

Short Communication

**In Vitro Anti-Trypanosomal Activity of Aqueous Extracts of *Viscum album* (Mistletoe) Leaf and Stem from Three Host Plants**

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ABSTRACT

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In sub-Saharan Africa, trypanosomosis is a constraint on livestock production. Thus, it is imperative to develop alternative strategies since current chemotherapy is bedevilled by high cost and limited access. This study investigated the *in vitro* anti-trypanosomal activity of aqueous extracts of *Viscum album* leaf and stem from three host plants namely *Acacia albida*, *Azadirachta indica* and *Psidium guajava*. Tail blood was collected from one of the donor rats and diluted with phosphate-buffered saline. An aliquot (2 $\mu$ L) containing the parasites (*Trypanosoma brucei*) was pipetted into the test tubes containing graded concentrations (40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, and 0.65 mg/ml) of the aqueous extracts and incubated at 37 °C for 15 minutes. The parasite count declined substantially with 100% clearance for *A. albida* leaf and stem, *P. guajava* stem at 10 mg/ml, *P. guajava* leaf at 40 mg/ml and lastly, *A. indica* leaf and stem. The results from the aqueous extract of *V. album* leaf and stem from three different host plants were comparable with Veriben®, a chemotherapeutic trypanocide.

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## INTRODUCTION

Trypanosomosis is still a major health threat to humans and livestock in tropical and subtropical areas of Africa (Umar *et al.*, 2010; Isaac *et al.*, 2017). This protozoan parasite is a eukaryotic organism belonging to the genus *Trypanosoma* and transmitted by the tsetse-fly of the genus *Glossina* during feeding. The haemolysis of red blood cells (Yusuf *et al.*, 2013) and the release of some biochemicals such as proteases and sialidases (Nok *et al.*, 1993; Nok and Balogun, 2003) have related to the *in vivo* presence of the parasite and have been established to play several roles in the pathogenesis of the disease, thereby making the management of the disease an enormous task.

The existing chemotherapeutic trypanocides are challenged with problems of drug resistance, toxicity, inaccessibility, and affordability (Bukachi *et al.*, 2017; Biu *et al.*, 2022). Thus, it is imperative to develop strategies to combat trypanosomosis. The use of herbal preparations still holds strong potential, as they are natural products derived from plants that have been reported to offer novel remedies (Shuaibu *et al.*, 2008; Ibrahim *et al.*, 2008; Mbaya *et al.*, 2010; Biu *et al.*, 2022).

*Viscum album*, commonly referred to as mistletoe, is a semi-parasitic plant which grows on the stems of other trees (Osadebe and Uzochukwu, 2006). In Nigeria, mistletoe has been reported as a novel remedy against inflammatory and degenerative diseases and has several biological properties such as antibacterial, anticancer, antiviral, apoptosis-inducing and immune-modulatory activities (Olapade, 1995; Nigerian Guardian Newspapers, 2007; Wassagwa *et al.*, 2018). In this study, we evaluated the *in vitro* anti-trypanosomal activities of aqueous extracts of mistletoe leaf and stem from three different

host plants namely *Azadirachta indica*, *Acacia albida* and *Psidium guajava*.

## MATERIALS AND METHODS

### *Collection, Preparation and Aqueous Extraction of Viscum album*

The leaf and stem of mistletoe (*Viscum album*) from three different host plants viz: *Azadirachta indica* (Neem), *Psidium guajava* (guava) and *Acacia albida* were freshly collected within the vicinity of the University of Maiduguri Campus. Collected leaves and stems were authenticated at the Department of Biological Sciences, University of Maiduguri, Nigeria by a botanist. The harvested leaf and stem of *Viscum album* were rinsed using clean water and dried for a period of one week under shade, and subsequently ground using mortar and pestle to obtain a 700-gram fine powder of each sample. Extraction was carried out using 1000 ml of distilled water at 60°C for 8 hours using a Soxhlet extractor (Quickfit, EnglandR) as previously described (Mittal *et al.*, 1981). The *Viscum album* extracts were concentrated using an oven overnight at 60°C. The drying process removed the water, leaving only the extract of *Viscum album* from *Azadirachta indica*, *Psidium guajava* and *Acacia albida* which weighed 200, 490 and 350 grams with percentage yields of 40%, 70% and 50% respectively. Extracts were stored at room temperature (27°C) until required.

