American Journal of Laboratory Medicine
2017; 2(4): 69-73
http://www.sciencepublishinggroup.com/j/ajlm
doi: 10.11648/j.ajlm.20170204.15
ISSN: 2575-3878 (Print); ISSN: 2575-386X (Online)

Evaluation of Trypanocidal Activity of *Bidens pilosa* and *Physalis peruviana* Against *Trypanosoma brucei rhodesiense*

Lilian Mwende Mwaniki[1], John Mokua Mose[2, *], Titus Mutwiri[2], James Mulinge Mbithi[2]

[1]Biotechnology Research Institute, in Biochemistry Division (Tissue Culture and Protozology Section), Kenya Agricultural and Livestock Research Organization (KALRO), Nairobi, Kenya

[2]Department of Medical Laboratory Science, School of Medicine and Health Sciences, Kenya Methodist University, Nairobi, Kenya

Email address:

mokuajohn4@gmail.com (J. M. Mose)

[*]Corresponding author

To cite this article:

Lilian Mwende Mwaniki, John Mokua Mose, Titus Mutwiri, James Mulinge Mbithi. Evaluation of Trypanocidal Activity of *Bidens pilosa* and *Physalis peruviana* Against *Trypanosoma brucei rhodesiense*. *American Journal of Laboratory Medicine*. Vol. 2, No. 4, 2017, pp. 69-73. doi: 10.11648/j.ajlm.20170204.15

Received: January 10, 2017; **Accepted**: January 20, 2017; **Published**: October 24, 2017

Abstract: Trypanosomiasis is a protozoan disease that causes death and morbidity to man, and also severely limits livestock productivity in endemic areas. It is caused by different species of trypanosomes that occur in Africa, South America and Asia. Although chemotherapy is the main method of trypanosomiasis control, the few drugs in the market are faced with challenges of drug resistance, high toxicity and very costly. There is also no likelihood of a vaccine to control new outbreaks and there are no new drugs in the market. Herbal medicines are increasingly being used as an alternative solution to the control of trypanosomiasis in endemic areas. These are cost effective and economic friendly. The purpose of this study was to evaluate trypanocidal activity of *Bidens Pilosa* and *Physalis peruviana* using *in-vitro* and *in-vivo* protocols. The *in-vitro* trials are carried out using 96 well plates where the trypanocidal activities of the plant extracts were evaluated by calculating the minimum inhibition concentration (MIC). Toxicity of the herbal extract was determined by monitoring survival, weight change, lethargy and difficulty in breathing. Parasitemia development after extract administration was used as parameters to test the test compound for trypanocidal activity. In vitro results revealed that *bidens pilosa* had the highest activity with an MIC of 125µg/ml after 48 hours of incubation against *Trypanosoma brucei rhodensiense* isolated from a patient in busia (KETRI 3684). Toxicity results showed that a dosage level of above 1000mg/kg body weight (highest toxicity trial dose used) of *bidens pilosa* caused clinical signs such as difficulty in breathing, lethargy, raised hair, loss of weight and death within five days. *In-vivo* results revealed that *Bidens pilosa* had some trypanocidal effect but did not perform better than the standard drugs. It is recommend that repeat therapy could be done to clear the parasites completely. Combined therapy of *bidens pilosa* and *physalis peruviana* was recommended to see if results can create a rationale for combination therapy in elimination of the parasites. This study has showed that *Bidens pilosa* and *Physalis peruviana* have trypanocidal potential.

Keywords: African Trypanosomosis, Medicinal Plants, Anti-trypanosomal Activity

1. Introduction

*S*leeping sickness is a disease caused by two subspecies of *Trypanosoma brucei*, *T. b. rhodesiense* and *T. b. gambiense*. The parasites live and multiply extracellularly in blood and tissue fluids of their human host and are transmitted by the bite of infected tsetse flies (*Glossina* spp). The occurrence of sleeping sickness is restricted to the distribution of tsetse flies which are exclusively found in sub-Saharan Africa between 14°N and 20°S [1]. More than 250 discrete active sleeping sickness foci in 36 African countries are recognized most of which are in rural areas [2].

