



**Anthelmintic activity of *Ocimum gratissimum* leaf extract in *Heligmosomoides bakeri* infected experimental mice**

<sup>1,3</sup>Udoha, M.U., <sup>1</sup>Okolie, N.J., <sup>2</sup>Ijioma, S.N

<sup>1</sup>Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Imo State, Nigeria.

<sup>2</sup>Department of Veterinary Physiology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

<sup>3</sup>Department of Medical Laboratory Services, Federal Medical Center, Umuahia, Abia state, Nigeria  
Correspondence email: [mudoha2014@gmail.com](mailto:mudoha2014@gmail.com)

**ABSTRACT**

The antihelmintic potential of leaf extract of *Ocimum gratissimum* in *H. bakeri* infected mice was studied using carefully controlled whole animal experimental model. Results obtained revealed an LD<sub>50</sub> value of 2075mg/kg body weight suggest some margin of safety as the extract could well be tolerated at low to moderate doses. High doses could be toxic. The antihelmintic study showed that *Ocimum gratissimum* leaf extract has strong antihelmintic activity as varying doses of the extract significantly lowered *H. bakeri* eggs and larvae counts in the infected treated mice ( $p < 0.05$ ) as 100, 200 and 400mg/kg of the extract lowered the eggs counts by 29.77%, 46.50% and 67.34% respectively at the end of 21 days of treatment, while 800mg/kg of the extract achieved 100% elimination of the eggs and larvae by the 12<sup>th</sup> day of treatment. The antihelmintic effect of *Ocimum gratissimum* leaf extract compared favourably with that of Albendazole (30mg/kg), a reference antihelmintic agent used. Results obtained from this study therefore suggest that *Ocimum gratissimum* leaf extract may contain principles with strong antihelmintic property and could be used in the treatment of worm infestation, particularly the *H. bakeri* type and may further provide template for the development of new synthetic antihelmintic agent.

**Key words:** Albendazole, Antihelmintic, *Heligmosomoides bakeri*, Mice, *Ocimum gratissimum*

**INTRODUCTION**

Herbal preparations are currently being used in many parts of the world for the treatment of diseases and the trend of handing down this knowledge from generation to generation means that the population of users can become the more tremendous tomorrow. In Africa, it is believed that effective healthcare cannot be achieved without complementing existing orthodox medicine with the traditional one [1] and may be the reason why at least 89% of Africans depend on plant medicine for their healthcare needs [1]. On the other hand, the availability of medicinal plants has been a source of inspiration and advancement for modern medicine as many of the currently available drugs have been derived directly or indirectly from them [2]. This seems to be the reason for the keen interest of modern scientists in herbal medicine, since in most cases, the search would lead to the discovery and development of new agents with high potency and less side effects for the treatment of diseases. Several medicinal plants are reported to be currently undergoing such scientific investigations with the purpose of finding scientific basis of their therapeutic actions [3]. *Ocimum gratissimum* is among the plants currently being studied.

*Ocimum gratissimum* also known as African Basil is a plant belonging to the order *Lamiales*, and family *Lamiaceae*, Genus: In Nigeria it is commonly called, Ncho-anwu or Ahuji (Igbo), Efinrin (Yoruba), Aramogbo (Edo) and Daidoya (Hausa) [4]. *Ocimum gratissimum* is an aromatic, perennial herb, 1-3 m tall; stem erect, round-quadrangular, much branched, glabrous or pubescent, woody at the base, often with epidermis peeling in strips [5]. The plant is indigenous to tropical areas, including India and West Africa. In Nigeria, It is found in the Savannah and coastal areas [6]. Its characteristic pleasant aroma is attributed to its volatile oil content [7]. The plant is well known in Nigeria folk medicine and has been used to manage various disease conditions, including rheumatism, paralysis, epilepsy, diarrhea, influenza and gonorrhoea [8, 9]. Leaves of the plant is used as a spice and condiment in the southern part of Nigeria to facilitate removal of blood clots from the female reproductive system after delivery [10]. Antidiarrheal effects [11], gastro-protective properties [12], anti-bacterial activity [13], anti-fungal properties [14], anti-mutagenic activity [15], have all been reported to be among the health benefits of *Ocimum gratissimum*. Treatment of skin diseases, pneumonia, tooth and gum disorder, fever, and as mosquito repellants have also been reported [16].