### *Source of Trypanosomes*

*Trypanosoma brucei brucei* Federe strain/NTR/14 was isolated from the blood of a naturally infected Ndama breed of cattle in Nasarawa State, Nigeria by the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Vom Plateau State Nigeria. Trypanosomes were stabilized in Swiss Wistar albino rats by serial passage in the parasitology laboratory, Department of Veterinary Parasitology and

Entomology, University of Maiduguri, Nigeria and maintained through the continuous passage until when required. All procedure was carried out following the guiding principles for biomedical research involving animals (C.I.O.M.S., 1985)

#### ***In vitro Trypanocidal Assay***

A serial dilution of the various extracts as graded concentrations of 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.625 mg/ml in phosphate-buffered saline solution was prepared into test tubes replicated 8 times for each concentration. An aliquot of 2µL of the parasite (*Trypanosoma brucei brucei*), which was prepared by taking the tail blood from a donor rat at peak *parasitaemia* and diluting with phosphate-buffered saline was pipetted into each test tube and incubated at 37 °C in a water bath for a period of 1 hour. Glucose D(R) (Glaxo Nigeria Ltd) was added to each test tube which provided a source of energy for trypanosome survival outside its natural

host. For reference tests, 2 control groups comprising a negative control with test tubes inoculated with 2 drops of infected blood in 1 mL of phosphate-buffered saline solution and added glucose D(R) and a positive control group with 2 drops of infected blood in phosphate-buffered saline added Glucose D(R) and treated with 1ml of veriben(R) (diminazene aceturate 3.5 mg/kg, Eagle Chemical Company Ltd Ikeja, Nigeria), was prepared by dissolving in 100 ml of distilled water. All tests were allowed to stand for a period of 15 minutes after which the improved Neubauer's chamber was used to quantify the number of trypanosomes per field under the light microscope at an interval of 5 minutes for the duration of 2 hours. Trypanosome mortality was determined by observing the cessation of motility of the parasite as described by Atawodi *et al.* (2003). It is to be noted that under *in vitro* testing trypanosomes in the negative control could survive for up to 4 hours

Percentage (%) mortality /inhibition/cessation of motility was calculated using the formula below: -

