

Insight to Antitrypanosomal and Anti-Anaemic Potency of Bitter Leaf (*Vernonia amygdalina*) Ethanol Extract on *Trypanosoma congolense*-Infected Wistar Rats

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Date Received: 29-11-2022

Date Accepted: 24-02-2023

DOI: <https://doi.org/10.48198/NJPAS/22.B15>

ABSTRACT

Introduction: Trypanosomiasis often caused by trypanosome is common among wildlife and domestic animals. **Aim and Objectives:** This study assessed the antitrypanosomal and anti-anemic activities of bitter leaf ethanol extract (BLEE) against *Trypanosoma congolense*. **Methodology:** Forty (40) healthy wistar rats of both sexes weighing 120-150 g were used at the beginning of the experiment. They were randomly divided into six groups (A, B, C, D, E and F). In A, B, D, E and F (n = 5) but group C was started with fifteen (15) rats till death reduced the population to five (5). Group A rats were uninfected and untreated; group B rats were uninfected but treated (500 mg/kgbw BLEE); group C rats were infected but not treated; groups D, E and F rats were infected and treated (100, 300 and 500 mg/kgbw BLEE, respectively). Infection of the rats was carried out by injecting 0.1 ml of blood containing approximately 1×10^3 trypanosomes intraperitoneally into each rat in the infected groups. The effects of BLEE on the infected rats were determined. Changes in haemoglobin level and packed cell volume (PCV) were determined prior, during and post infection. **Results:** The results obtained revealed that the percentage values for haemoglobin level and PCV of infected rats in this study were remarkably reduced compared with the uninfected rats. A marked association between the prevalence of *Trypanosoma congolense*, haemoglobin level and PCV was observed in this study. The extract immobilized the trypanosome in the treated groups as compared to the infected but untreated group. The BLEE was able to cause a remarkable reduction of parasitaemia level, improved haemoglobin level and PCV as compared to the infected-untreated rats. **Conclusion:** Our findings thus has given an indication of *in vitro* and *in vivo* antitrypanosomal and anti-anemic activities of *Vernonia amygdalina* against *Trypanosoma congolense*.

Keywords: Anaemia, Hemoglobin level, Packed cell volume, Trypanocidal agent, *Vernonia amygdalina*

Introduction

According to Tom-Otu *et al.* (2022); Ari-egoro *et al.* (2019), it is estimated that 66-85% of the world's population depends directly on plants as medicine

while the search for drugs from plants is on the increase presently (Mahmoodally *et al.*, 2010). The leaves of *Vernonia amygdalina* are good appetizers and are used to aid digestion. The leaves have been reported to be beneficial for human healthcare and

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treatment of several microbial and parasitic infections or diseases such as amoebic dysentery, typhoid fever, rubeola amongst others (Oyeyemi *et al.*, 2018).

Page | 4532 Parasitic infection such as trypanosomiasis caused by trypanosome is common among wildlife and domestic animals. Earlier studies have indicated the association of trypanosomiasis with anaemia, and the interplay of synergetic factors account for the development of haemolytic anaemia in human and animal trypanosomiasis (Fajinmi *et al.*, 2021; Kumela and Behabtom, 2021).

T. congolense is a causative agent of trypanosomiasis (sleeping sickness) in animals. It is the major pathogen responsible for nagana disease in cattle and other livestock animals including, sheep, goats, dogs, horses, camels and pigs (Deschamps *et al.*, 2016). Research has revealed that *T. congolense* is the major cause of nagana in West and East Africa (Kumela and Behabtom, 2021). The parasite is spread by tsetse fly. According to Deschamps *et al.* (2016), *T. congolense* lives in blood vessels and particularly causes anemia. Other trypanosomes include *T. evansi*, *T. vivax*, *T. brucei brucei* which together with *T. congolense* are responsible for African trypanosomiasis (AAT) called nagana. In West Africa, *Trypanosoma brucei brucei* causes sleeping sickness or the human African Trypanosomiasis (HAT) while *T. evansi* and *T. equiperdum* cause sura and douvine respectively (Deschamps *et al.*, 2016).