Fig. 1: *Ocimum gratissimum* plants source: ([www.onlyfoods.net/ocimum-gratissimum.html](http://www.onlyfoods.net/ocimum-gratissimum.html))

Gastrointestinal nematodes including all forms of worm infestations is fast becoming a global problem as a larger population of persons in the developing countries being at great risk due to several factors including overcrowding, poor hygiene and sanitation, inadequate toilet facilities and poverty. Systemically, worm infestation leads to impaired animal productivity through reduction in voluntary food intake and/or inefficient use of nutrients. Disturbances in protein metabolism and reduced absorption and/or retention of minerals are significant during parasite infection [17]. Worm infestations indeed contribute to the prevalence of undernourishment, anaemia, eosinophilia, pneumonia, bloody diarrhea, weight loss, vomiting, neurological problems, constipation and skin symptom [18]. The development of resistance to existing treatment agents [19] and high cost of the synthetic drugs have necessitated the search for alternative means of effectively curbing the menace of worm infestation [20, 21, 22]. Plants and/or their products have today become the source of a major breakthrough.

The aim of this current study is to investigate the traditionally acclaimed antihelminthic property of *Ocimum gratissimum* leaves in *H. bakeri* infected mice.

## MATERIALS AND METHODS

### 2.1 Collection of plant material

Fresh leaves of *Ocimum gratissimum* were collected from a local settlement in Umuahia south Local Government Area of Abia state, Nigeria. The leaves were authenticated as *Ocimum gratissimum* leaves by a Botanist in the Department of Plant Science and Biotechnology, College of Natural Sciences, Micheal Okpara University of Agriculture, Umudike Abia State. A voucher number MOUAU/CVM/026 was given and a sample was deposited at the Departmental herbarium.

### 2.2 Preparation leaf extract

The fresh leaves of *Ocimum gratissimum* were dried under shade for ten days, after which they were milled to fine powder using manual blender. Fifty(50) grams of the powdered sample was introduced into the extraction chamber of soxhlet extractor and extraction was done using ethanol as a solvent. Temperature was maintained at 70°C throughout the extraction period of 48hours. At the end of the period, the extract was dried in a laboratory oven at

40°C to obtain dried dark green oily extract which weighed 11.25g and represented a yield of 22.50%, which was preserved at 4°C till use.

### **2.3 Animals**

A total of 95 adult albino mice (30 to 40g) were used for this study. The animals were obtained from the animal unit of the college of Veterinary Sciences, Michael Okpara University of Agriculture Umudike and were housed in aluminum cages and allowed to acclimatize for one week for proper adaptation to the new environment and living conditions. They were feed at liberty with standard feed(vital feed Nigeria) and clean water but starved for 12hours prior to the commencement of the experiment. All animal experiment were conducted in compliance with NIH guidelines for care and use of laboratory animal (Pub.No 58-23, Revised 1985) as reported by Akahet *al*(2009). This work was carried out at the Physiology Laboratory of the Department of Veterinary Physiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

### **2.4 Acute Toxicity Studies (LD<sub>50</sub> determination)**

For acute toxicity study, 35 mice of both sexes weighing 30-40g were divided into 7 groups of 5 mice each. The groups were assigned graded oral doses of *Ocimum gratissimum* in the order 500,750,1000,1500,2000,2500, and 3000mg/kg body weight respectively. After the administrations the mice were kept in aluminum cages and allowed free access to feed and water and were observed for toxicity signs and number of deaths within a period of 24hours. The LD<sub>50</sub> value for the extract was determined using the arithmetic method of Kerbaras reported by Enegide *et al.*, (2013) [23].

### **2.5 Helminth material**

A mouse adapted strain of *Heligmosomoides bakeri* larva (3<sup>rd</sup> stage larva) supplied by Dr. Ngongeh of the Department of Parasitology, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike was used. The droppings of mice artificially infected with *Heligmosomoides bakeri* were collected and soaked in water for 4 hours. Excess water was then decanted, the faeces crushed in a glass mortar, transferred to plastic containers in lots of about 5.0 g and shaken with glass beads. The material was then mixed with about 1 liter of water and strained through several layers of gauze. The filtrate was transferred to jars. The supernatant solution was then discarded and the sediment mixed with vermiculite in labeled plastic petri dishes and incubated at 4°C for 7 days. The infective 3<sup>rd</sup> stage larvae (L<sub>3</sub>) were recovered from 7 to 14 day old vermiculite faecal cultures using modified Baermann apparatus. The L<sub>3</sub> obtained were washed several times with distilled water and their number determined by dilution counting. The volume was then adjusted to give 200 L<sub>3</sub> in 0.2 ml.

