).

Treatments	Dose(mg/kg b.w)	Abdominal constriction (X + SEM)	Inhibition (%)
C. senegalensis	75	39.039± 2.24	37.05
Acetylsalicylic acid	150	8.57± 2.35	82.25
Normal saline	20ml/kg	48.28±4.02	-

n= 5

Table 3: Effects of *C. senegalensis* extracts on rat paw oedema (antiinflammation).

Treatments	Dose(mg/kg b.w)	Paw oedema (mm^3)	Inhibition (%)
C. senegalensis	75	0.34	60.92
Acetylsalicylic acid	150	0.16	81.61
Normal saline	20ml/kg	0.87	-

n=6

Effect of long term dose of crude *C. Segenalenses* extract in mice

A progressive decline in weight was obtained for mice dosed over five weeks with the extract, (Fig1). The packed cell volume (PCV) of the animals also declined (Fig 2).

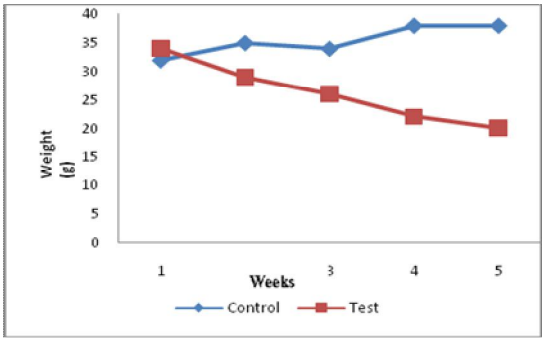


Fig. 1: Weight Variation in mice dosed with *C. .senegalensis* extracts.

Results for serum glucose, total proteins and triacylglycerides are in Table 4. A significant ($p<0.05$) elevation was obtained for glucose. Total proteins exhibited insignificant variation ($p>0.05$) but triacylglycerides significantly ($p<0.05$) decreased in test mice.

Table 4: Serum Glucose, Total Proteins and Triacylglycerides dosed wit *C. Senegalensis* extracts in mice.

Parameters		Weeks 1	2	3	4	5
Glucose (mg/dL)	Control	95.24±1.25	97.44±2.50	96.13+3.85	98.33±2.47	100.28±4.44
	Test	94.28±1.47	99.01+1.89	115.07±1.11*	116.41±2.22*	118.37±1.76*
Protein (mg/dL)	Control	6.30±1.55	5.42±1.85	7.18±2.00	7.59±1.46	6.81±1.17
	Test	5.88±0.19	3.58±1.13*	4.22±1.50*	6.05±1.66	6.75±2.15
TAGs (mg/dL)	Control	156.04+4.00	155.11±4.23	145.62±1.88	155.24±1.14	153.25±3.05
	Test	140.22+10*	142.13±0.13*	140.00±1.58*	145.89±1.62*	148.35±3.08*

n= 20; Values are X±SEM; *= p<0.05.

Table 5: Serum AST, ALT and ALP in mice dosed with *C. senegalensis* extracts.

Serum Enzymes (µ/L)		Weeks 1	2	3	4	5
AST	Control	50.00±1.93	44.50±1.25	42.00+3.96	48.00±4.11	52.50±2.39
	Test	48.50±0.88	46.00+3.36	49.10±5.00*	56.08±3.25*	53.51±2.56
ALT	Control	29.50±1.56	31.50.±3.56	30.00±2.47	33.00±2.78	32.00±3.37
	Test	30.00±2.22	33.00±3.62	32.00±2.31	40.50±4.23*	45.00±3.56*
ALP	Control	105.0+3.59	125.50±4.55	110.50±6.13	130.00±4.79	110.50±5.55
	Test	141.00±2.56*	144.50±3.00*	110.00±4.22	126.00±2.69	126.00±5.81*

n= 20 ; Values are X±SEM; *= p<0.05

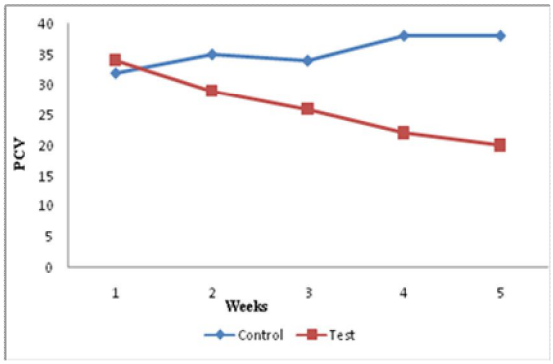


Fig. 2: Packed Cell Volume (PCV) in mice dosed with *C. senegalensis* extract.

