EFFECT OF ETHYL ACETATIC EXTRACT OF *Cymbopogon citratus* LEAF ON *Trypanosoma brucei* INFECTION IN ALBINO RATS

ABSTRACT

Ethyl acetate extracts of *Cymbopogon citratus* leaves were evaluated for their phytochemical constituents and anti-trypanosomal activity in *Trypanosoma brucei* infected rats. The albino rats were treated for ten days with 200mg/Kg, 100mg/Kg and 50mg/Kg plant extracts per body weight. Treatment with ethylacetatic extract of *Cymbopogon citratus* at 200mg/kg, 100mg/kg and 50mg/kg extracts per body weight had an effect on the parasite. Significant difference ($P<0.05$) was observed in the parasitaemia levels of *Trypanosoma brucei* infected rats treated with 200mg/kg, 100mg/kg and 50mg/kg extracts per body weight compared with the infected untreated ones. The result of the haematological study showed that significant difference ($P<0.05$) was observed in the packed cell volume (PCV) of treated rats when compared with the infected untreated group. Also, the mean weight and survival rate of the infected treated rats showed a significant difference ($P<0.05$) when compared to the infected untreated rats. From this study, it was observed that ethyl acetatic extract of *Cymbopogoncitratus* leaf is effective against *Trypanosoma brucei*.

Keywords: Ethyl Acetatic, *Cymbopogon citrates*, *Trypanosoma brucei*

INTRODUCTION

African Trypanosomiasis, also called sleeping sickness in humans and *Nagana* in domestic animals, is a parasitic disease caused by protozoa which affect both human and livestock.

It is estimated that about55 million people are at risk of the infection in which only 3.5 million are under surveillance in endemic countries [1]. Trypanosomiasis consists of a group of important human and animal diseases caused by parasitic protozoa of the genus Trypanosoma [2].

Trypanosomes are classified under the kingdom protista, sub-kingdom protozoa, phylum sarcom astigophora, order kinetoplastida, family Trypanosomatidae, and genus Trypanosoma. This genus has two groups, s tercoraria and salivarian [3].-The main genera in this group are: *Duttonella* spp (*T. vivax, and T. uniforme*); *Nannomonas spp* (*T. congolense* and *T. simiae*); *Pycnomonas spp* (*T. suis*); and *Trypanozoon spp* (*T. brucei; T. brucei brucei*, *T. b. rhodosiense,* a nd *T. b. gambiense; T. evansi;* and *T. aquiperdum*)[3]. The disease, human African Trypanosomiasis (HAT) is exclusively African and is more prevalent in the rural areas[4]. Plants used in traditional medicine are considered to be potential sources for the development of alternative therapies [5]. It is, therefore, against this background that the plant was investigated for its trypanocidal efficacy in this research.

There are over fifty species of lemongrass but the scientific names for the ones more commonly used for cooking and healing are *Cymbopogon citratus* and *Cymbopogon flexuosus*. In India, it is more popularly referred to as choomana poolu [6]. In the Caribbean, it is known widely as fever grass, attesting to its traditional use to relieve the symtoms of fever [7]. The main chemical component found in lemongrass is citral, an aromatic compound, also known as lemonal[8]. It is an antimicrobial plant and therefore effective in cidal and static microorganisms.

Lemongrass has rubefacient property, meaning that it may be able to improve blood circulation [7].

The health benefits of Lemongrass Essential Oil can be attributed to its many beneficial properties as an analgesic, antidepressant, antimicrobial, antipyretic, antiseptic, astringent, bactericidal, carminative, deodorant, diuretic, febrifuge, fungicidal, galactogogue, insecticidal, nervine, sedative and tonic substance [8]. Lemongrass essential oil is extracted through the process of steam distillation of dried lemongrass. Lemongrass is known by the scientific names *Cymbopogon Citratus* or *Andropogon Citratus*. The main constituents of its essential oil are Myrcene, Citronellal, Geranyl Acetate, Nerol, Geraniol, Neral, Limonene and Citral [6,9]. As the name implies, lemongrass smells just like lemons, but it is milder, sweeter, and far less sour. This grass is used in countless beverages (including tea), desserts and other forms of culinary creations as a flavoring agent, where fresh lemon is not available or is not to be used because of its more potent flavor [10]. It is widely used in Chinese and Thai recipes. It grows and spreads veryfast like any other grass and fetches a good price in the market, which makes it a profitable and common item in organic and mainstream markets.