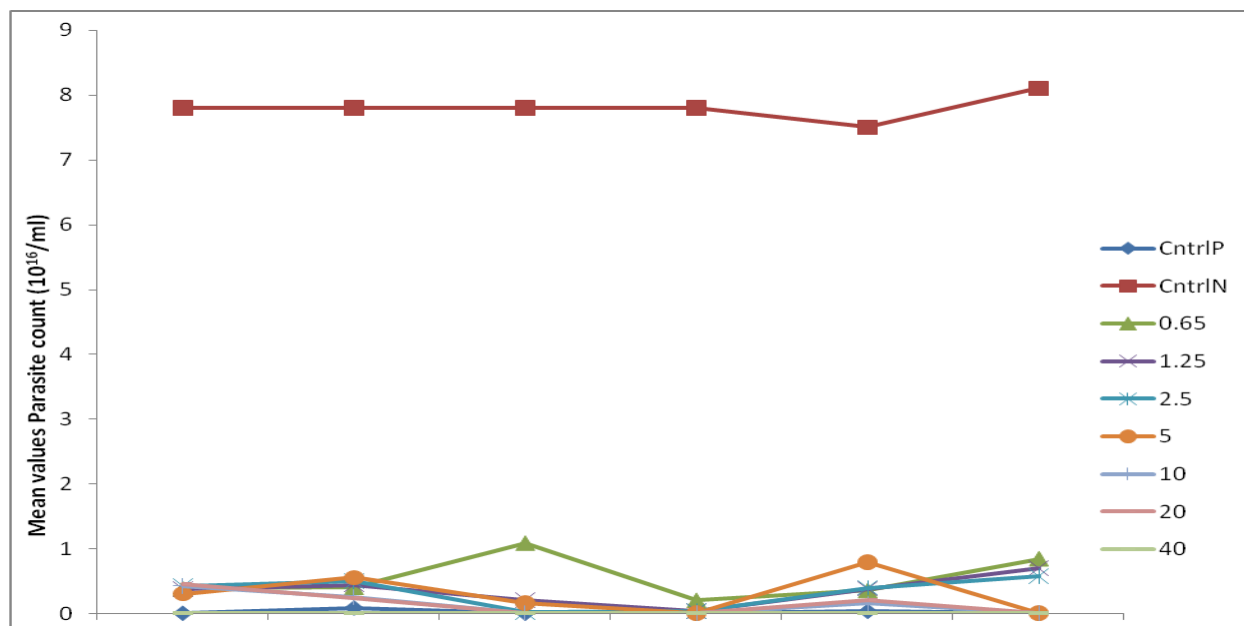
$$\% \text{ inhibition} = \frac{\text{Parasite count of control} - \text{Parasite count of treated}}{\text{Parasite count of control}} \dots\dots\dots \text{equation 1}$$

Atawodi and Ogunbosala (2009)

## **RESULTS AND DISCUSSION**

The *in vitro* efficacy of graded concentrations of *Viscum album* aqueous extracts from *P. guajava*, *A. indica* and *Acacia albida* host plants against *Trypanosoma brucei brucei* is shown in Figure 1. *In vitro* efficacy was observed across all the graded concentrations. There was 100% trypanosome mortality for

the positive control (Veriben®), *A. albida* leaf and stem and *P. guajava* stem at 10 mg/ml, and *A. indica* leaf and stem and *P. guajava* leaf at 40 mg/ml. The *in vitro* extract assays were compared positively with Veriben®, a chemotherapeutic trypanocide and there was a positive correlation between trypanosome mortality with graded aqueous extract of *Viscum album* leaf and stem.



Leaf - *A.indica* Stem- *A.indica* Leaf- *A.albida* Stem- *A.albida* Leaf-*P.guajava* Stem-*P.guajava*  
**Figure 1:** In vitro antitrypanosomal efficacy of graded concentrations of *Viscum album* leaf and stem from three host plants *A. indica*, *A. albida* and *P. guajava*

This study has revealed the *in vitro* efficacy of graded concentrations of *Viscum album* aqueous extracts from *P. guajava*, *A. indica* and *Acacia albida* host plants against *Trypanosoma brucei brucei*. Earlier studies have indicated that natural products resulting from plants and plant products offer novel remedies against trypanosomiasis, and that plant extracts contain some potent trypanocidal constituents (Atawodi *et al.*, 2003; Haruna *et al.*, 2003).

Wassagwa *et al.* (2018) also reported that *Viscum album* leaf and stem extracts contain active biotic components such as anthraquinones, flavonoids, saponins, terpenes, tannins and alkaloids and could remedy inflammatory and degenerative diseases (Nigerian Guardian Newspapers, 2007). Bioactivity of flavonoids, alkaloids, anthraquinones, tannins, saponins, and phenols is peroxidative damage to trypanothione reductase, which is very sensitive to alterations in redox balance, impediment of glucose catabolism, polyamine synthesis,

inhibition of ornithine decarboxylase, and intercalation with kinetoplast DNA of trypanosomes (Alli and Gower, 2010; Biu *et al.*, 2015). They possess structures capable of generating intra-parasitic toxic radicals through 5-nitofuryl moiety and inhibition of cruzipain through the thiosemicarbazone pharmacophore (Alli *et al.*, 2011; Biu *et al.*, 2016). Though an *in vivo* and *in vitro* study of parasites provides avenues that allow their physiology, behavior and metabolism to be assessed (Ahmed, *et al.*, 2014). However, specific phytochemical and mechanistic studies are required to determine the active phytochemical components responsible for the trypanocidal activities of these extracts.

