



# International Journal of Pharmacology

ISSN 1811-7775

**science**  
alert

**ansinet**  
Asian Network for Scientific Information

## The Butanol Extract of *Mitragyna ciliata* Root: Potential as a Trypanocide

<sup>1</sup>H.A. Ogbunugafor, <sup>2</sup>V.I. Okochi, <sup>3</sup>J. Okpuzor, <sup>2</sup>O.S. Odesanmi and <sup>1</sup>F.J. Ebigwe

<sup>1</sup>Department of Applied Biochemistry,

Faculty of Natural Sciences, Nnamdi Azikiwe University, Awka, Nigeria

<sup>2</sup>Department of Biochemistry, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria

<sup>3</sup>Department of Cell and Genetics, Faculty of Science,  
University of Lagos, Yaba, Lagos, Nigeria

**Abstract:** Bioassay guided-fractionation of *Mitragyna ciliata* Aubrev and *Pellegr* (Rubiaceae) ethanolic root extract at 100 mg kg<sup>-1</sup> in *T. brucei* infected rats indicated that the bioactive constituent reside is present in the butanol fraction (inhibition = 66.61%). *In vitro* investigation of the extract revealed that it had low (25.55%) antioxidative property. Chemical analysis of the active fraction showed that it consists of alkaloids. The extract's fraction had no effect on the hematological profile of treated rats which remained consistent with the major characteristics of trypanosomiasis-anaemia, leucocytopenia and thrombocytopenia. Results of the *in vivo* evaluation of calcium concentration showed a significant difference ( $p < 0.05$ ), between the active butanol fraction ( $2.53 \pm 0.036$  mmol L<sup>-1</sup>) and the untreated/infected ( $17.79 \pm 0.034$  mmol L<sup>-1</sup>). A correlation existed between calcium concentration and parasitaemia in the active fraction ( $r = 0.40$ ,  $p = 0.488$ ) and the infected/untreated ( $r = -0.60$ ,  $p = 0.29$ ). These observations suggest that the active agent had an effect on the calcium metabolism in the animals which was deleterious to the parasites.

**Key words:** *Mitragyna ciliata*, trypanocidal, bioactive, calcium

### INTRODUCTION

Combating trypanosomiasis or sleeping sickness is still a major health challenge in sub-Saharan Africa. Trypanosomiasis caused by flagellated protozoa parasites-trypanosomes; infects both humans and livestock and is a reemerging disease (WHO, 2000; Scovil *et al.*, 2001; Mishina *et al.*, 2007). Animal trypanosomiasis causes an estimated economic loss of US\$4.5 billion each year (TDR, 2005). The search for vaccines remains elusive and the prospects of prophylactic immunisation are poor since the parasites change their surface coat to evade the host immune system in a process known as antigenic variation (Burchmore *et al.*, 2002). Existing trypanocides are faced with drug resistance, toxicity and a lack of guaranteed supply (Burchmore *et al.*, 2002; De Koning, 2001). Drugs remain the principal means of intervention. Many potentially good biochemical targets for drugs have been identified, some of these have been validated and lead compounds developed (Mottram and Coombs, 1999). The infection contributes to poverty more through its effect on livestock than through its effects on humans. This makes it expedient for scientists to intensify the

search for leads from medicinal plants used in ethno medicine for the treatment of trypanosomiasis. Finding trypanocides from medicinal plants offers a real hope for the control and management of this disease, within the context of the economies of the countries to which this disease is endemic.

Traditional medicine practitioners in Nigeria use many plants for the treatment of sleeping sickness. These plants need to be exploited for new and efficient medicines that are less expensive and therefore affordable to the poor and affected population. A number of these plants have been reported in literature to exhibit trypanocidal activity (Wurochekke and Nok, 2004; Atidehou *et al.*, 2004; Atawodi, 2005), but most of these plant extracts have not been evaluated for their various biochemical effects *in vivo*.