MATERIALS AND METHODS

PLANT MATERIAL

The plant was collected from University of Jos senior staff quarters, Jos Plateau State of Nigeria. The plant was identified in the herbarium department, federal College of Forestry Jos.

EXTRACTION

A freshly collected plant leaves were cut into small pieces, and dried for 24 hours in an

oven at 30^0C to dry. The dried particles were blended in an electronic blending machine into powder form. About 100g of the powdered drug (powdered plant) was weighed and transferred into 250ml conical flask capacity and soaked with 75 ml of Ethyl acetate. This was allowed to stand overnight (24 hours) and then warmed on the water bath at 40^0C and filtered. The filtration was repeated in three parts with continuous addition of fresh solvent. The collective filtrate was evaporated to dryness on a water bath at about 60^0C. The percentage yield was determined. The dry extract was transferred into clean sterile sample container and kept in desiccators till the phytochemical screening and trypanocidal screening.

INOCULATION OF RATS

Experimental rats were infected with *Trypanosoma brucrei*. Highly inoculated blood as observed under light microscope was obtained from the tail of an infected rat directly into phosphate saline glucose (PSG), p^H 7.5 without anticoagulant at $1x10^4$ trypanosomes per ml, 0.2ml of suspension was injected into the experimental albino rats intraperitoneally.

ADMINISTRATION OF THE EXTRACT

Trypanosoma brucei infected rats were treated with ethylacetatic extract of *cymbopogon citratus* leaf intraperitoneally at 200mg, 100mg, and 50mg/kg body weight. Infected rats were administered once daily with this extract from the first day parasites were sighted in the blood and continued until the infected animals died. Treatment continued daily with continuous monitoring of parasitaemia.

DETERMINATION OF PARASITE

Parasitaemia was monitored in blood obtained from the tail, pre-sterilized with methylated spirit.

The number of parasite was determined microscopically at x40 magnification using the "Rapid Matching" method. The method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS, pH 7.2) [11].

IN-VIVO TEST FOR TRYPANOCIDAL ACTIVITY

Rats inoculated with *Trypanosoma brucei* were intraperitoneally treated with 200mg, 100mg and 50mg/kg body weight of the extracts when the parasites started manifesting. The treatment continued daily with continuous monitoring of parasitaemia. The rats were grouped in group of three except the positive and negative controls which had five rats each.

Group 1 rats were uninfected and untreated.

Group 2 rats were infected and untreated.

Group 3 rats were infected but treated with 200mg of the extract.

Group 4 rats were infected but treated with 100mg of the extract.

Group 5 rats were infected but treated with 50mg of the extract.

Experimental Animals

The animals were monitored with care and all the experimental procedure with the animals was in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols were duly approved by the ethical committee of Animal House of University of Jos, Nigeria.

DETERMINATION OF PACKED CELL VOLUME (PCV) (Microhaematocrit method)

Principle: This is the percentage of the volume of blood occupied by packed red blood

cells, when a known volume of blood is centrifuged at a constant speed for a constant period of time.

PHYTOCHEMICAL EVALUATION

The ethylacetatic extract was screened for its phytochemical constituents.

a. Test for alkaloids

About 0.5g of the extract was stirred with 3ml of 1% aqueous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with few drops of Dragendorff's reagent. Precipitation with this reagent was taken as preliminary evidence for the presence of alkaloids in the extract [12,13].

b. Test for saponins

About 0.5g of the extract was shaken with water in a test tube. The absence of frothing which persist on warming was taken as preliminary evidence for the absence of saponins [13,14].

c. Test for tannins

About 0.5g of the extract was stirred with 1ml of distilled water, filtered, and ferric chloride reagent added to the filtrate. A blue-black, precipitate was taken as evidence for the presence of tannins [13].

d. Test for anthraquinones

Borntrager's test was used for the detection of anthraguinones. About 0.5g of the extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5 minutes. The ex tract was filtered, and the filtrate shaken with an equal volume of 100% ammonia solution. The a bsence of pink, violent or red colour in the ammonical layer (lower layer) indicated the absence o

f free anthraquinones [13].