Trypanosomiasis is transmitted by a bite of the vector, 'tsetse fly' of *Glossina* species. Trypanosomiasis, especially in Nigeria seems to be re-emerging as an important livestock disease, thus causing significant clinical importance in small ruminants with possible extension to previously designated tsetse fly zones, causing annual loss of about 5 billion US dollars in African livestock production and mixed agriculture (D'Archivio *et al.*, 2011).

According to Tom-Otu *et al.* (2022), *Vernonia amygdalina* is reported to have a catholic use in treating amoebic dysentery gastrointestinal discomforts and have antiparasitic properties. The findings of Udochukwu *et al.* (2015) revealed that the leaf extracts of *Vernonia amygdalina* possess inhibitory potentials against parasites and some bacteria such as *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Proteus mirabilis*. Moreover, not much information has been given on the antitrypanosomal activity of bitter leaf ethanol extract against *T. congolense*. These limitations necessitate the development of novel trypanocidal agents from natural and affordable sources so as to mitigate the deleterious effects of *T. congolense*. Hence, the reason for this study was to investigate the antitrypanosomal and anti-anaemic activities of ethanol extract of bitter leaf in *T. congolense*-infected rats.

Materials and Methods

Study area

This research work was carried out in the Advanced Microbiology and Biochemistry Laboratories for Postgraduates, Salem University, Lokoja, Kogi State, Nigeria. Lokoja is the capital of Kogi State which was created in 1991. Kogi State is located in the North Central part of Nigeria. The State lies between latitudes 7° 49'N and 8° 10'N and Longitudes 6° 01'E and 7° 50'E, covering an area of about 27,747 km². It shares common boundaries with Niger, Kwara, Nasarawa and The Federal Capital Territory to the North. In the East, the state is bounded by Benue and Enugu States; in the South by Enugu and Anambra States; and in the West by Ondo, Ekiti and Edo States. Lokoja, the Niger/Benue confluence town is the state capital. The 1991 census in Nigeria puts the population of the state at 2,147,756 (Egbunu *et al.*, 2022).

Collection of plant samples

Fresh leaves of *Vernonia amygdalina* were harvested from a local garden in Ajaokuta Local

Government Area of Kogi State, Nigeria. The botanical identification and authentication of the plants (SU-H-0056) were done by a Botanist in the Biological Department of Salem University, Lokoja. The leaves were carefully detached and air-dried until crispy to touch and then pulverized into a fine powder using a sterile electrical blender (Euro premium: 1500-1799 W).

Preparation of plant extracts

The bitter leaf powder was weighed using a Camry automatic scale, model No: Ek3250 (A & D Company Limited, Japan) into a clean dry flat-bottom flask. Twenty-five grams (25 g) of the pulverized sample was cold macerated in 250 ml ethanol (1: 10 w/v) for 48 hrs with constant rocking using an electronic shaker, model No: 001 (AOAC, 2010). The ethanol extract was filtered using Whatman No. 1 filter paper, a pore size of 100 (195 mm by 195 mm). The filtrate was concentrated using a rotary evaporator and placed on a water bath at 60°C to allow evaporation of the solvents as described by AOAC (2010). The obtained jelly-like extract was stored in a sterile Petri dish (well rapped with paper tape) in the refrigerator at 4°C until required for use.

Sterility of plant extracts

The sterility of the ethanol extract was conducted by inoculating a small quantity of the extract on a sterile nutrient agar and incubated at 37°C for 24 hrs. Absence of microbial growth on the extracts after the incubation confirmed the sterility of the extract.

Experimental animals

Forty (40) healthy wistar rats of both sexes, weighing 120-150 g were purchased from Nigerian Institute of Trypanosomiasis Research (NITR), Jos, Plateau State, Nigeria, and were used at the beginning of the experiment. The animals were kept in the animal house of Salem University, Lokoja, Nigeria, and maintained under 12 hrs (light/dark) cycle. The experimental animals were allowed free access to pellet diet and clean water *ad libitum*.