*Mitragyna ciliata* Aubrev and *Pellegr* (Rubiaceae) is a plant used by traditional healers in South-Eastern Nigeria for the treatment of trypanosomiasis (Ogbunugafor *et al.*, 2007). Our earlier studies of the *in vivo* trypanocidal activity of the root extract of this plant corroborated this practice (Ogbunugafor *et al.*, 2005, 2007). The aim of this present study is to evaluate the effect of fractions of *M. ciliata* root extract on

hematological parameters and determine the effect of the active fraction on serum  $\text{Ca}^{2+}$  concentration, in order to understand the basis for its activity.

## MATERIALS AND METHODS

**Plant preparation:** The fresh root of the plant was collected in August and authenticated at the Forestry Research Institute (FRIN) Ibadan, Nigeria, by Mr. Felix Isang and Mr. T.K. Odewo, respectively. Plant specimen with No. FHI: 106999 *Mitragyna ciliata* Aubrev Pellergr (*Rubiaceae* local name *Abura*) was deposited at the FRIN herbarium.

**Animals:** In this study, 25 male Swiss albino mice weighing about 20.0 g and 60 Wistar rats of mixed sexes, weighing between 200-250 g purchased from the animal house of College of Medicine, University of Lagos, were used for the experiment. The animals were housed in standard mouse and rat cages and were maintained on standard pellet diet and water *ad libitum* and were acclimatised for 2 weeks.

**Chemicals and solvent:** All chemicals and solvents were supplied by Sigma-Aldrich Chemical Company, Germany. Reference drug Somorenil<sup>®</sup>-diminazine diacetate was supplied by Eagle Chemical Company Ltd., Ikeja, Nigeria.

**Extraction of plant material:** Three hundred grams of air-dried powdered root bark were extracted with ethanol/ $\text{H}_2\text{O}$  (8:2) by cold extraction for 72 h. This process was repeated several times to remove the extractable components. Combined extracts was evaporated to dryness under reduced pressure below 40°C to yield 20.34 g.

**Phytochemical analysis:** Phytochemical analysis of the crude extract of the plant was performed by the method of Sofowora as described by Edeoga *et al.* (2005). Test for presence of alkaloids, glycosides, tannins, saponins, flavonoids, were carried out.

**Free radical scavenging activity of *M. ciliata*:** The scavenging activity of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical by *M. ciliata* extract was determined according to the method reported by Gyamfi *et al.* (1999). *M. ciliata* ethanolic root extract was prepared in 50 mL methanol, to yield concentrations of 0.02, 0.04, 0.08 and 0.1 mg mL<sup>-1</sup>. Each concentration was mixed with 100 mL of 0.1 mM DPPH-ethanol solution and 450 mL of 50 mM Tris-HCl buffer (pH 7.4). Methanol (50 mL) alone was used as negative control and Vitamin E was used as positive control in this experiment. The reaction mixture was

incubated for 30 min at room temperature. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm.

**Fractionation of extract:** The extract was fractionated by the method of Wu *et al.* (2005). This method involved successive extraction by increasing polarity with n-Hexane, chloroform, ethyl acetate, saturated n-butanol and water. Each extract was evaporated to dryness under reduced pressure to yield n-Hexane fr (4.16 g),  $\text{CHCl}_3$  fr (5.59 g) EtOAc fr (6.90 g), BuOH fr (1.46 g) and  $\text{H}_2\text{O}$  fr (2.0 g).

### Animals studies

**Acute toxicity test of crude extract:** Determination of the dose of root extract of *M. ciliata* that would kill 50% of animal population ( $\text{LD}_{50}$ ) was undertaken according to the method of Litchfield and Wilcoxon as described by Aji *et al.* (2001). Five groups of 5 mice each of male albino mice were used for the study. They were fasted overnight with water *ad libitum*. Doses of 500, 1000, 2000, 4000 and 8000 mg kg<sup>-1</sup> were administered orally with the aid of a cannular. Animals were observed for 24 h and mortality was recorded. The  $\text{LD}_{50}$  was calculated by probit analysis method.