e. Test for cardiac glycosides

About 100mg of the extract was taken in a test tube and 2.5ml of dilute sulphuric acid w as added and boiled in a water bath for 15 minutes. This was cooled and neutralized with 20% potassium hydroxide solution. 5ml of a mixture of Fehlings solution A and B was added and boiled for 3 minutes. A brick red precipitate indicated the hydrolysis of a reducing sugar, which is indication of cardiac glucoside[13].

f. Test for steroids

About 100mg of the extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface indicated the presence of steroidal ring [14].

g. Test for flavonoids

About 2g of the extract was completely detanned [15] with acetone. The residue was extr acted in warm water after evaporating the acetone on a water bath. The mixture was filtered whil e hot. The filtrate was cooled and used for the following test.

Lead acetate test for flavonoids

About 5ml of the filtrate was added to lead acetate solution. A yellow coloured precipitate indicated the presence of flavonoids.

Sodium hydroxide test for flavonoids

About 5ml of 20% sodium hydroxide was added to equal volume of the detanned water e xtract. A yellow solution indicated the presence of flavonoids.

h. Test for Carbohydrate

About 100mg of the extract was dissolved in 3ml of distilled water and mixed with a few drops of Molisch reagent (10% solution of α-naphthol in alcohol). Then 1ml of concentrated sulphuric acid was carefully added down the side of the inclined tube so that the acid form a layer beneath the aqueous solution without mixing it. A violet ring at the junction of the liquids was observed indicating the presence of carbohydrate.

Also, about 5mg of the extract was heated with 1ml of concentrated sulphuric acid. Blackening and effervescence occurred indicating the presence of carbohydrate.

RESULTS

PHYTOCHEMICAL SCREENING

Table 1: Phytochemical constituents of *Cymbopogon citratus* leaf

Phytocemical constituents	Inference
Alkaloids	+++
Saponins	_
Tanins	++
Flavonoids	+
Steroids	++
Carbohydrates	++
Cardiac glycosides	+++
Anthraquinones	_

Key

- = absent

+ = slightly present

++ = moderately present

+++ = highly present

Table 1 presents the results recorded for the phytochemical analysis (screening) conducted on ethylacetatic extract of *Cymbopogon citratus* leaf. The plant extract exhibited high concentrations of alkaloids and cardiac glycosides. The concerentrations of tannins, steroids and carbohydrates were moderate. The concerntration of flavonoids was low, while saponins and anthraquinones were absent in the extract.