Infection of the experimental rats was carried out by injecting 0.1 ml of blood containing approximately 1×10^3 trypanosomes intraperitoneally into each rat in the infected groups. Ndungu *et al.* (2019). All procedures used complied with the guide for the care and use of laboratory animals in accordance with good laboratory practice (GLP) regulations of the World Health Organization (WHO). The principles of laboratory animal care were also duly followed.

Table 1: Experimental design

Group	Treatment	Number of Animals	Period
A	UNINFECTED-UNTREATED (Negative control)	5	14 days
B	UNINFECTED-TREATED (500 mg/kgbw of BLEE)	5	14 days
C	INFECTED-UNTREATED (Positive control)	15	14 days
D	INFECTED-TREATED (100 mg/kgbw of BLEE)	5	14 days
E	INFECTED-TREATED (300 mg/kgbw of BLEE)	5	14 days
F	INFECTED-TREATED (500 mg/kgbw of BLEE)	5	14 days

Note: The number of rats in the positive control (group C) was 15 because of the expected high mortality rate. This population reduced to 5 at the end of the experiment.

Determination of parasitaemia level

Parasitaemia level was determined according to the method described by Mergia *et al.* (2014), with a slight modification. The parasitaemia level was monitored daily by microscopic examination of blood obtained from the tail of each rat. The tip of each rat's tail was gently agitated and then pierced with a sterile needle to produce a droplet of the infected blood. The droplet was placed on a clean, dry and grease-free glass slide, and covered with a copper slip. Microscopic examination of this preparation was done under $\times 400$ objective magnification. Wet preparation of the infected blood was done in triplicates from each *T. congolense* infected rat. The mean value of slide counts was taken per sample examined. The log values of the counts obtained were achieved by matching with Herbert and Lumsden's table, (Herbert and Lumsden, 1976).

Determination of haemoglobin level and packed cell volume

Haemoglobin (Hb) and packed cell volume (PCV) were determined by measuring the Hb and PCV of each experimental animal, before infection, during infection and during treatment periods, using the method of “Rule of Three” by Tallquist method as described by Olalekan and Emmanuel (2016).

Determination of bodyweight change

Bodyweight of each experimental rat in all the groups was taken using an electronic weighing scale (SDW series) before infection, during infection and every day of the treatment.

Data analysis

Data were analyzed using graph prism (V: 20) and one-way analysis of variance together with post hoc Duncan’s multiple range tests. A statistically significant association between variables was said to exist if the p value < 0.05 at 95% confidence level.

Results

In vitro antitrypanosomal activity of bitter leaf ethanol extracts (BLEE) on *T. congolense*

Figure 1 shows the *in vitro* antitrypanosomal activity of BLEE on *T. congolense* in a dose-dependent fashion; 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml, with extract of 1 mg/ml exerting the greatest antitrypanosomal effect.

In vivo antitrypanosomal activity of bitter leaf ethanol extract on *T. congolense*

Figure 2 shows the *in vivo* antitrypanosomal activity of BLEE on *T. congolense* level in the blood of the *T. congolense* infected rats for 7 days. Groups A and B were infected-free rats, with zero parasitaemia level throughout the experimental period. A periodical increase in parasitaemia level was observed in infected-untreated group (group C) by microscopic examination of the rats’ blood samples. Treatment with BLEE (100 mg/kgbw, 300 mg/kgbw and 500) caused a reduction in the

parasitaemia level in dose and time-dependent patterns.

Effect of bitter leaf ethanol extract (BLEE) on haemoglobin level and packed cell volume of experimental rats

Figures 3 and 4 show the *in vivo* effects of BLEE on haemoglobin level and packed cell volume (PCV) of the experimental rats, respectively. No remarkable change in the haemoglobin and PCV levels of group A; low haemoglobin and PCV levels were observed in the infected animals. In comparison with the infected-untreated group, infected groups treated with BLEE showed gradual improvements in haemoglobin and PCV levels irrespective of the concentration of the extract, from day 3-7 of treatment period but a marked improvement was observed in the groups that received 300 and 500 mg/kgbw of BLEE.