### In vivo antitrypanosomal evaluation

**Test organism:** Parasites *T. brucei brucei* were obtained from field isolates from cattle at the Department of Veterinary Parasitology and Entomology, University of Nigeria Nsukka. They were maintained by continuous passaging in the laboratory until needed.

**Trypanocidal screening of crude extract:** Eighteen Wistar rats grouped into 3 groups of 6 rats each were used for trypanocidal screening of *M. ciliata* crude root extract. The animals were inoculated with approximately  $10^6$  trypanosomes per milliliter of blood using phosphate buffer saline and infection was allowed to establish for 5 days. Two groups served as test and positive control (i.e., Reference drug-diacetate). A third group served as negative control (untreated). The extract was dissolved in 5% carboxyl-methyl cellulose and administered at a dose of 50 mg kg<sup>-1</sup> for 5 days.

**Trypanocidal evaluation of fractions:** Seven groups of 6 rats each and were inoculated with  $10^6$  trypanosomes per milliliter of blood using phosphate buffer saline. After 5 days, the animals were administered with the test compounds (extract fractions) and reference drug (diacetate). A group served as negative control (i.e., infected/untreated). The fractions administered were n-Hexane, Chloroform, Ethyl Acetate, saturated n-Butanol

and Water. The n-Hexane and Chloroform fractions were dissolved in 1% tween 20, while other fractions were dissolved in 5% CMC. The fractions were administered orally, to the infected rats at a dose of 100 mg kg<sup>-1</sup> for 5 days. Reference drug was administered at 3.5 mg kg<sup>-1</sup> also for 5 days.

**Monitoring and parasiteamia estimation:** Parasiteamia of each rat was monitored during the administration of the extracts and drug and the levels were estimated by Rapid Matching Method of Herbert and Lumsden as described by Atawodi (2005). Parasiteamia levels were compared with untreated control animals and percent inhibition of parasites assessed by the formula 100- [parasiteamia of test/parasiteamia of untreated × 100]. Assessment of the extracts and fractions was also based on survival periods of the animals.

**Biochemical analysis**

**Heamatological evaluation:** The heamatological analysis of infected and treated animals was carried out using automated heamatology analyser (Coulter A<sup>c</sup>. T.diff., Beckman coulter, Miami, USA.). The Packed Cell Volume (PCV) was determined using standard micro-haematocrit method. White blood cell count was determined by haemocytometer method.

**Determination of calcium concentration:** Calcium concentration in mmol L<sup>-1</sup> was estimated by the method of Moorehead and Briggs as described by Ledwaba and Roberson (2003) using commercially available kit supplied by Biolab S.A. UK.

**Statistical analysis:** Data was analyzed with Medcalc statistical package of Microsoft Excel. Values are means±SEM. The level of significance was determined using student's t-test and correlation test was by Rank's correlation.

**RESULTS**

The drugs/extract ratio of ethanolic root bark extract *M. ciliata* was 1:15, while the phytochemical screening revealed that it consisted of alkaloids, cardiac glycosides,

flavonoids and saponins. Evaluating the extract for the free radical scavenging activity revealed a low antioxidative property with average inhibition of 25.25±0.090 compared to vitamin E 82.84±0.334 (p<0.05) (Fig. 1).

Preliminary screening of the root extract showed a significant inhibitory effect (54.24%) at a dose of 50 mg kg<sup>-1</sup> in *T. brucei* infection. The bioassay guided-fractionation at a dose of 100 mg kg<sup>-1</sup> of *M. ciliata* root extract in the infected rats, as indicated in Fig. 2 showed that the butanol fraction was the most active (66.61%) Table 1 in comparison with the reference drug group (77.03%). As also shown in Fig. 2, the n-hexane, chloroform and the aqueous fractions were inactive while ethyl acetate fraction showed slight activity.