The values for serum Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline Phosphatase (ALP) are given in Table 5. AST was significantly (p<0.05) elevated in weeks three and four, ALT in weeks four and five, while ALP was weeks one, two and five.

DISCUSSION

Crude *C. senegalensis* moderately suppressed *P. berghei* in mice. This potential is in conformity with earlier findings by Benoit-Vical *et al* (2008). Reports also abound of the antimicrobial activity of the plant against a variety of bacteria species including pathogens (Usman *et al*., 2007; Benoit-Vical, 2008 and Yusha'u *et al*., 2011). Some antimicrobial agents e.g tetracycline and clindamycin are reported to be used as antimalarials, thus establishing a link between the two effects (Bloland, 2001). The antiplasmodial efficacy of crude *C. senegalensis* may be enhanced by further purification. It has been suggested that crude plant extracts tended to have better plasmodistatic than plasmodicidal effects as unpurified bioactive principles require initial conversions which time lag allows for parasite proliferation (Jigam *et al*., 2011[b]). The significant anti-inflammatory and moderate analgesic effects of the plant further signifies its suitability as an antimalarial agent. These additional pharmacological phenomena to antiplasmodial action are better in the resolution of the disease than bioactive agents that clear parasites only (Tona *et al*., 1999).

The decline in whole body weights and packed cell volume in mice chronically dosed with *C. senegalensis* are noteworthy. These can be attributed to antinutritive factors in the crude extract. There could be loss of apetite and even poor feed utilization by such animals. Tannins inhibit growth by decreasing the digestion coefficient of most nutrients and the coagulation of proteins. Essential minerals such as calcium, iron, magnesium etc can be chelated and adversely affect vital processes such as hemopoiesis (Sotohy *et al*., 1997; Jigam *et al*., 2011[a]). Serum glucose, AST, ALT and ALP were significantly (p<0.05) elevated in the test animals and could indicate some tissue damage.

Alterations in serum glucose levels other than those associated with stress are uncommon, hence the present finding could point to some destructions of pancreatic islets of langerhans responsible for insulin synthesis (Gad, 2001).. Serum ALT level is of greater diagnostic specificity in respect to the liver than AST. AST exists in high levels in many tissues such as kidney, heart, liver, skeletal muscles etc (Atangwho *et al*., 2007; Sodipo *et al*., 2011). Raised serum ALP is usually encountered in billiary disorders such as obstructive jaundice and cirrhosis, bone and intestinal disorders (Tietz, 1983). The insignificant variation in total proteins at the end of week five is in contrast with the consistent hypolipidemic effect of the plant extract in mice. Triglycerides are rich alternative sources of metabolic energy and can readily be mobilized on demand. The present study has shown the antimalarial potency of *C. senegalensis* but the major problem

is associated with the elevation of some biochemical parameters and enzymes due to long term consumption of the crude plant extract. Histopathological studies of vital organs are however required to confirm the safety of *C. senegalensis*

CONCLUSION

Chrozophora senegalensis could be a source of an effective malarial medicament. However, this potential will be better harnessed by further purification in an attempt to isolate the bioactive principle(s) and minimize the toxicity inherent in the crude extracts.

ACKNOWLEDGEMENT

This research was financed by a PhD (2000-2008) and University Board of Research (2009) grants to Dr A. A. Jigam Courtesy Federal University of Technology, Minna NIGERIA. Chloroquine Sensitive *P. berghei* (NK65) strain was obtained by kind permission from Mr. Zakarrayya of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja NIGERIA.

REFERENCES

Annoni GG, Bottasso RM, Ciaci D, Donato MF and Tripoli A. Clinical Investigation of triglycerides. *J. Res. Lab. Med*. 1982; 9:115.

Atangwho IJ, Ebona PE, Ebbung GE, Eteng MU and Eyong EU. Effects of *Veronia amygdalina* Del. on liver function in alloxan-induced hyperglycaemic rats. *J. Pharmaceut Biores*. 2007. 4(1): 25-31.

Benoit-Vical F, Soh PN, Salery M, Harguem L, and Poupat C. Evaluation of *senegalensis* plants used in malaria treatment: Focus on *Chrozophora senegalensis*. *J Ethanorpharmacol*.2008; 116(1):43-48.

Bloland PB. Drug resistance in malaria. Background Document for WHO Global strategy for Containment of antimalarial Resistance. WHO, Switzerland. 2001: 3-27.

CCAC. Canadian Council of Animal Care Guidelines and Protocol Review. 1997.