#### **2.5.1 Parasite Inoculations**

The mice used for anthelmintic studies were dewormed using albendazole in their drinking water (7 mg/ml) for three days, one month prior to the experiment. The mice were infected orally with *Heligmosomoides bakeri* larvae, 100 L<sub>3</sub> per 0.1 ml per mice using an oral gavage

#### **2.5.2 Recovery of eggs and confirmation of infection**

After 7days of post infection, one gram of freshly passed out faeces of the experimentally infected mice were collected using a tea spoon and was homogenized in 60 ml of saturated Sodium Chloride solution. The mixture was filtered using a tea sieve and 150 microlitre sieve. The filtrate was transferred into a test tube and filled until the formation of an upper meniscus. A cover slip was used to cover each of the tubes and allowed to stand for 3minutes. This was to enable eggs of the parasite to move upward, float and attach to the cover slip. The latter was removed and placed on a slide for observation using microscope to confirm the presence of *H. bakeri* eggs.

#### **2.5.3 Chemotherapeutic trials**

Heavily infected mice *H. bakeri* were randomly assigned into six groups of ten mice each. Group I served as the negative control and was given no treatment except normal feed and water. Group 2 mice were treated with a standard drug, Albendazole (30 mg/kg). Groups 3, 4, 5 and 6 were given *Ocimum gratissimum* and served as the test groups. All treatments were given by the oral route using gavage and lasted for period of 21 days during which stool samples were collected every 3 days for analysis and egg counts. In-vitro parasite egg output was counted from 1-3 fecal pellets that were collected every 3 days from each group of mice (starting from the 8<sup>th</sup> day post *H. bakeri* inoculation). Helminth eggs were recovered and examined qualitatively by flotation using saturated Sodium Chloride solution and egg output per gram of faeces was calculated. For each mice sample, two grids of a McMaster slide were counted and the average was used as the eggs per gram of faeces for the parasite.

## 2.6 Statistical Analysis

Data were presented as means standard errors of means (SEM) and analysed using the one way analysis of variance. P values less than 0.05 for test versus control were considered as being statistically significant. Computer software SPSS version 17 was employed.

## RESULTS

### 3.1 Acute toxicity (LD<sub>50</sub>) study and effective dose (ED<sub>50</sub>) values

Toxicity signs and deaths were observed in some groups during the acute toxicity study period. No deaths were recorded in groups 1 and 2 treated with 500 and 750mg/kg. Groups treated with 1000, 1500, 2000, 2500 and 3000 mg/kg body in addition to obvious signs of toxicity recorded 1, 1, 2, 3 and 5 deaths respectively (Table 3). LD50 value by Karber's arithmetic method was thus obtained:

$$LD_{50} = LD_{100} - \frac{\sum(DD \times DM)}{N}$$

$$3000 - 4625/5$$

$$3000 - 925 = 2075 \text{ mg/kg}$$

### 3.2. Antihelminthic effects of *Ocimum gratissimum* leaf extract in *H. bakeri* infected mice

Albendazole at a dose of 30mg/kg significantly ( $p < 0.05$ ) reduced the number of the parasites in the infected treated control animals and completely eliminated them by the end of the 6<sup>th</sup> day of treatment. All doses of *Ocimum gratissimum* leaf extract also significantly ( $p < 0.05$ ) lowered the parasites in all treatment groups as 100, 200 and 400mg/kg of the extract lowered the number of parasites by 29.77%, 46.50% and 67.34% respectively at the end of 21 days of treatment. However, at a dose of 800mg/kg, *Ocimum gratissimum* leaf extract completely eliminated the parasites by the end of the 12<sup>th</sup> day of treatment, thus achieving 100% antihelminthic activity when compared to untreated group 1 animals in which the number of parasites increased progressively (Table 1, Figure 2). The antihelminthic effect of *Ocimum gratissimum* leaf extract at 800mg/kg compared favourably with that of albendazole, the standard treatment agent used (Table 1).