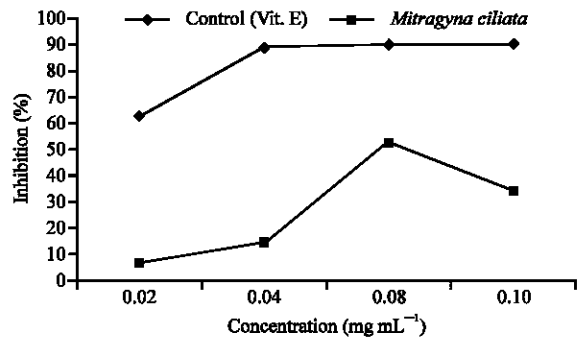


Fig. 1: Free radical scavenging activity of *M. ciliata*

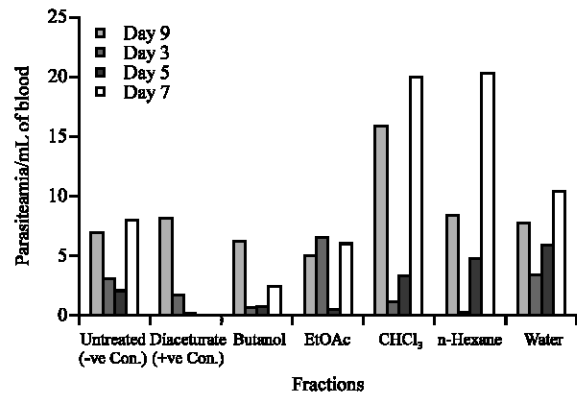


Fig. 2: Parasitemia of fractions treated rats

Table 1: Heamatological indices of fractions *M. ciliata* extract in *T. brucei* infection

Fractions	PCV (%)	HB (g dL <sup>-1</sup> )	WBC (mm <sup>3</sup> )	Platelets (×10 <sup>3</sup> )	Lymphocytes	Neutrophils
Uninfected/Untreated	38.00±2.40	13.80±2.30	2900±132.40	200.07±2.30	50.02±3.40	49.08±2.90
Untreated	21.00±2.35*	6.98±0.77*	4300±254.90 <sup>†</sup>	170.20±4.90*	46.40±1.44	53.60±1.44
Pet ether	19.20±2.20*	6.40±0.73*	3360±156.80 <sup>†</sup>	138.00±2.55*	47.40±1.60	52.40±1.63
Chloroform	25.00±2.15*	8.34±0.72*	4100±291.55 <sup>†</sup>	115.00±7.07*	42.20±2.27	57.40±2.46
Ethyl acetate	22.40±1.54*	7.47±0.51*	3400±187.00 <sup>†</sup>	117.00±7.68*	43.80±2.63	56.20±2.63
Butanol	19.20±2.20*	6.47±0.29*	3940±337.05 <sup>†</sup>	118.00±9.03*	43.40±2.10	56.60±2.06
Water	18.40±1.57*	6.13±0.52*	3340±187.10 <sup>†</sup>	146.60±1.57*	45.60±2.06	54.40±2.46

<sup>†</sup>Significantly higher, \*Significantly lower

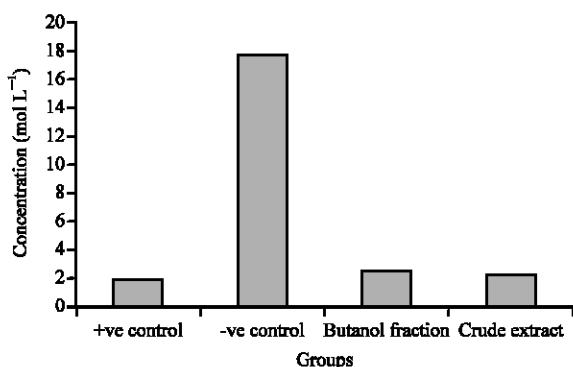


Fig. 3: Serum calcium concentration (mmol L<sup>-1</sup>) in *M. ciliata* treated rats