Trypanosoma b. rhodesiense is found in East and Southern Africa whereas *T. b. gambiense* occurs in West and Central Africa. The course of sleeping sickness is different depending on the subspecies. Infections with *T. b. rhodesiense* lead to an

acute form of the disease while infections with *T. b. gambiense* give rise to a chronic infection.

The symptoms of the first stage of the disease, defined by the restriction of trypanosomes to the blood and lymph system, include fever, headache, joint pain and itching. The clinical signs of the second stage of the disease, characterised by the invasion of trypanosomes into the central nervous system, are neurological and endocrinal disorders [3, 4]. If left untreated, sleeping sickness patients infected with *T. b. rhodesiense* will die within months whereas those infected with *T. b. gambiense* usually survive for several years.

In the late 19th Century, Africa experienced several sleeping sickness epidemics the most devastating of which was an epidemic with 300,000 to 500,000 deaths between 1896 and 1906 which mainly affected the Congo Basin and the Busoga focus in Uganda and Kenya [5, 6, 7]. The disastrous effect of this epidemic persuaded the various colonial administrations to call for their medical scientists to develop a cure for sleeping sickness. At that time, the field of chemotherapy was developing and had begun to make use of the novel methods of medicinal chemistry, i.e. the identification, synthesis and development of new chemical entities suitable for therapeutic use. In fact, it was for the development of early anti-sleeping sickness drugs that medicinal chemistry was first used [8, 9].

The current study aimed at investigating the trypanocidal effect and toxicity of *Bidens pilosa* and *Physalis peruvian* against *T.b. rhodesiense* isolates. This will provide valuable information about the possible alternatives considering that the drugs used for treatment of sleeping sickness e.g Melarsoprol often have severe side effects, are few, costly and production may be discontinued [10]. There is an urgent need for therapeutic agents that are effective, affordable and accessible to the rural poor in Africa who bear most of the disease burden [11, 12]. Most of the drugs used for treatment of sleeping sickness e.g Melarsoprol are very toxic and high therapeutic failure has been reported recently in several foci.

2. Materials and Methods

2.1. Study Area

This work was done at the Biotechnology research institute of Kenya Agricultural and Livestock research organization (KALRO) in biochemistry division Tissue culture and Protozology section.

2.2. Experimental Animals

BALB/c white mice (both sexes) were obtained from the small animal laboratory of Biotechnology Research Institute of Kenya Agricultural and Livestock research organization (KALRO) (6–8 weeks of age, weighing 20-30g). The mice were housed in plastic cages (medium size cages; Length 16.9 inches, Width 10.5 inches, and height 5 inches) with wood shaving bedding and nesting material and kept in a 12:12 h light-dark cycle. Food (Pellets from Unga feeds) and water were provided *ad libitum*. They were kept under a natural light-dark cycle of 12/12 hours, at ambient-temperature (21-25°C) and relative humidity of between 50 and 60%.

2.3. Study Design

2.3.1. In-vitro Studies

The herbs were prepared in three different concentrations and then in a 96 well plate each drug dilution was put in duplicate in wells (column 11), and was serially diluted as we move across the rows up to column 4 and a standard concentration of trypanosomes (5x 10^5 trypanosomes) was added. The experiment was run alongside the Standard drugs suramine and malarsoprol.

2.3.2. In-vivo Studies

The experiment comprised of 11 groups of 4 mice each giving a total of 44 mice. Groups 1 to 4 were for toxicity studies which were treated with the herbal extract 1000mg/kg, 500mg/kg and 250 mg/kg respectively and the fourth group was the control. They were monitored for two weeks.

The 5^{th} to 10^{th} groups of mice were infected with *Trypanosoma brucei rhodensience* stabilate (KETRI 3684) originally obtained from a patient at 1 x 10^4 trypanosomes/ml intraperitoneally and they were monitored by wet blood film for presence of parasites in blood. The extract which was most effective *in-vitro (Bidens pilosa)* was tested in three different concentrations 500mg/kg, 250mg/kg and 125mg/kg. The infected mice were treated at the onset of parasitaemia, the experiment was alongside the standard drugs suramine and malarsoprol. The 10^{th} group was infected and not treated which was the positive control while the 11^{th} group was not infected and not treated which was the negative control.