Cordell GA. Biodiversity and Drug Discovery- a Symbiotic Relationship. *Phytochem*. 2000; 55:463-480

Dalziel JM. The useful plants of West Tropical Africa. Vol. 1 Millbank, London, S.W.: Crown Agents for Oversea Government and Administration (1955). 271-273.

Etkin NL. Antimalarial Plants used by Hausa in northern Nigeria. Tropical Doctor. 1997; 27(1):12-16.

Fidock DA, Rosenthal PJ, Croft SL, Brun R and Nwaka S. Antimalarial drug discovery: Efficacy Models for Compound Screening. Supplementary Documents. *Trends Parasitol*. 2004; (15): 19-29.

Gad SC. Principles and methods of Toxicology. Taylor and Francis, Philadelphia. (2001): P. 351-353.

Gamaniel KS. Toxicity from medicinal plants and four products. *Nigerian J natural Prodt. and Med*.2000; 4:4-8.

Green JH. An Introduction to Human Physiology. *Oxford Medical Publications London*. (1976). 128-130

Jigam AA and Akanya HO, Phytochemical Antimicrobial and Antiplasmodial Screening of *Cohlospectrumum tinctorium*. *J sci. Edu*.2007; 1(1): 4-8.

Jigam AA, Akanya HO, Dauda BEN and Ogbadoyi EO. Antiplasmodial, analgesic and anti-inflammatory effects of crude *Guiera senegalensis* Gmel (Combretaceae) leaf extracts in mice infected with *Plasmodium berghei*. *J Pharmacog. and Phytother*. 2011[b]; 3 (10):150-154

Jigam AA, Bukar END, Jimoh T, Hauwa NY, and Umar ZT. Determination of Copper, Zinc, Lead and some Biochemical Parameters in fresh cow milk from different locations in Niger State, Nigeria. 2011[a]; *Afr. J. Fd. Sci*. 5(3): 156-160.

Koster R, Anderson M and Debeer EJ. Acetic acid Method of Analgesic Screening. *Curr. Opin. Immunol*. 1959; 18:412.

Matushcheski K. Vaccine development against malaria. *Curr. Opin. Immunol*. 2009; 18(4): 449-457.

Rahman AU and Choudhary MI. Recent Studies on Bioactive Natural Products. *Pure App Chem*.1999; 71(6): 1079-1081.

Sodipo OA, Abdulrahman FI, SAndabe UK and Akinniyi JA. Biochemical liver function with aqueous fruit extract of *Solanum macrocarpum Linn.* In albino rats acutely administered triton-x to induce hyperlipidaemia. *J. Appl. Pharmaceut. Sci*. 2011; 01(08): 89-93.

Sofowora A. Medicinal Plants and traditional medicine in Africa. 2nd ed. Sunshine House, Ibadan, Nigeria Spectrum Books Ltd. (1993). 134-156.

Sotohy SA, Sayed AN, and Ahmed MM. Effects of Tannin-Rich plant (*Acacia nilotica*) on some Nutritional and Bacteriological parameters in Goats. *German J. Sci*., 1997; 104(10): 432-435

Tietz NW. A reference method for the measurement of ALP activity in Human serum. Study group on ALP. *J. Clin. Chem*. 1983; 29: 751.

Tignokpa M, Laurens A, Mboup S and Sylla O. Popular Plants of Dakar Markets. *Int J Crude Drug Res. 1986; 24(2): 75-80*.

Tona L, Ngera NP, Nsakala M, Cimanga C and Vietnick AJ. Antimalarial activity of 20 crude extracts from African Medicinal Plants used in Kinshasa, Congo. *J. Ethnopharmacol*. 1999; 68: 193-203

Usman HW, Musa YM, Ahmadu AA and Tijjani MA. Phytochemical and Antimicrobial Effects of *Chrozophora Senegalensis*. *Afri J. Tradit., Complement and Altern Med*. 2007; 4(4): 488-494.

Winter CA, Risley EA and Nuss GV. Carrageenin induced oedema in hindpaw of rats as an assay for anti-inflammatory drugs. *Proc. Soc Exptl Biol. Med*. 1962; 3:544-547.

Wolf S. Assay of SGPT. *Clin. Chem. Acta*. 1980; 105:147-172.

World Health Organization (WHO). Tropical Report Series Expert Committee on Malaria: 32nd Report Geneva 2008.

Yusha'u M. Phytochemistry and inhibitory activity of *Chrozophora senegalensis* extracts against some clinical Bacterial isolates. *Bayero J Pure and Appl. Sci.; 2011:* 4(1): 153-156.