There was a significant decrease ( $p < 0.0001$ ) in PCV in all fractions. WBC showed a significant increase ( $p < 0.003$ ) and a decreased ( $p < 0.0001$ ) platelet count in all the fractions (Table 1). Calcium concentration in the active fraction and the crude extract treated rats was significantly lower than the untreated (negative control) (Fig. 3). There was a correlation between calcium concentration and parasitaemia in the active fraction treated animals ( $r = 0.400$ ,  $p = 0.488$ ) and the infected/untreated ( $r = -0.60$ ,  $p = 0.29$ ).

## DISCUSSION

The type of *M. ciliata* root extract prepared and evaluated in this experiment was informed by the method of preparation used by traditional healers, which is the ethanolic cold extraction. The low antioxidative property shown by the crude extract of *M. ciliata* suggests that it might have acted as a prooxidant *in vivo*. This is supported by studies that plant extract effect their trypanocidal activity by creating oxidative stress (Moura *et al.*, 2001; Atawodi *et al.*, 2003; Hoet *et al.*, 2004).

*Mitragyna* species have been reported to be rich in alkaloids especially those with conjugated double bonds like the indoles and oxindoles. Many of these alkaloids such as mitraphylline, mitragynine, ciliaphylline, rotundifoline, etc., have been isolated (Dongmo *et al.*, 2003; Matsumoto *et al.*, 2005).

The hematological indices of the fractions treated rats revealed that they did not have a major effect on the main features of trypanosomiasis. The observed anaemia (Naessens *et al.*, 2005) in all fractions is consistent with the disease and might also be due to oxidative stress created by the fractions (Ogbunugafor *et al.*, 2007). The

increased WBC count (leucocytopenia) and decreased platelets count (thrombocytopenia) are also major characteristics of trypanosomiasis (Kagira *et al.*, 2006) that were not affected by the fractions.

The 8-fold increase in Ca<sup>2+</sup> concentration in the infected rats compared to uninfected, crude extract and active fraction treated rats underscores the important role Ca<sup>2+</sup> which is compartmentalized in organelles called acidocalcisomes (Buchanan *et al.*, 2005), plays in *T. brucei* infection (Nikolskaia *et al.*, 2006). Indirect lines of evidence implicate Ca<sup>2+</sup> as an important component in the control of *T. brucei* infection (Decampo and Morenso, 2001). The effect of the butanol fraction on serum Ca<sup>2+</sup> concentration in the infected animals might partly explain its trypanocidal activity. This is further substantiated by previous experimental evidences that Ca<sup>2+</sup> potentiate the lethal effect of certain trypanocides such as melansoprol, SHAM and HDL (Gustavo and Vincenza, 2000). Furthermore, Ca<sup>2+</sup> binding proteins have been purified from *T. brucei*, where they serve as intracellular Ca<sup>2+</sup> buffers and mediate cellular response to Ca<sup>2+</sup> signals (Maldonado *et al.*, 1999; Gustavo and Vincenza, 2000; Buchanan *et al.*, 2005).

According to Hool and Corry (2007), oxidative stress could affect calcium channels in mammals. Our previous studies revealed that oxidative stress was created in the active fraction due to a decline in catalase activity (Ogbunugafor *et al.*, 2007). This is further buttressed by our studies that showed a weak correlation ( $r = 0.400$ ,  $p = 0.488$ ) between calcium concentration and parasitaemia in the active fraction treated animals. There was also moderate negative correlation ( $r = -0.60$ ,  $p = 0.29$ ) between calcium concentration and parasitaemia in the infected/untreated.

The conclusion from these findings is that *M. ciliata* active fraction reduced calcium concentration in *T. brucei* infected animals which might be one of the reasons for its trypanocidal activity.