PARASITAEMIA COUNT

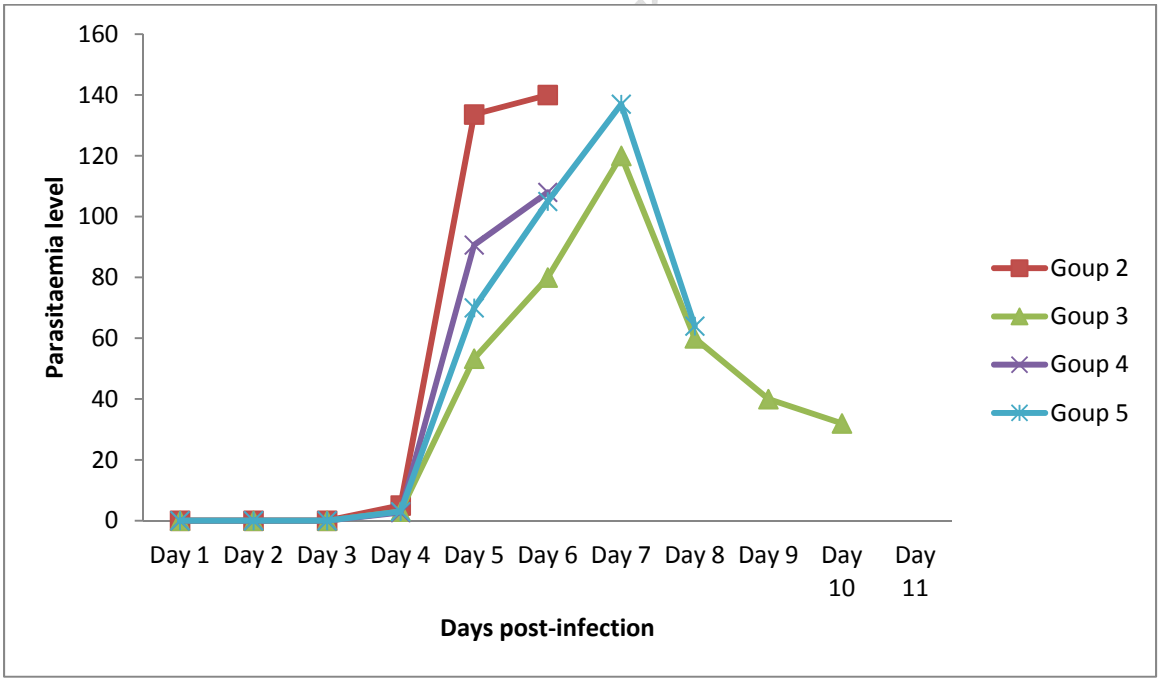

Figure 1: Parasitaemia levels of *T. brucei* infected rats treated with 200mg/kg, 100mg/kg and 50mg/kg ethylacetatic extract of *Cymbopogon citratus* leaf.

From figure 1, the amount of parasitaemia for group two was zero from day 1 to 3, it grows from day 4 and all died on day 6 . For group three, amount of parasitaemia was zero from day 1 to 3, it grows from day 4, attaining its peak on day 7 and then begins to depreciate afterwards upto day 10. Group 4 group, all died on day 6. Group 5 similar to group 3 only that the animals in this group all died after day 8 unlike group 3 where the animals died after day 10.

MEAN WEIGHT

Table 2:Mean weight of

Trypanosoma brucei infected rats treated with 200mg/kg,100mg/kg and 50mg/kg ethylacetatic extract of *Cymbopogon citratus* leaf.

Gp	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
Gp 1	109±26.92	110.4±24.44	111±24.32	112.9±24	116.8±23.36	112.7±23.78	111.8±24.36	109.1±23.72	114.8±27.82	116.1±27.54	122±28.20
Gp 2	115.7±11.56	116.5±11.99	116.8±11.91	112.4±14.50	125.7±18.50	110.2±0	-	-	-	-	-
Gp 3	140±3.82	140.7±3.40	143.1±3.82	129.8±4.11	113.6±9.51	107.9±0	105.3±0	109.9±0	110.5±0	117.7±0	-
Gp 4	130±5	132.4±3.7	133±3.72	125.7±3.59	107.3±3.01	104.2±2.47	-	-	-	-	-
Group 5	120±3.61	121.7±3.26	122.5±2.81	109.1±8.52	95.7±7.72	94.9±11.74	93±13.65	97±14.78	97.5±15.13	-	-

As presented in table 2, it can be observed that the changes in daily mean weight of the uninfected/untreated rats (groups 1) showed steady increase in weight from day 1 to 5, decreased from day 6 to 8, and finally increased from day 9 to 11. The mean weight of the infected/untreated rats (group 2) showed steady increase from day 1 to 3, decreased in day 4, increased on day 5, and finally decreased on day 6 before joining their ancestors. Group 3, 4 and 5 also recorded initial increase in weight from day 1 to 3, decreased from day 4 to 7, and finally increased from day 8 upward. While group 4 ended their life time on day 6, group 3 and 5 on day 10 and 9 respectively.

PARCKED CELL VOLUME (PCV)

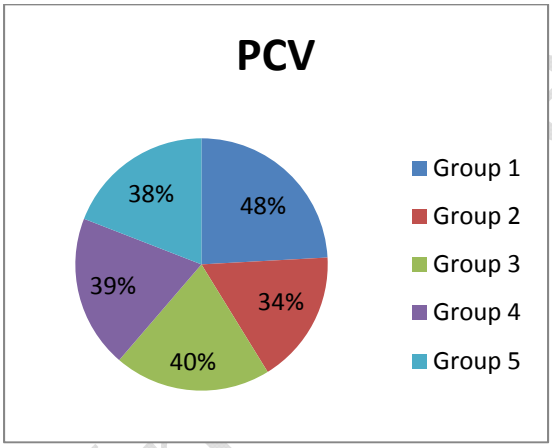

Figure 2: Packed cell volume (PCV) levels of *Trypanosoma brucei* infected rats treated with 200mg/kg, 100mg/kg and 50mg/kg ethylacetatic extract of *Cymbopogon citratus* leaf.