## CONCLUSION

Findings from this study indicate that the aqueous extract of *Viscum album* leaf and stem from host plants *Psidium guajava*, *Azadirachta indica*, and *Acacia albida* exhibited graded concentration-dependent

activity against *Trypanosoma brucei brucei* by causing 100% clearance of the parasite.

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#### REFERENCES

- Ahmed, N.H. (2014). Cultivation of parasites. *Tropical Parasitology*. **4**(2): 80–89.
- Alli, A. A. and Gower, W. R. (2010). Molecular approaches to examine the phosphorylation state of the C type natriuretic peptide receptor. *Journal of Cellular Biochemistry*. **110**(4): 985-994.
- Alli, L.A., Okochi, V.I. and Adesokan, A.A. (2011). Antitrypanosomal Activity and haematological effects of aqueous extracts of leaves *Morinda lucida* on *Trypanosoma brucei brucei* infected rats. *Asian Journal of Pharmaceutical and Health Sciences*. **1**:111-115.
- Atawodi, S.E., Bulus, T., Ibrahim, S., Ahmed, D.A., Nok, A.J., Mamman, M. and Galadima, M. (2003). *In vitro* trypanocidal effect of methanolic extract of some Nigerian savanna plants. *African Journal of Biotechnology*. **2**: 313-321.
- Atawodi, S.E. and Ogunbusola, F. (2009). Evaluation of antitrypanosomal properties of four extracts of leaves, stem and root barks of *Prosopis africana* in laboratory animals. *Biokemistri* **21**:101-108.
- Biu, A.A., Buratai, L.B., Hambali, I.U., Auwal, M.S., Lawan, F.A., Mustapha, F.B., Yakaka, W., Ruth, P.A., Ibrahim, M.M. and Mbaya, A.W. (2015). *In vitro* trypanocidal efficacy of *Balanites aegyptiaca* DEL (*Zygophyllaceae*) leaf aqueous extract. *Annals of Borno*. **25**:171-177.
- Biu, A.A., Buratai, L.B., Hambali, I.U., Auwal, M.S., Lawan, F.A., Mustapha, F.B., Yakaka, W., Ruth, P.A., Ibrahim, M.M. and Mbaya, A.W. (2016). Studies on the

is greatly appreciated throughout the course of the experiment

#### CONFLICT OF INTEREST

No conflicts of interest exist among the authors.

Phytochemistry, Acute Toxicity and *in vitro* Trypanocidal

Efficacy of *Guiera senegalensis* Leaf Aqueous Extract. *Annals of Borno*. **26**:201-208.

- Biu, A. A., Auwal, M. S., Maina, U. A., Salihu, S. I., Kashimbe, I. B., Buratai, L. B., Ibrahim, A., Lawan, F. A. and Ruth, P. A. (2022). *In vitro* Trypanocidal Activity of Aqueous Leaves Extract of *Allium cepa* Linn in Experimentally Infected Wistar Rats. *Sahel Journal of Veterinary Science*. **19**(2): 1-6.
- Bukachi, S. A., Wandibba, S. and Nyamongo, I. K. (2017). The socio-economic burden of human African trypanosomiasis and the coping strategies of households in the South Western Kenya foci. *PLoS Neglected Tropical Diseases*. **11**(10), e0006002.
- C.I.O.M.S. (1985). Council for International Organization of Medical Sciences. International Guiding Principles for Biomedical Research Involving Animals. Pp. 98-134.
- Haruna, U., Kwanashie, H.O., Anuka, J.A., Atawodi, S.E. and Hussaini, I.M. (2003). Bioassay fractionation and antitrypanocidal effects of fractions and crude methanol root extracts of *Securidaca longependunculata* in mice and rats. *International Journal of Modern Biochemistry*. **2**: 1-4.
- Ibrahim M.A., Njoku, G.C. and Sallau, A.B. (2008). *In vivo* activity of stem bark aqueous extract of *Khaya senegalensis* against *Trypanosoma brucei*. *Africa Journal of Biotechnology*. **7**: 661-663.