2.4. Study Sample

The sample was *Trypanosome brucei rhodensience* parasites isolates (KETRI 3684) isolated from a patient in Busia. The stabilates were retrieved from liquid nitrogen -198°C in trypanosome bank at Biotechnology research institute.

2.5. Plant Extraction Procedure

The plant extracts provided in powder form by Mr. Chege from the Technical University of Kenya (TUK) were used. An aqueous extract was prepared by soaking 10mg powder in 100mls of water. The solution was heated on a water bath for 2 hour at 60°C and then left at room temperature for 10 hours. The extract was filtered using Whatman's filter paper, and then poured in Petri dishes and dried in an oven at 37°C. The dried extracted was weighed and stored in a cool dry conditions until required.

2.6. In-vitro Screening

The sample was collected from trypanosome bank (Biochemistry division) in capillary tube and then diluted in 0.4mls of phosphate saline glucose (PSG) pH 8.0). Donor mice were infected and parasitemia was monitored daily until the parasitemia got to log 8.4 per field. The infected mouse was put in an enclosed container leaving the tail outside; the

tail was washed with water and rinsed with 70% ethanol. The mouse was taken to the laminar flow hood; blood was collected from the tail using a micro pipette, and a 50ul tip.

The blood was put in the flask containing media. The flask was kept in an incubator for 2 hours. Everything was pipetted out of the flask into a centrifuge tube and centrifuged at 1000 revolutions per minute (rpm) for 10 minutes. The supernatant was then poured in a sterile container and centrifuged at 3000rpm for 10 minutes. The supernatant was poured out and the sediment resuspended in 1ml of the culture media. The parasites in the suspension were counted using an haemocytometer and a microscope. Dilution of the parasites was made to achieve the desired concentration of 5 x 10^5 trypanosomes per ml of media.

Reference drugs and the plant extracts were prepared and diluted to achieve the needed concentration using culture media (Each made into three different concentrations (Melarsopro 100ng/ml, 10ng/ml, 1ng/ml. Suramin, *bidens pilosa* and *physalis peruviana* 1000µg/ml, 100 µg/ml and 10 µg/ml). 100ul of the prepared media was put into each well of 96 well plates leaving all the outer wells round. Three different concentrations (test extracts and standard drugs) in duplicates were used per plate. 100ul of each concentration was put in column 11.

Serial dilution was done using a multi channeled pipette from column 11 picking 100ul dilution up to column 4 and the final 100 was discarded leaving 100ul in each column. Leaving column number 2 and 3 for controls. 100ul of 5*10^5 concentrations of trypanosomes was put in all wells containing the diluted drug including the wells without drugs. Plates were placed in moist modular chamber which was gased with neat CO_2. Then incubated at 37°C the assay was read after 48 hours and the concentration with no motile trypanosomes was noted. The most effective herb of the two used *in-vitro* was used for the *in-vivo* work.

2.7. Toxicity

Four groups of 4 mice each was treated with 3 different concentrations of the Bidens pilosa: 1000mg/kg body weight; 500mg/kg body weight and 250 mg/kg body weight. The 4th group was the controls (not treated). The animals were monitored for clinical signs such as dyspnea, raised far, and lethargy and were recorded daily. The animals were weighed after every 2 days for two weeks.

2.8. Drug Sensitivity

Mice were divided into 7 groups of 4 mice each. Six groups were intraperitoneally infected with the trypanosomes at 1 x 10^4trypanosomes. At the onset of parasitemia groups 1 to 3 were treated with 3 different concentration of the herbal extract, group 4 and 5 were treated with the standard drug. And they were kept under close observation and fed on pellet diet and water. Clinical signs such as dyspnea, raised far and lethargy were observed and recorded daily for two weeks. The animals were monitored for parasitemia after every three days for 33 days post treatment. All the parameters were recorded.

2.9. Ethical Issues

All protocols and procedures used in the current study were reviewed and approved by both the biotechnology research institute and Kenya Methodist University.