Figure 3 shows that group 1 has the highest PCV followed by groups 3, 4, 5 and 2.

DISCUSSION

This research work presents an experimental studies on African trypanosomiasis in rats treated for ten days with ethylacetatic extract of *Cymbopogon citratus* leaf post infection with *Trypanosoma brucei*. In this research, the parasite became detectable in the tail blood of experimental rats on the fourth day after infection. The findings is in line with that of other investigators [16] who reported similar results on rats inoculated with *Trypanosoma brucei*. Once inside the body, the parasite is completely exposed to the host's immune system, but in many instances the y survive and proliferate, resulting in characteristic waves of parasitaemia every three to five day s. The immune system kills subpopulations of the parasites but a population of the parasites that escape the immune system proliferate and another relapse of parasite is observe in the blood [17].

From the parasitaemia count (Fig. 2), it can be seen that the plant extract may have activated the immune system of the rats prior to infection with the parasites. The result suggest that administration of ethylacetatic extract of *Cymbopogon citratus* leaf at 50mg/kg and 200mg/kg body weight of rats considerably reduced the parasitaemia. This reduction in parasitaemia may be attributable to the anti-proliferative activity of iron chelation. The iron chelating activity of *Cymbopogon citratus* have been suggested to contribute to its antimicrobial activity [18], and it has been shown in a previous experiment that the trypanocidal action of *Cymbopogon citratus* is related to this property. A pilot study carried out on rats infected with *T. b. b.* using similar concerntration resulted in clearance of the parasites from the blood. Furthermore, the

drastic reduction of parasitaemia in group 3 (Fig. 2), and their longer period of survival may suggest that the higher the concerntration of the plant extract administered, the higher the rate of immune response against the trypanosome parasite.

Haematologically, the result obtained in this studies showed that there was a severe drop in the packed cell volume (PCV) of group 2 (Fig. 3). This drop is an indiction of anaemia which is a consistent haematological feature in trypanosomiasis. The exact cause of anaemia is as yet unknown but certain mechanisms have been posited. These include dyshaemopoiesis, haemodilution, and haemolysis. Trypanosome infection may cause anaemia as a result of massive erythrophagocytosis by an expanded and active mononuclear phagocytic system (MPS) of the host [19]. It has been established that the measurement of anaemia gives a reliable indication of the disease status and productive performance of trypanonsome infected animals [20]. The PCV result obtained in this study are consistent with earlier studies by Ekanem *et al.*[21]. The low PCV observed in the infected/untreated group may be as a result of acute haemolysis due to growing infection. Previous studies have shown that infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably as a result of depletion of reduced glutathione on the red blood cell [22]. The degree of oxidative damage may have been reduced in the infected/treated rats by the antioxidant property of *Cymbopogon citratus* which prevented the depletion of reduced glutathione on the red blood cell in contrast to infected/untreated rats with low PCV.

As seen in table 2, the experimental rats (group 2, 3, 4 and 5) all experienced weight loss after day 3 before recovering their weight after some times. A notable lack of appetite and decrease in food in-take always preceded the decrease

in body weight. Similar findings have been reported in rats infected with *Trypanosoma brucei*. From the daily body weight recorded for rats in group 3 and 5, the recovery of the weight may be attributed to the fact that group 3 and 5 were treated with extract after infection when compared to group 2

CONCLUSION

The results obtained from this studies evince that Ethyl acetate extract of *Cymbopogon citratus* leaf at 50mg/kg and 200mg/kg body weight of rats considerably reduced the level of parasitosis in *Trypanosoma brucei*-infected rats. Thus, it can be concluded that Ethyl acetate extract of *Cymbopogon citratus* leaf is appreciably effective in the therapeutic management of *Trypanosoma brucei* infection.

Competing interests

The authors declare that they have no competing interests

Ethical Approval:

As per international standard or university standard ethical approval has been collected and preserved by the authors.

References

WHO Media centre. Fact sheet N°259: Trypanosomiasis, Human African (sleeping sickness). *World Health Organization*. 2014.

Barrett, M. P., Burchmore, R. J. and Stich, A. The trypanosomes. *Lancet.* 2003; 362 (9394):1469-80.

Hoare CA, Wallace FG. Developmental stages of trypanosomatid flagellates: a new terminology. Nature. 1966;212(5068):1385–6.