Figure 5 showed the *in vivo* effect of BLEE on bodyweight of the experimental rats. A reduction in bodyweight was observed in the infected animals. In comparison with the infected-untreated group, infected groups treated with BLEE showed gradual improvements in bodyweight irrespective of the concentration of the extract, from day 3-7 of treatment period but a marked improvement was observed in the groups that received 300 and 500 mg/kgbw of BLEE.

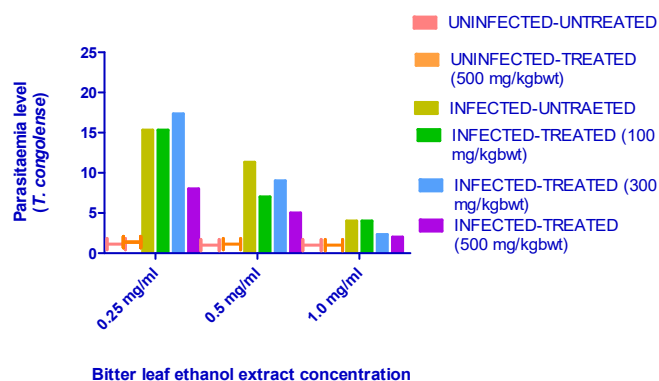


Figure 1: *In vitro* antitrypanosomal effects of bitter leaf ethanol extract on *Trypanosoma congolense*. All determinations were conducted in triplicates; data were expressed as mean±SEM

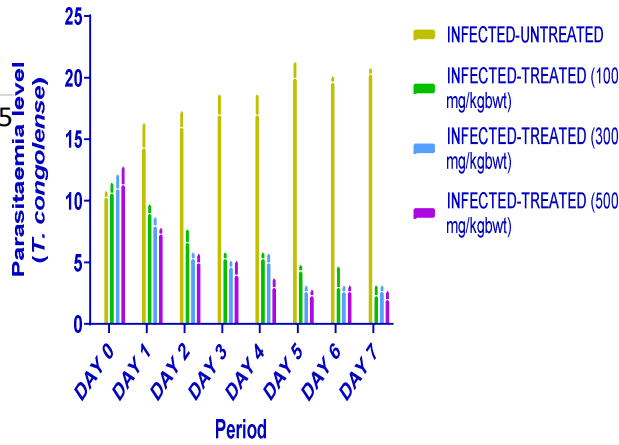


Figure 2: *In vivo* effect of bitter leaf ethanol extract on *T. congolense* level in the blood of the *T. congolense* infected rats. All determinations were conducted in triplicates; data were expressed as mean±SEM. DAY 0: during infection, which lasted for 4 days before treatment commenced; DAYS 1-7: during treatment.

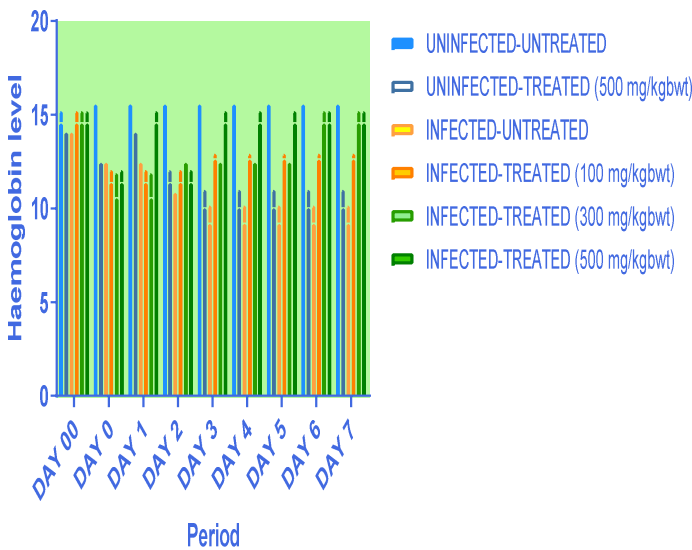


Figure 3: Effect of bitter leaf ethanol extract on haemoglobin level of the experimental rats. All determinations were conducted in triplicates; data were expressed as mean±SEM. Day 00: Before infection; day 0: The very day of infection; Day 1-7:

Treatment days.