## REFERENCES

- Aji, B.M., P.A. Onyelili and U.A. Osunkwo, 2001. The central nervous effects of *Mitragyna africanus* (Willd.) stem bark extract in rats. *J. Ethnopharmacol.*, 77 (2-3): 143-149.
- Atawodi, S.E., T. Bulus, S. Ibrahim, D.A. Ameh, A.J. Nok, M. Mamman and M. Galadima, 2003. *In vitro* trypanocidal effect of methanolic extract of some Nigerian savannah plants. *Afr. J. Biotechnol.*, 2 (9): 317-321.

- Atawodi, S.E., 2005. Comparative *in vitro* trypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian savannah plants. *Afr. J. Biotechnol.*, 4 (2): 177-182.
- Atidehou, K.K., C. Schmid, R. Brun, M.W. Kone and D. Traore, 2004. Antitrypanosomal and antiplasmodial activity of medicinal plants from Cote d'Ivoire. *J. Ethnopharmacol.*, 90 (2-3): 221-227.
- Buchanan, K.T., J.B. Ames, S.H. Asfaw, A. Wingard, C.L. Oslon, P.T. Capana, A.U. Araujo and D. Egman, 2005. A flagellum-specific calcium sensor. *J. Biol. Chem.*, 280 (48): 40104-40111.
- Burchmore, R.J.S., P.O.J. Ogbunude, B. Enanga and M.P. Barrett, 2002. Chemotherapy of human African trypanosomiasis. *Curr. Pharm. Des.*, 8 (4): 257-267.
- Decampo, R. and S.N.J. Morenso, 2001. The acidocalcisomes. *Mol. Biochem. Parasitol.*, 114 (2): 151-159.
- De Koning, H.P., 2001. Transporter in African trypanosomes: Role in drug action and resistance. *Int. J. Parasitol.*, 31 (5): 512-522.
- Dongmo, A.B., A. Kamanyi, G. Dzikouk, N.B. Chungag-Anye, P.V. Tan, T. Nguelefack T. Nole, M. Bopellet and H. Wagner, 2003. Anti-inflammatory and analgesic properties of stem bark extract of *Mitragyna ciliata* (Rubiaceae) *Aubrev. and Pellegr.* *J. Ethnopharmacol.*, 84 (1): 17-21.
- Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian plants. *Afr. J. Biotechnol.*, 4 (7): 6885-6888.
- Gustavo, B. and C. Vincenza, 2000. Intracellular calcium homeostasis and signaling in trypanosomatids. *Elect. J. Path History*, 6 (1): 423-426.
- Gyamfi, M.A., M. Yonamine and Y. Aniya, 1999. Free radical scavenging action of medicinal herbs from Ghana: *Thomingia sanguinea* on experimentally induced liver injuries. *Gen. Pharmacol.*, 32 (6): 661-667.
- Hoet, S., Opperdoes, B. Brun and J. Quetin-Leclereq, 2004. Natural products active against African trypanosomes: A step towards new drugs. *Nat. Prod. Rep.*, 21 (3): 353-364.
- Hool, L.C. and B. Corry, 2007. Redox control of calcium channels: From mechanism to therapeutic opportunities. *Antioxidants and Redox Signaling*, 9 (4): 409-435.
- Kagira, J., J.K. Thuita, M. Ngotho, R. Ndachi, D.M. Mwangangi and J.M. Ndung'u, 2006. Hematology of experimental *Trypanosoma rhodesiense* infection in vervet monkeys. *Afr. J. Health Sci.*, 13 (3-4): 59-65.
- Ledwaba, M.F. and K.D. Roberson, 2003. Effectiveness of Twenty-five hydroxycholecalciferol in prevention of Tibial dyschondroplasia in Ross cockrels depends on dietary calcium level. *Poult. Sci.*, 82 (11): 1769-1777.