- Isaac, C., Ohiolei, J.A., Ebhodaghe, F., Igbinsosa, I.B. and Eze, A.A. (2017). Animal African Trypanosomiasis in Nigeria: a long way from elimination/eradication. *Acta Tropica*. **176**: 323–331.
- Mbaya, A. W., Ibrahim, U. I., God, O. T., & Ladi, S. (2010). Toxicity and potential anti-trypanosomal activity of ethanolic extract of *Azadirachta indica* (Maliacea) stem bark: An in vivo and in vitro approach using *Trypanosoma brucei*. *Journal of Ethnopharmacology*. **128**(2), 495-500.
- Mittal, G. C., Aguwa, C. N., Ezeiru, V. U. and Akubue, P. I. (1981). Preliminary pharmacological studies on antivenin action of *Diodi siandens* leaves. *Nigerian Journal of Pharmacology*. **12**: 432–436.
- Nigerian Guardian Newspapers, (2007). Herbal routes to beating diabetes. Lagos, Nigeria 13: 12.07.
- Nok, A.J., Esievo, K.A.N., Longdet, I., Arowosafe, S., Onyenekwe, P.C., Gimba, C.E. and Kagbu, J.A. (1993). Trypanocidal Potentials of *Azadirachta indica* In Vivo Activity of Leaf Extract Against *Trypanosoma brucei brucei*. *Journal of Clinical Biochemistry and Nutrition*. **15**(2):113-118.
- Nok A.J. and Balogun, E.O. (2003). Blood Stream *Trypanosoma congolense* sialidase could be involved in anaemia during experimental trypanosomiasis. *Biochemistry*. **133**:725-730.
- Olapade, E. O. (1995). Food and herbs on *Diabetes mellitus*. Ibadan: NARL *Specialist Clinicians Publication*. Pp. 1-5.
- Osadebe, P.O. and Uzochukwu, M I.C. (2006). Chromatographic and antimotility studies on extracts of *Loranthus micranthus* Linn. *Journal of Pharmaceutical and Allied Sciences*. **3**(1): 263-268.
- Shuaibu, M.N., Ponchang, T., Wuyep, A., Yanagi, T., Hirayama, K., Ichinose, A., Tanaka, T. and Kouno, I. (2008). Trypanocidal activity of extracts and compounds from the stem bark of *Anogeissus leiocarpus* and *Terminalia avicennoides*. *Parasitology*. **102**(4):697-703.
- Umar, I. A., Ibrahim, M. A., Fari, N. A., Isah, S. and Balogun, D.A. (2010). *In vitro* and *in vivo* anti-*Trypanosoma evansi* activities of the extracts from different parts of *Khaya senegalensis*. *Journal of Cell and Animal Biology*. **4**:91–95.
- Wassagwa, J., Buratai, L.B., Biu, A.A., Luka, J., Ngulde, I.S. and Onyiche, T.E. (2018). Phytochemistry and toxicity studies on aqueous extracts of leaf and stem of Mistletoe (*Viscum album*) in albino rats. *International Journal of Homeopathy and Natural Medicines*. **4**(1):1-5.
- Yusuf, L., Oladunmoye, M.K., Ogunbare, A.O., Akinyosoye, F.A., Daudu, O.A. and Hassan, G. A. (2013). Antimicrobial and antioxidant properties of mistletoe (*Viscum album*) growing on cola (*Cola nitida*) tree in Akure North, Nigeria. *International Journal of Medical and Medicinal Science*. **3**(5):9-11.