3. Results

3.1. In-vitro Trypanocidal Activity

The two crude plant extracts from two plants were analyzed *in-vitro* for trypanocidal activity against *Trypanosoma brucei rhodensiense.* The test was run in 48 hours with the two extracts and two reference drugs and the results obtained are shown in Table 1. Minimum inhibition concentration (MIC) of reference drugs and the herbal extracts were determined. Efficacy of the drug is determined by the MIC. Average MIC results are shown in Table 2 representing the trypanocidal effects after 48 hours incubation with standard drugs and test extracts. Bidens pilosa had a lower MIC compared to *Phasalis peruvian* and therefore it was used for in-vivo study

Table 1. *Trypanocidal activity for the reference drug and the herbal extracts for against Trypanosoma brucei rhodensiense.*

Condition of the control moderate density		
Plant extracts/Reference drugs	**Concentrations of stock solutions**	**Results**
Melarsoprol	100ng/ml	0.39ng/ml
	10ng/ml	0.16ng/ml
	1ng/ml	0.13ng/ml
Suramine	1000µg/ml	31.25 µg/ml
	100µg/ml	25µg/ml
	50µg/ml	25µg/ml
Bidens pilosa	1000µg/ml	125µg/ml
	500µg/ml	125µg/ml
	250µg/ml	125µg/ml
Physalis peruviana	1000µg/ml	500 µg/ml
	500µg/ml	>250µg/ml
	250µg/ml	>125µg/ml

Table 2. *MIC for the reference drug and the herbal extract for isolate KETRI 3486.*

Drug and herbal extract	**MIC KETRI 3486**
Suramin	27.08µg/ml
Melarsoprol	0.23ng/ml
Bidens pilosa	125 µg/ml
Physalis peruviana	500 µg/ml

MIC calculation

$MIC = C/D*2^n$

Where

MIC = Minimum inhibition concentration

D = Dilution of drug by medium in the wells of column 11 (maximum concentration).

N = Steps of dilution to the first well with no living trypanosomes

C = Concentration of drug solution

3.2. In-vivo Toxicity of Bidens Pilosa Extract in Mice

Treatment of mice with a dosage level of 1000mg/kg body

weight resulted in 100% death of mice in 5 days post treatment. Dosage level 500mg/kg and 250mg/kg resulted to no death (Table 3).

Table 3. *Invitro Toxicity of Biden pilosa extract in mice.*

Group	Dosage (mg/kg bw)	No. died	% died
I	1000	4/4	100
II	500	0/4	0
III	250	0/4	0
II	Control untreated	0/4	0

3.3. Clinical Response

Observation on mice after injection with 1000mg/kg body weight was that after the first 15minutes 3 of the four mice were found to be lethargic with difficulties in breathing and the hair was raised. All four mice died within the first five days. The results after injecting the mice with 500mg/kg body weight showed two mice becoming weak after 15 minutes with difficulty in breathing and raised hair. They recovered after one hour. Mice injected with 250mg/kg body weight showed no clinical signs.

3.4. Change of Body Weight

There was significance difference between all the groups where $p< 0.05$. The toxic effect of highest concentration of *Bidens pilosa* resulted into death of all animals in that group by day five as shown in Figure 1. Mice treated with 1000mg/kg of *Bidens pilosa* had a decline in mean body weight while those treated with 500mg/kg and 250mg/kg lossed some body weight.

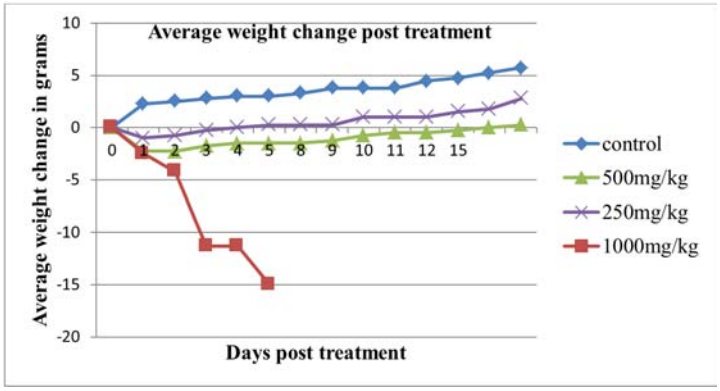

Figure 1. *In-vivo evaluation of efficacy of Bidens pilosa against Trypanosoma brucei rhodensiense.*

Group comparison: There was significant where ($p<0.05$) differences between all groups except between group 1 and 6, also groups 4, 5 and 7 are not significantly different from each other. Progression of parasetemia between mice treated with Group 4, 5 and 7 no significance difference (Figure 2). There was no significance difference between group 1 and group 6. Signficance difference was observed between group 2 and 3 from day 1 to day 15. From day 18 there was no significance difference.