Atouguia, J. and Costa, J. Therapy of human African trypanosomiasis: current situation. *Memoorias do Institute Oswaldo Cruiz Rio de Janeiro.* 1999; **94:** 221-224.

Atawodi, S.E. and Alafiatayo, A.A. Assessment of the phytochemical and antitrypanosomal properties of some extracts of leaves, stem and root bark of *Landolphia* sp., P. Beauv. *J. Ethnopharmacol.* 2007; **114:** 207-211.

Leite, J. R., Seabra, M. L. and Maluf, E. Pharmacology of lemongrass (*Cymbopogon citratus* Stapf). III. Assessment of eventual toxic, hypnotic and anxiolytic effects on humans. *J Ethnopharmacol.* 1986; **17** (1): 75–83.

Takeguma, A. Gowing Citronella 2013.

Edmon, A. Lemon grass as mosquito repellent - WorldNgayon® 2013.

Ferguson, M. A. and Homans, S. W. (1988). Parasite glycoconjugates: towards the exploitation of their structure. *Parasite Immunol.* 1988; 10 (5): 465-7

Toshiya, M., Yuka, O., Natsuko, O., Katsuo, N. and Hideki, K. *Journal of Agricultural and Food Chemistry.* 2008; *56* (2), 597-601.

Laura, P., Tovar, G., César, B. B. and Rubens, M. *Industrial & Engineering Chemistry Research.* 2011; *50* (13), 8185-8194.

Atawodi, S.E., Ameh, D.A., Ibrahim, S., Andrew, J.N., Nzelibe, H.C., Onyike, E., Anigo, K.M., Abu, E.A., James, D.B., Njoku, G.C. and Sallau, A.B. Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. *J.Ethnopharmacol.* 2002; **79:** 279-282.

Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. London, UK: Chapman & Hall Ltd.; 1973.

Trease GE, Evans WC. *Pharmacognosy*. 11th edition. Brailliar Tiridal Can Macmillian Publishers; 1989

Wall, M. E., Eddy, C. R., McClenna, M. L. and Klump, M. E. *Anal Chem.* **1952**. 24: 1337.

Wall, M. E.,Krider, M. M., Krewson, C. F., Wilaman, J. J., Cordell, D. S. and Gentry, H. S.'Steroidal sapgenins X111. Supplementary table of data for steroidal sapogenins V11'.1954; 363 pp.

Sofowora A. *Medicinal Plants and Traditional Medicine in West Africa*. New York, NY, USA: John Wiley and Sons; 1982.

Segelman, A.B., N.R. Farnsworth and M.D. Quimby. False negative saponins test results induced by the presence of tannins. Lloydia, 1969; 32: 52-58.

Lundkvist, G. B., Kristensson, K. and Bentivoglio, M. (2004). Why Trypanoso mes Cause Sleeping Sickness. *Physiology* .2004; 19: 198–206.

Pays, E., Coquelet, H., Pays, A., Tebabi, P. and Steinert, M. *Trypanosoma brucei*: posttranscriptional control of the variable surface gl ycoprotein gene expression site. *Mol. Cell. Biol.* 1989; 9 (9): 4018–21.

Grenier D, Huot MP and Mayrand D. Iron-chelating activity of tetracyclines and its impact on the susceptibility of Actinobacillus actinomycetemcomitans to these antibiotics. Antimicrob Agents Chemother. 2000; 44(3):763-6.

Igbokwe, Ikechukwu and Nwosu, Chukwunyere. Lack of correlation of anaemia with splenomegaly and hepatomegaly in Trypanosoma brucei and Trypanosoma congolense infections of rats Journal of comparative pathology. 1997;(97)80020-5.

Ekanem JT, Majolagbe OR, Sulaiman FA, Muhammad NO. Effects of honey supplemented diet on the parasitaemia and some enzymes of *Trypanosoma brucei*- infected rats. Afr. J. Biotechnol. 2006; 5: 1557-61.

Ekanem JT, Yusuf OK. Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *T.brucei*-infected rats. Afr. J. Biomed. Res. 2008; 11: 79–85.

Akanji MA, Adeyemi OS, Oguntoye SO, Sulyman F. *Psidium guajava extract* reduces trypanosomosis associated lipid peroxidation and raises glutathione concentrations in infected animals. EXCLI J.2009; 8: 148-154.

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