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In vitro anti-trypanosomal activity of *Thysanophora penicilloides, Aureobasdium pullulans,* and *Sporangia spp* extracts

Tukur, S. T.[1]*, Jega, Y. N.[2] and Faragai, S. R.[3]

[1]Department of Science Laboratory Technology, Federal Polytechnic Kabo, Kano State, Nigeria.
[2]REACH Organisation, Malaria Case Management office, Kebbi State, Nigeria.
[3]Department of Environmental Sciences, Federal Polytechnic Kabo, Kano State, Nigeria.

*Corresponding author. Email: sanitajo88@gmail.com/sanitajo@fedpolykabo.edu.ng

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ABSTRACT: The genus *Trypanosoma,* a flagellated protozoan, causes trypanosomiasis. The disease occurs in humans and animals in Africa and South America. African trypanosomiasis is a debilitating disease affecting humans and domestic animals in the humid and sub-humid zones of Africa. It's estimated that over 60 million people and 50 -70 million animals are exposed to the infection. Chemotherapy for African trypanosomiasis remains unsatisfactory due to unacceptable toxicity, increasing parasite resistance, high cost, and unavailability. There is an urgent need for effective, affordable, and accessible therapeutic agents for the rural poor in Africa who bear most of the disease burden. This study aimed to determine the *in vitro* antitrypanosomal activity of *Thysanophora penicilloides*, *Aureobasidium pullulans*, and *Sporangia spp* extracts against *T. brucei brucei*. Fungal ethanolic extracts from *M. oleifera* stem, *M. oleifera* leaf, and *Vachellia nilotica* leaf were investigated for therapeutic effects in experimental African trypanosomiasis. Effects were compared with *Diminazene aceturate.* Phytochemical screening and blood incubation infectivity tests were conducted using the Drug Incubation Survival Test (DIST). The high concentration (1.0 mg/ml) of *Sporangia spp* from *Vachellia nilotica* leaf showed potential *in vitro* activity, immobilising trypanosomes within 2-8 hours and abrogating infectivity to rats. *Thysanophora penicilloides* and *Aureobasidium pullulans* extracts from *M. oleifera* showed no trypanocidal effect but may prolong life span due to antioxidant activities. *Sporangia spp* from *Vachellia nilotica* leaf have potential antitrypanosomal activity, making it a potential source for new drugs against Human African Trypanosomiasis.

Keywords: *Anti-trypanosome, Aureobasidium pullulans, Diminazene aceturate, Sporangia spp*, *Thysanophora penicilloides.*

INTRODUCTION

Trypanosomiasis is a parasitic disease caused by a flagellated protozoan belonging to the genus Trypanosoma. This genus causes potentially fatal human and animal trypanosomiasis in Africa (African trypanosomiasis) and South America (American trypanosomiasis). African trypanosomes cause Human African Trypanosomiasis (HAT), a debilitating disease of humans and domestic animals in the humid and sub-humid zones of Africa (Assefa, 2017). It is estimated that over 60 million people and 50 – 70 million animals are exposed to the infection (Ogbadoyi *et al*., 2011). The disease is mainly transmitted through the bite of the tsetse fly (*Glossina species*), which introduces the causative agent, trypanosome, into the blood. The tsetse belt extends from the Sahara in the North to South Africa in the South. Large areas of Africa, approximately 4 million Km^2, have been rendered unsuitable for livestock production by trypanosomes. This makes African Animal Trypanosomiasis (AAT) one of the vector-borne parasitic diseases which are causing major health and economic

problems in rural sub-Saharan Africa (Assefa, 2017). Trypanosomiasis, though neglected sub-Sahara African tropical infectious disease, its medical and veterinary importance cannot be overemphasized as it poses threat to 36 countries with the estimated population of 55million people at risk for the period of 2016 2021. It is caused by a protozoan parasite of the genus Trypanosoma, transmitted by the tsetse fly (WHO, 2019). Human African Trypanosomiasis (also called sleeping sickness) is caused by *T. b. rhodesiense* and *T. b. gambiense* while *T. brucei brucei* is responsible for African Animal Trypanosomiasis (AAT) (also called Nagana) in West Africa (Medina *et al*., 2011).

Therefore, the objective of this study is to determine the *in vitro* antitrypanosomal activity of *Thysanophora penicilloides*, *Aureobasidium pullulans*, and *Sporangia spp* extracts against *Trypanosoma brucei brucei.*

MATERIAL AND METHODS

Plant collection

Samples of *Moringa oliefera* stems and leaves, and *Vachellia nilotyca* leaves were collected from the botanical garden in the Department of Biological Science, Bayero University Kano. The plant samples were collected in black polythene bags and labelled appropriately with appropriate labelling and taken directly to the laboratory for analysis.