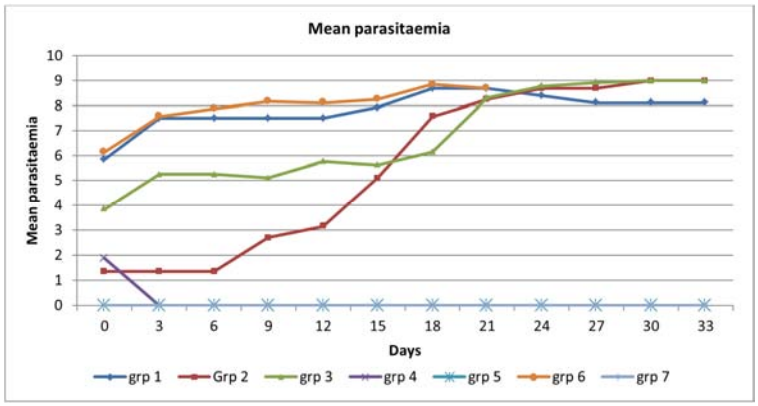

Figure 2. *Mean parasitaemia.*

Group 1 -125mg/kg; Group 2 -250mg/kg; Group 3-500mg/ kg; Group 4- suramin; Group 5- melarsoprol; Group 6- infected and not treated; Group 7-Non infected

4. Discussion

In-vitro studies showed that between the two herbal extracts tested *bidens pilosa* had the best trypanocidal effects, with MIC of 125µg/ml with isolates KETRI 3486 after 48 hours of incubation. *Physalis peruviana* had a very high MIC of 500µg/ml. Isolate KETRI 3486 was more susceptible to the two standard drugs compared to *Bidens pilosa* with

melarsoprol having the lowest MIC of 0.27ng/ml. Similar antitrypanosomal activity of the standard drugs were observed by Sara *et al.,* [14].

Toxicity results revealed that mice treated with dosage level 1000mg/kg body weight of *Bidens pilosa* extract expressed clinical signs of lethargy, difficulty in breathing and raised hair within the first fifteen minutes and all mice died within five days. This implied that the dosage was very toxic to mice hence that concentration could not be used in this study. Mice treated with dosage 500mg/kg and 250mg/kg showed very little change in weight and clinical signs. It implied that this dosage was not toxic to the experimental animal. Similar results were achieved by [15, 16, 17] with extracts of *strychnos spinosa.* Literature from other studies on medicinal plants revealed both extracts contain similar compounds.

In-vivo results showed that extract *Bidens pilosa at* dosage level 500mg/kg and 250mg/kg expressed antitrypanosomal activity, with dosage level of 250mg/kg being the most active extract. Maintaining low parasitemia levels as compared to the positive control up to the day 15, then a very fast increase from day 18. Dosage 500mg/kg reduced the level of parasites as compared to the positive controls where the numbers of parasites remained high. Dosage 250gm/kg showed a better trypanocidal activity as compared to 500mg/kg. Suramin and melarsoprol eliminated the parasites completely from day 3 post treatment to the end of 33 days of observation period. Similar results were obtained in another related study [18].

5. Conclusion

Bidens pilosa is found to have a high trypanocidal effect to *Trypanosoma brucei rhodensiense* isolate as compared to *Physalis Peruvian*. But both were found to have antitrypanosomal activity at different concentration. They were not as effective as the standard drugs (Suramin and melarsoprol). Water was used as the only solvent since it is the only solvent a farmer can get. 250mg/kg showed a better trypanocidal effect as compared to 500mg/kg which is an indication from the toxicity study that it was abit toxic since the weight decreased than that one of 250mg/kg. This study has demonstrated a lot of trypanocidal potential of Kenya medicinal plants.

References

[1] F. A. Kuzoe, "Current situation of African Trypanosomiasis," *Acta tropica*. Vol. 54, no. (3-4), pp. 153-162, 1986.