**Table 1: Antihelminthic activity of *Ocimum gratissimum*(OG) leaf extract**

Group	1	2	3	4	5	6
Agent	Water	Albendazole	OG	OG	OG	OG
Dose	0.2ml	30mg/kg	100mg/kg	200mg/kg	400mg/kg	800mg/kg
Day 0	171.20±5.98	137.20±3.60*	288.20±3.22*	337.20±9.69*	188.60±4.08	222.40±6.85*
Day 3	210.60±5.37	86.20±2.92*	286.20±9.19*	338.60±7.00*	163.40±2.62*	182.60±2.54*
Day 6	288.00±3.87	2.60±0.93*	263.20±15.08*	269.20±2.84*	132.40±3.66*	20.80±1.59*
Day 9	320.00±1.81	0.00±0.00*	262.00±3.32*	254.80±3.15*	129.80±2.65*	3.60±1.10*
Day 12	404.00±4.09	0.00±0.00*	247.40±2.33*	230.80±4.52*	105.20±3.02*	0.00±0.00*
Day 15	472.20±3.87	0.00±0.00*	228.40±2.68*	209.00±5.93*	100.00±3.90*	0.00±0.00*
Day 18	490.80±3.30	0.00±0.00*	209.00±3.94*	200.60±4.06*	76.60±4.61*	0.00±0.00*
Day 21	491.00±7.58	0.00±0.00*	202.40±3.50*	180.40±3.37*	61.60±3.04*	0.00±0.00*

\*=  $P < 0.05$  when compared to untreated control group

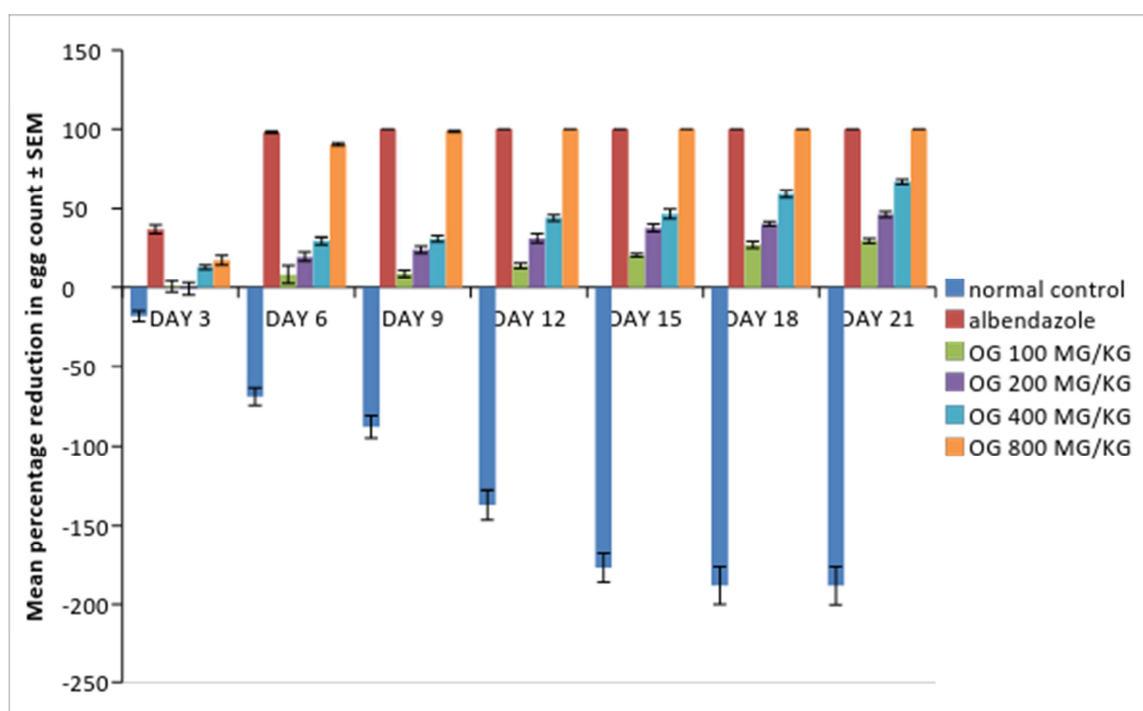


Figure 2: Percentage inhibition of *Ocimum gratissimum* leaf extract in *H. bakeri* egg counts in infected mice

## DISCUSSION

The high percentage yield obtained after the soxhlet extraction of *Ocimum gratissimum* leaves suggests that the plant is heavily enriched with bioactive substances which can be harnessed into useful forms to help surmount the numerous health challenges of man and other animals. The presence of these bioactive substances may be why *Ocimum gratissimum* leaves have been used over the years for the management of diseases [7, 24] and may be the cause of observed responses in this current work. Similar result was obtained after subjecting the leaves of *Ocimum gratissimum* to soxhlet extraction using ethanol as solvent and was reported [10].