Isolation and identification of entophytic fungi

40 g of Potatoes Dextrose Agar (PDA) was measured and mixed with 0.04 g of streptomycin. The mixture was later transferred into a 1000 ml conical flask and made up to one litre by adding distilled water. The mixture was dissolved by gentle shaking with the opening of the conical flask wrapped with cotton wool and foil paper. The mixture was autoclaved at 121°C for 15 minutes. Then sterilised and allowed to cool, and then poured into the petri dishes and allowed to solidify for 3 days. The plant materials were rinsed gently in running water to remove dust and debris. After proper washing, the stem and leaf samples were cut into small pieces, and leaves were selected for further processing under aseptic conditions. Highly sterile conditions were maintained for the isolation of endophytes. All the work was performed in an aseptic condition. Sterile glassware and other materials such as scissors, forceps, razor blades, etc., were sterilised before the experiments. The leaves were cut into 3-4 mm and 0.5-1cm in length, with and without midribs. Stem samples were cut into 0.5-1cm lengths. The isolation of endophytic fungi was done according to the method described by Petrini. (1986). The surface sterilisation was done by a mixture of 100% sodium hypochlorite (NaOCl) (10 ml of hypo bleach and 90 ml of distilled water), and treated with 70% ethanol (70 ml of ethanol and 30 ml of distilled water) and finally washed with 100 ml of distilled water. The time for sterilisation was 3-10 minutes. Each set of plant materials was treated with 70% ethanol for one minute, followed by immersion in sodium hypochlorite for 30 seconds and again in 70% ethanol for 30 seconds. Later, the segments were rinsed 3 times with sterile distilled water. The plant pieces were blotted in sterile blotting paper and inoculated aseptically in prepared media. The medium, which was prepared and poured into each petri dish, 1 segment was placed on the medium supplemented with antibiotic streptomycin 100 µg/ml concentration. The petri dishes were sealed with paraffin and kept under dark conditions. Most of the fungal growths were initiated within 2 weeks of inoculation. The incubation period for each fungus was recorded. The day of the first visible growth was observed, and an incubation period was noted. The pure cultures were prepared from the master plate by transferring the hyphal tips to another fresh potato dextrose agar plate (PDA) without the addition of any antibiotic to obtain the pure culture, which was observed after 7-10 days of inoculation. The pure cultures were then taken to the BUK central laboratory for identification.

Fungal identification

The fungi were identified on the basis of their morphological and cultural characteristics. Fungi were grown on a prepared medium at specified culture conditions for identification. The mycelia were placed on a grease-free, clean slide in a drop of distilled water and covered with a cover slip. Micrographs were taken using a microscope (Olympus, Bx3m, Japan) using a x40 objective and compared with the standard fungal atlas.

Fermentation in liquid media

Czapex dox media was constituted by using 30 g sucrose, 3 g $NaNO_3$, 1 g di-potassium phosphate, 0.5 g Magnesium sulphate, 0.5 g potassium chloride and 0.0 1 g of ferrous sulphate in 1 litre of distilled water. Three hundred millilitres of media were added to a 1 litre conical flask and autoclaved at 121°C for 15 minutes. A sample of 10 days' fresh mycelia from the fungus grown on PDA in a Petri dish at 27°C was aseptically inoculated into a flask. The flask was kept stationary for 30 days and examined periodically for contamination.

Extraction of the metabolite from the liquid media

Extraction of the metabolites from the liquid media was performed as described by Kar *et al.* (2003). The culture media and mycelia were separated by filtration. The mycelia were extracted in methanol and later in ethyl

acetate. The extract of the mycelia was collected after 7-10days. The liquid broth was extracted with ethyl acetate 3-4 times. The organic extract was evaporated using a rotary evaporator at 40°C to obtain solid residues. The residues were weighed, and the results were recorded.

Brine shrimp lethality bioassay

One spatulaful of eggs of Artemia salina (about 50 mg) was placed into a hatching chamber containing seawater and kept under a light for 48 hours. After the eggs have hatched, 3 mls of seawater containing 20 larvae were placed in vials containing extract, positive and negative control. The Positive control was prepared by weighing 0.6 g of caffeine in 1ml of seawater in each 3 ml of the solution. Whereas, the negative control and prepared 10% methanol in 1000, 100 and 10 µg/ml concentrations, respectively. All vials containing the dosages and the controls were left overnight for the methanol to evaporate, leaving only the fungal extract as residue. Twenty- four hours after the inoculation, the number of surviving shrimp larvae at each dosage was counted and recorded.

Determination of the LC_{50} (lethal concentration)

The LC_{50} results obtained by the BSLA was categorized according to Clarkson's toxicity criterion for the toxicity assessment of fungal extracts, classifies extracts in the following order: extracts with LC_{50} above 1000 µg/ml are non-toxic, LC_{50} of 500 - 1000 µg/ml are low toxic, extracts with LC_{50} of 100 - 500 µg/ml are medium toxic, while extracts with LC_{50} of 0 - 100 µg/ml are highly toxic (Araújo *et al*., 2022).