[2] H. W. Thomas and A. Breinl, "Trypanosomes, Trypanosomiasis and Sleeping sickness; pathology and Treatment," *Liverpool School. Trop. Med. Memoir*. Vol. 16, pp. 1-96, 1905.

[3] E. pays and B. Vanhollebeke, "Human innate Immunity against African trypanosomes," *Current Opinions in Immunology.* Vol. 21, pp. 493-498, 2009.

[4] M. Bentivoglio, G. Grassi-Zucconi and K. Kristensson, "From trypanosomes to the nervous system, from molecules to behavior: a survey, on BMHJGNMHGFNMHGF the occasion of the 90th anniversary of Castellani's discovery of the parasites in sleeping sickness," *Ital. J. Neurol. Sci.* vol. 15, pp. 77–89, 1994.

[5] P de Raadt, "The history of sleeping sickness," *World Health Org*. 2005. http://www.who.int/trypanosomiasis_african/country/history/en/print.html

[6] G. Hide, "History of sleeping sickness in East Africa," *Clin Microbiol Rev*. vol. 12, pp. 112–125, 1999.

[7] World Health Organization, "African trypanosomiasis (sleeping sickness)," *World Health Organ Fact Sheet*; 2006. http://www.who.int/mediacentre/factsheets/fs259/en/

[8] D. Steverding, "The history of African trypanosomiasis," *Parasites & Vectors*. Vol. 1, no. 3, 2008.

[9] D. H. Molyneux, V. Pentreath and F. Doua, "African trypanosomiasis in man. In: Cook GC, editor. *Manson's Tropical Diseases*. 20. London: W. B. Saunders Company Ltd; pp. 1171–1196, 1996.

[10] T. M. Leach and C. J Roberts, "Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the eastern hemisphere, "*Pharmacology & Therapeutics.* Vol. 13, pp. 91–147, 1981.

[11] N. Nwude, "Ethnoveterinary Pharmacology and Ethnoveterinary practices in Nigeria- An overview. Paper presented at the Inaugural Review and Planning Workshop on Nationally Co-or-dinated Research Programme on Livestock Diseases NVRI, Vol, 24 March 1997.

[12] N. Nwude, M. A. Ibrahim, "Plants used in traditional veterinary in Nigeria," *Journal of Veterinary Pharmacology and Therapeutics* vol. 3, pp. 261–273, 1980.

[13] H. Sara, O. Frederik, B. Reto, A. Victor, and Q. L. Joelle, "In vitro Antitrypanosomal Activity of Ethnopharmacologically Selected Beninese Plants," *J. Ethnopharmacol*. Vol. 91, pp. 37–42, 2004.

[14] M. A. Ibrahim, A. Mohammeda, M. B. Isah, A. B. Aliyu, "Anti-trypanosomal activity of African medicinal plants: A review update," *Journal of Ethnopharmacology* vol. 154, no. 1, pp. 26–54, 2014.

[15] S. Hoet, F. Opperdoes, R. Brun, V. Adjakidjé, J. Quetin-Leclercq, "Invitro antitrypanosomal activity of ethnopharmacologically selected Beninese plants," *Journal of Ethnopharmacology*. Vol. 911, pp. 37–42, 2004a.

[16] S. Hoet, F. Opperdoes, R. Brun, J. Quetin-Leclercq, "Natural products against African trypanosomes: a step towards new drugs," *Natural Product Reports*. Vol. 21, pp. 353–364, 2004b.

[17] S. Hoet, L. Pieters, G. G. Muccioli, J. L. Habib-Jiwan, F. R. Opperdoes, J. Quetin- Leclercq, "Antitrypanosomal activity of triterpenoids and sterols fromthe leaves of Strychnos spinosa and related compounds," *Journal of Natural Products* vol. 70, pp. 1360–1363, 2007.

[18] P. C Kimani, PK Gathumbi, K Ndungu, J Auma, JJ Ngeranwa, DK Masiga, "Evaluation of trypanocidal activity of selected medicinal plants in Kenya against Trypanosoma Evansi," *Kenya Veterinarian.* Vol 37, no 1, 2013.