The LD<sub>50</sub> result indicates that *Ocimum gratissimum* leaf extract have low toxicity and could be well tolerated at low to moderate doses which accounts for its use in most part of the world for the management of diseases. This result agree with Omorbuwajor *et al.*, (2011)[25], who reported that animals administered *Ocimum gratissimum* showed no overt sign of distress or toxicity even at a dose of 800mg/kg body weight. However the rats that died during the acute toxicity (LD<sub>50</sub>) tests may have received toxic doses of *Ocimum gratissimum* leaf extract indicating that *Ocimum gratissimum* leaf extract could be toxic at high doses with an LD<sub>50</sub> value of 2075mg/kg. The OECD guidelines on acute oral toxicity test [26], had reported that non observation of mortality within an animal population treated with a dose range at which mortality is expected indicates tolerance or lack of acute toxicity. This is because mortality is the expected end point of acute oral toxicity test [26]. *Ocimum gratissimum* is therefore well tolerated at low doses but could be toxic at higher doses. The toxicity level of *Ocimum gratissimum* may be due to the presence of saponins, cyanogenetic glycosides and tannins [12], which are reported to be part of its phytochemistry and with high possibility of causing toxicity at high doses [27].

Anthelmintic studies revealed that all mice treated with the extract had significantly dose dependent inhibition in the number of *H. bakeri* ova counted and totally eliminated the parasites by the 12<sup>th</sup> day of treatment with 800 mg/kg dose of *Ocimum gratissimum*. This suggests that *Ocimum gratissimum* may contain phytochemicals active against *H. bakeri*. The anthelmintic activity shown by *Ocimum gratissimum* may be due to the presence of phenolic compounds in the extract. Phenolic compounds have been reported to exact strong anthelmintic activity [28]. The presence of high amounts of phenolic compounds in leaf extracts of *Ocimum gratissimum* have also been reported [29, 30]. The mechanisms for the inhibition of egg hatching and larval development of *Ocimum gratissimum* may be



related to the inhibition of cell division in the organisms and consequently, inhibiting the formation and development of vital structures of the parasites. This has been reported to be a major mechanism for the antihelminthic effects of several extracts [28]. *Ocimum gratissimum* may also have acted like albendazole, a standard antihelminthic agent whose primary mode of action is to inhibit the polymerisation of the parasitic tubulin into microtubules. The high affinity of albendazole to the tubulin causes the loss of the cytoplasmic microtubules which leads to impaired uptake of glucose by the larval and adult stages of the parasites and making the parasite to be unable to maintain energy production. This chain of events leads to immobilization and eventual death of the parasite [31]. *Ocimum gratissimum* leaves extract may in addition to the above mechanism have acted by inhibition of the enzyme fumarate reductase - a secondary mechanism for albendazole action. The antihelminthic effect of *Ocimum gratissimum* compared favourably with that of the standard drug albendazole (Table 1) and suggests that *Ocimum gratissimum* leaf extract can be used as a substitute for albendazole in the treatment of intestinal worms infestation thereby avoiding the adverse effects associated to the use of albendazole, including headaches, nausea, vomiting, abdominal pain and in some cases fever, thrombocytopenia and hepatotoxicity [31]. This result tends to agree with Pessoa *et al.*, (2002)[32] who reported that *Ocimum gratissimum* showed significant anthelmintic activity when tested on laboratory animals and also validates the use *Ocimum gratissimum* leaf extract in traditional medicine for the treatment of worm infestation.

### CONCLUSION

In this study, the ethanol leaf extract of *Ocimum gratissimum* was found to exhibited strong antihelminthic activity with total elimination of the ova and larvae of *H. bakeri* from infected treated mice by the 12<sup>th</sup> day of treatment and suggest that *Ocimum gratissimum* leaf extract could be harnessed into a potent agent for the treatment of intestinal worms with less side effects when compared to existing orthodox ones. This is in addition to providing fresh templates for the development of new synthetic antihelminthic agents. The use of *Ocimum gratissimum* leaves extract in traditional medicine for the treatment of helminthiasis is hereby validated.