- Maldonado, R.A., S. Mirzoera, L.M. Godsel, T.J. Lukas, S. Goldenberg, D.M. Watterson and D. Egman, 1999. Identification of calcium binding sites in the trypanosome flagellar calcium-acyl switch proteins. [ABST] [www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed).
- Matsumoto, K., L.T. Yamamoto, K. Watanabe, S. Yano, J. Shan, P.K.T. Pang, H. Takayama and S. Horie, 2005. Inhibitory effect of mitragynine, an analgesic alkaloid from Thai herbal medicine, on neurogenic contraction of the vas deferens. *Life Sci.*, 78 (2): 187-194.
- Mishina, Y.V., S. Krishna, R.K. Haynes and J.C. Meade, 2007. Artemisinin inhibits *Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense in vitro* growth. *Antimicrob. Agents Chemother.*, 51 (15): 1852-1854.
- Mottram, J.C. and G.H. Coombs, 1999. Recent advances in identifying and validating drug targets in trypanosomes and leishmaniasis. *Trends Microbiol.*, 7 (2): 82-87.
- Moura, K.C.G., F.S. Emery, C. Pinto-Neves, F.R.M. Pinto, A.P. Dantas, K. Saloma, S.L. Castro and A.V. Pinto, 2001. Trypanocidal activity of isolated naphthaquinones from *Tabebuia* and some heterocyclic derivatives: A review from an interdisciplinary study. *J. Braz. Chem. Soc.*, 12 (3): 325-338.
- Naessens, J., H. Kitani, Y. Nakamura, Y. Yagi, Sekikawa and F. Iraq, 2005. TNF- $\alpha$  mediated the development of anaemia in a murine *Trypanosoma brucei rhodesiense* infection, but not the anemia associated with a murine trypanosome congenital infection. *Clin. Exp. Immunol.*, 139 (3): 405-410.
- Nikolskaia, O.U., A.P.C. Lima, Y.V. Kim, J.D. Lonsdale-Eccles, T. Fukuma, B. Scarfstein and D.J. Grab, 2006. Blood-brain barrier traversal by African trypanosomes requires calcium signaling induced by parasite cysteine protease. *J. Clin. Invest.*, 116 (10): 2739-2747.
- Ogbunugafor, H.A., V.I. Okochi, J. Okpuzor, S. Ogbonna and P. Emeka, 2005. Antitrypanosomal activity of *Mitragyna ciliata* and *Ritchea longipedicellata*. Society for Medicinal Plant Research (GA). 53rd Annual Meeting and International Congress, Florence, pp: 171.
- Ogbunugafor, H.A., V.I. Okochi, J. Okpuzor, S. Esue and T. Adedayo, 2007. *Mitragyna ciliata* and its trypanocidal activity. *Afr. J. Biotechnol.*, 6 (20): 2303-2309.

- Scovil, J., E. Blank, M. Konnick, E. Nenortas and T. Shapiro, 2001. Antitrypanosomal activities of tryptanthrins. *Antimicrob. Agents Chemother.*, 46 (3): 882.
- TDR, 2005. Making health research for poor people. Seventeenth Programme Progress Report, 2003-2005.
- WHO, 2000. Report on global surveillance of epidemic-prone infectious disease. [www.who.int/csr/resources/publications/surveillance/s\\_tryps](http://www.who.int/csr/resources/publications/surveillance/s_tryps).
- Wu, J., Y. Tung, S. Wang, L. Shyur, Y. Kwo and S. Chang, 2005. Phenolic antioxidant from the heartwood of *Acacia confusa*. *J. Agric. Food Chem.*, 53 (15): 5917-5921.
- Wurochekke, A.U. and A.J. Nok, 2004. *In vitro* antitrypanosomal activity of some medicinal plants used in the treatment of trypanosomiasis in Northern Nigeria. *Afr. J. Biotechnol.*, 3 (9): 481-483.