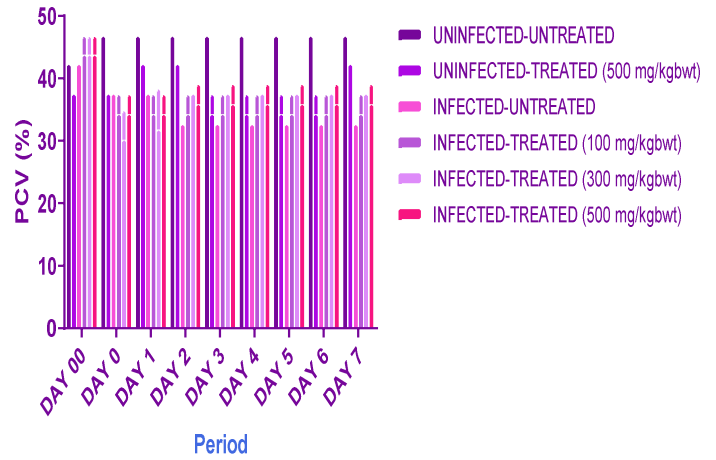


Figure 4: Effect of bitter leaf ethanol extract on packed cell volume (PCV) of the experimental rats. All determinations were conducted in triplicates; data were expressed as mean±SEM. Day 00: Before infection; day 0: The very day of infection; Day 1-7: Treatment days.

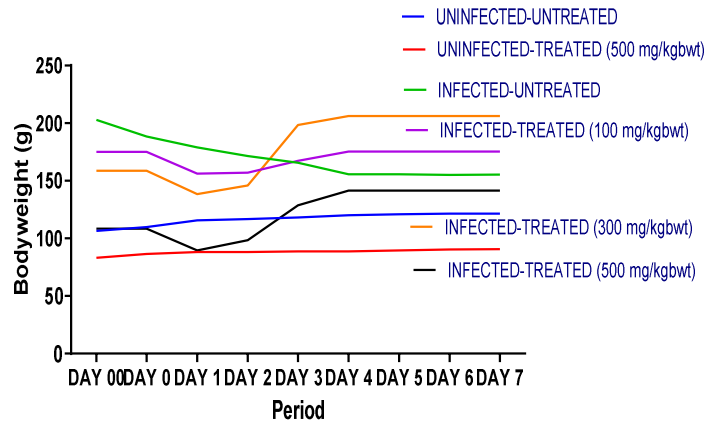


Figure 5: Effect of bitter leaf ethanol extract on bodyweight change of the experimental rats. All determinations were conducted in triplicates; data were expressed as mean±SEM. Day 00: Before infection; day 0: The very day of infection; Day 1-7: Treatment days.

Discussion

Studies and several literatures have shown that plants are used in traditional medicine (Evbomwan *et al.*, 2018; Mbaya and Ibrahim, 2011). In spite of the diverse antiparasitic and antitrypanosomal drug usage against trypanosomes for both livestock and humans, there seems to be no sharp noticeable reduction in the prevalence of some of the trypanosome species. Therefore, the need for natural, cheaper, more effective and readily available treatment options cannot be over emphasized.

According to Fajinmi *et al.* (2021); hematological parameters such as haemoglobin and PCV are indicative of true health status as well as indicators for anaemic conditions. There was an observed anaemia indicated by a marked reduction in haemoglobin and PCV levels in the infected-untreated rats in this study, which validates the reports of Fajinmi *et al.* (2021); Deschamps *et al.* (2016); Faremi and Ekanem (2011) that *T. congolense* causes anaemia in animals. The loss of bodyweight observed in the infected rats (Group C), groups D, E and F validates the reports of Madaki *et al.* (2016) who observed reduction in the weight of *T. congolense* infected mice and attributed their observation to progression of infection followed by appetite loss, resulting in bodyweight reduction of the animals (Figure 5). Low haemoglobin, PCV, fatigue, weakness and lethargy were observed in the infected groups. This agrees with the report of Fajinmi *et al.* (2021) that wistar rats experimentally infected with *T. congolense* developed acute form of trypanosomiasis associated with observed decrease in bodyweight, anaemia, low haemoglobin and low PCV.