### REFERENCES

- [1]. Panda, T. *British Journal of Pharmacology and Toxicology* **2010**,1(2): 67-71
- [2]Krentz, A.J., Balley, C.J. *Drugs*,**2005**,65:385-411.
- [3]Gupta, Y.K. and Briyal, S. *Indian J Physiol Pharmacol.***2004**,48(4)379-94.
- [4]Effrain, I. O., Salami, H. A. and Osewa, T. S., *Afri. J. Biomed Res.*,**2000**, 175 – 179.
- [5]Orwa, C. A., Mutua, K. R., Jamnadass, R., S.A., **2009**. Agroforestry Database: a tree reference and selection guide version 4.0 (<http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp>)
- [6]Nogochi, N.E., *Free Rad. Biol. Med.*,**2000**, 28:1538-1546.
- [7]Prabhu, K.S., Lobo, A.A., Shirwaikar, A.A., Shirwaikar, A. *The Open Complementary Medicine Journal*, **2009**, 1:1-15.
- [8]Dalziel, J.M. *Plants Description, Useful Plants of West Tropical Africa*, **1993**, 3:431-433.
- [9]Dhawan, B.N., Patnik, G.R., Rastogy, R.A.T., Singh, K.K., Tandol, T.S. *YL India Exp.***1997**, p15:108.
- [10]Akomas, S C. and Ijioma, S.N. *Comprehensive Journal of Medical Sciences*,**2014**,20 2(1): 9-13.
- [11] Sofowora, L.A. *Medicinal plants and traditional medicine Africa*. Spectrum Books, Ltd, Ibadan. **1993**.55-71.
- [12] Offiah, V.N., Chukwuedu, U.A. (1999). *Ethnopharmacol.***1999**, 68(1-3): 27-30.
- [13] Akah, P.A., John-Africa, L., Nworu, C.S. *International Journal of Pharmacology*,**2007**,3:461-467.
- [14] Orafidiya, O.O., Elujoba, A.A., Iwalewa, F.O., Okeke, I.N. *Pharm. Pharmacol. Lett*, **2000**, 10:9-12.
- [15] Njoku, C.J., Asuzu, I.U. *Phytomedicine*,**1998**, 5(6):485-8.
- [16] Ilori, M., Sheteolu, A. O., Omonigbehin, E. A., Adeneye, A. A. *J. Diarrheal Dis. Res.*,**1996**, 14: 283 – 285.
- [17] Waller, P.J. *Vet. Parasitol*,**1987**, 25:171-191
- [18] Tagbota, S. and Townson, S. *Adv Parasitol*, **2001**, 50:199-205.
- [19] Bowman, D.D., Lynn, R.C., Eberhard, M.L. and Lcaraz, A. A. (1999). *Georgi's Parasitology for Veterinarians*. Saunders, Missouri, USA p.142-161.
- [20] Al-shaibani, I.R.M., Phulan, M.S. and Shiekh, M. *Int J Agric Bio.*,**2009**, 11:431-436.
- [21] Bachaya, H.A. (2009). Screening of some indigenous plants for anthelmintic activity with particular reference to their condensed tannin content, PhD thesis, University of Agriculture, Faisalabad-Pakistan.
- [22] Deeba, F., Muhammed, M., Iqbal, Z. and Hussan, I. *Int J Agric Bio*,**2009**, 11:535-541.
- [23] Enegide, C., David, A. and Fidelis, S.A. *Toxicol International*, **2003**, 20(3):224-226.

- 
- [24] Olusegun, R. J., Josiah O., Luqman, A. O., Ayokunle, O. and Sikiru A. B. *An international journal of Nigerian Society for Experimental Biology. BIOKEMISTRI*, **2008**, ,20(1):33-37.
- [25] Omobuwajor, O.R., Alade, G.O., Akanmu, M.A., Obuotor,E.M. and Osasan, S.A.*African Journal of pharmacy and pharmacology*, **2011**, 5(1):12-17.
- [26] OECD (**2001**). Guidelines for the Testing of Chemicals/Section 4: Health Effects Tests no, 423: Acute oral Toxicity – Acute Toxic Class method.Organization for Economic Cooperation and Development, Paris..
- [27] Nweze, E.I., Eze, E.E.*BMC Complementary and Alternative Medicine* ,**2009**, 9 (37) Article Number 1472
- [28] Feireira,L.E., Castro, P.M.M. Chagas, A.C.S., Franca, S.C., Belebani. R.O. *Experimental parasitology*.**2013**, 134(3): 327-332.
- [29] Awah, F.A. and Winkor V.A. *Journal of Medicinal Plant Research*, **2010**, 01(4): 2429-2437.
- [30] Mahapatra, S.K., Chakraborty, S.P., Das, S., and Roy, S. *Oxid Med Cell Longev*,**2009**, 2(4): 22-230.
- [31] Dayan, A.D. *Acta Trop.* **2003**, 86 (2-3): 141-59.
- [32] Pessoa, L.M., Morais, S.M., Bevilaqua, C.M.L. and Luciano, J.H., (**2002**).*Veterinary Parasitology*, **2002**, 109(1-2): 59-63.