In addition, anaemia is a major clinical feature of African trypanosomiasis in both humans and animals. The measurement of anaemia in this study gives a reliable indication of the status and productive performance of the trypanosome infected animals earlier reported by Ekanem *et al.*

(2005; 2006). The observed low haemoglobin and PCV in infected-untreated group could be due to haemolysis as a result of rising parasitaemia infection, oxidative damage of red blood cells and proliferation of the parasite (Fajinmi *et al.*, 2021; Akanji *et al.* 2009; Ekanem and Yusuf, 2008).

The gradual recovery of the haemoglobin and PCV of the infected and BLEE-treated rats is indicative of the efficacy of bitter leaves in clearing trypanosomes from the peripheral blood as earlier documented by Egbe-Nwiya *et al.* (2020).

Conclusion

This study has given an indication of *in vitro* and *in vivo* antitrypanosomal and anti-anemic activities of bitter leaf ethanol extract against *Trypanosoma congolense*. The extract exhibited antitrypanosomal activity in a dose-dependent pattern. This study has shown that bitter leaf (*Vernonia amygdalina*) could have trypanocidal potential and as such it should be considered as a formidable source for the search for new drugs against African animals' trypanosomiasis.

Conflict of interest disclosure

The authors declare no conflicts of interest

References

- Akanji, M.A., Adeyemi, A.O., Oguntoye, S.O. and Sulyman, F. (2009). *Psidium guajava* extract reduces trypanosomiasis associated lipid peroxidation and raises glutathione concentrations in infected animals. *EXCLI Journal*, 8: 900-903.
- Amana, G.U., Omowaye, O.S. and Ibrahim, M.A. (2020). *In vitro* sensitivity of anti-trypanosomal activities of leaves and roots of extracts of *Moringa oleifera* against *Trypanosoma brucei brucei* of *Rattus norvegicus* (Wistar Rats). *Salem Journal of Life Sciences*, 1(1):52-62.

- Ari-egoro, Y.E., Ayodele, P.F. and Oyeleke, O.M. (2019). Effects of graded levels of *Moringa oleifera* leaf-meal in albino rat diet on some hematological parameters. *Journal of Analytical Technique and Research*, 1(2): 37-46.
- Association of Official Analytical Chemists. (2010). Official methods of analysis of AOAC international, 18th edition, Washinton DC.
- D'Archivio, S., Medina, M., Cossan, A., Chamond, N., Rotureau, B. and Miriopro, P. (2011). Genetic engineering of *Trypanosoma vivax* and *in vitro* differentiation under axenic conditions, *PLOS Neglected Tropical Disease*, 5: 1461-1465.
- Deschamps, J.Y., Desquesnes, M., Darso, L., Ravel, S., Bossard, G., Charbonneau, M., Garaud, A. and Roux, F.A. (2016). Refractory hypoglaemia in a dog infected with *Trypanosoma congolense*. *Parasite*, 23: 1-7.
- Egbe-Nwuyi, T.N., Abalaka, S.E., Sani, N.A., Tenuche, O.Z. and Idoko, I.S. (2020). Individual and combined antitrypanosomal effects of artether and diminazeneqceturate in the treatment of experimental *Trypanosomabruceibrucei* infection in rats. *Veterinary World*, 22: 31-36.
- Egbunu, A.A., Makolo, D. and Alkali, D.E. (2022). A spatial analysis of health care accessibility and utilization among rural households in Kogi State, Nigeria. *International Journal of Life Science Research Archive*, 3(1):101-109.
- Ekanem, J.T., Majolagbe, O.R., Sulaiman, F.A. and Muhammad, N.O. (2006). Effects of honey supplemented diet on the parasitaemia and some enzymes of *Trypanosoma brucei*-infected rats. *African Journal of Biotechnology*, 6:280-289.
- Ekanem, J.T., Sulyman, F.A. and Adeyemi, O.S. (2005). Therapeutic properties and serum iron in *T. brucei* infected rats treated with amodiaquine and mofloquine. *Biokemistri*, 17(2): 115-121.
- Ekanem, J.T. and Yusuf, K. (2008). Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *T. brucei*-infected rats. *African Journal of Biomedical Research*, 7(2): 153-157.
- Evbuomwan, L., Chukuka, E. P., Obazenu, E. I., and Ilevbare, L. (2018). Antibacterial activity of *Vernonia amygdalina* leaf extracts against multidrugs resistant bacteria isolates. *Applied Sciences and Environmental Management*, 22(1): 17-21.
- Fajinmi, A.O., Faleke, O.O., Magaji, A.A., Chafe, U.M., Kassim, M.A., Musa, U.B, Ormaga, M.T., Tese, T. and Hussaini, M. (2021). Haematological changes in wistar rats experimentally infected with *Trypanosoma congolense* and *Trypanosoma brucei brucei* obtained from North-west Nigeria. *Sokoto Journal of Veterinary Sciences*, 19(2): 73-80.
- Herbert, W.J. and Lumsden, W.H.R. (1976). *Trypanosoma brucei*: A matching method for estimating the host's parasitaemia. *Experimental Parasitology*, 40:427 – 431.
- Kumela, L. and Behabloom, M. (2021). Anaemia associated with trypanosomes infections in cattle of West Gojjam, zone, Northwest Ethiopia. *Veterinary Medicine International*, 21: 1-7.
- Madaki, F. M., Kabiru, A. Y., Mann, A., Abdulkadir, A., Agadi, J. N. and Akinyode, A. O. (2016). Phytochemical analysis and *in vitro* antitrypanosomal activity of selected medicinal plants in Niger State, Nigeria. *International Journal of Biochemistry Research and review*, 11(3): 1-7.

Mahomoodally, M.F., Gurib-Fakin, A. and Subratty, A.H. (2005). Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius. *Pharmacological Biology*, 43: 237-242.

Mahomoodally, M.F., Gurib-Fakin, A. and Subratty, A.H. (2010). Screening for alternative antibiotics: An investigation into the antimicrobial activities of medicinal food plants of Mauritius. *Journal of Food Science*, 75(3): 173-177.

Mbaya, A.W. and Ibrahim, U.I. (2011). *In vivo* and *in vitro* activities of medicinal plant on haemic and humoral trypanosome: a review. *International Journal of Pharmacology*, 7(1):1-11.

Mergia, E., Shibeshi, W., Terefe, G. and Teklehaymanot, T. (2014). Phytochemical screening and *in vitro* antitrypanosomal activity of aqueous and methanol leaf extract of *Verbascum sinaiticum* against *Trypanosoma congolense* field isolate. *Journal of Clinical Experiment and Pathology*, 4: 183-187.

Ndungu, K., Thungu, D., Wamuriri, F., Mireji, P., Ngae, G., Gritonga, P., Mulinge, J., Auma, J. and Thuita, J. (2019). Route of inoculation influences *Trypanosoma congolense* and *Trypanosoma brucei brucei* virulence in Swiss white mice. *PLoS One*, 14(6):e0218441

Olalekan, A.W. and Emmanuel, A.O. (2016). How valid is the tallquist method in screening

pregnant women with anemia in poor rural setting of South Western Nigeria?

Oyeyemi, I.T., Akinlabi, A.A., Adewumi, A., Alshinloye, A.O. and Oyeyemi, O.T. (2018). *Vernonia amygdalina*: A folkloric herb with anthelmintic properties. *Beni-Suef University Journal of Basic and Applied Sciences*, 7(1): 43-49.

Tom-Otu, M.O., Omowaye, O.S., Makolo, D., Ayodele, P.F., Ogunyemi, O.M. and Fabusiwa, M. (2022). *In silico* and *in vitro* antimicrobial testing of aqueous extract of *Vernonia amygdalina* against *Escherichia coli* isolated from wistar rats infected with *Trypanosoma congolense*. *Open Journal of Bioscience Research (OJBR)*, 3(2): 1-16.

Udochukwu, U., Omeje, F.I., Uloma, I.S.F. and Osiewe, D. (2015). Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activities on some drug resistant bacteria. *American Journal of Research Communications*, 3(5): 225-235.