Test organisms and its maintenance (*Trypanosoma brucei brucei)*

The test organism used for this study was *Trypanosoma brucei brucei* (Federe strain). Stabilate of the trypanosome species was obtained from the cryobank maintained at the Vector and Parasitology Department of the National Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria. After thawing the stabilate at room temperature, trypanosomes were screened for viability by examining wet smears prepared from the stabilates using a light microscope at x400 magnification. The presence of a motile trypanosome was taken as an indication of trypanosome viability.

Donor animals

Two Swiss albino mice were utilised as donor animals. They were fed with standard animal feed and given water *ad libitum* at room temperature, 23-25°C, with relative humidity of 60-65%. All procedures complied with the Guide for the Care and Use of Laboratory Animals (Bulus *et al.,* 2012). Each of these was intraperitoneally inoculated with blood suspension prepared from the stabilate. The inoculum was prepared by the addition of phosphate-buffered saline-glucose (pH 7.4) to a small amount of the blood from the stabilate until the trypanosome count was 2 per microscopic field. The mice were inoculated with 0.1 ml of the prepared blood suspension. Parasitemia was monitored in the inoculated mice two days post-inoculation to determine the establishment of active infection. Following infection with the trypanosomes, the donor mice were sacrificed when the trypanosome count was about 10^9 per millilitre of blood.

Determination of parasitemia

The trypanosome count in the infected mice was determined by the rapid matching method. Briefly, a drop of whole blood collected by tail snip or cardiac puncture was placed on a clean, grease-free glass slide, and a cover glass was placed over it; the blood was then spread into a thin circular film. The slide was placed on a microscope and examined at x400 magnification. The distribution of trypanosomes among the red blood cells (RBCs) was matched using Lumsden's chart, and the approximate number of trypanosomes per millilitre of blood was estimated (Saldanha *et al.,* 2024).

Collection of parasitised blood

Trypanosome containing blood was collected into a 2 ml syringe from the donor mice by the cardiac puncture technique after chloroform anaesthesia. The blood was dispensed into an ethylene diamine tetra acetate (EDTA) sample container and gently mixed together to prevent clotting of the blood.

***In Vitro* anti-trypanosomal activity**

Assessment of *in vitro* trypanocidal activity was performed in triplicate in 96 Wells micro titer plates. Infected mice with high parasitemia were sacrificed, and blood was collected in EDTA coated tubes mixed with phosphate buffered saline glucose (PBSG). Twenty-five micro liters of blood containing about 9-17 organisms per field were mixed with 5 microliters of each of the test substances at final concentrations of 1.0, 0.75, 0.50, 0.25 and 0.1 mg/ml.

Phytochemical screening

The ethanolic fungal extracts were tested for the presence of bioactive compounds like saponin, flavonoid, alkaloid, steroids and phenols by using standard tests (Peiris *et al*., 2023).

Table 1. Isolated and identified fungi

S/N	Plant name	Name of fungal isolated
1	*Moringa Oliefera* stem	*Thysanophora penicilloides*
2	*Moringa Oliefera* leaf	*Aureobasdium pullulans*
3	*Vachellia nilotica* leaf	*Sporangia spp*

Table 2. Effects of *Thysanophora penicilloides, Aureobasdium pullulans* and *Sporangia spp* extract on Brine shrimp larvae.

Concentration of the extract (µg/ml)	Initial	*Thysanophora penicilloides*		*Aureobasdium pullulans*		*Sporangia spp.*		0.6 Caffeine		Control Distiled water	
		M	S	M	S	M	S	M	S	M	S
10	20	17	3	18	2	15	5				
100	20	15	5	20	0	17	3	18	2	3	17
1000	20	20	0	20	0	20	0				

M = Mortality and S = Survival.

Table 3. Determination of LC_{50} of fungal extract.

S/N	Fungal Isolates	LC50 values (µg/ml)	Level of toxicity
1	*Thysanophora penicilloides*	1.16	highly toxic
2	*Aureobasdium pullulans*	0.85	highly toxic
3	*Sporangia spp*	0.24	highly toxic

NB: The LC_{50} was evaluated at 95% CI using probit analysis.

Statistical analysis

Statistical Package for Social Science (SPSS) version 23 was used for data analysis. Analysis of variance (ANOVA, one-way) was employed to test the statistical difference of the extract; the Tukey test was used to test for significant difference between two groups. Mean Significance was determined at 95% confidence interval (CI), and all results were expressed as Mean ± SD. P-values less than 0.05 were considered statistically significant.

RESULTS

Fungal species isolated from plant samples

Table 1 shows the species of fungi isolated from the plant samples. *Thysanophora penicilloides and Aureobasidium pullulans* were isolated from *Moringa oleifera* stem and leaves, respectively, while *Sporangia* were isolated from *Vachellia nylotica* leaf.

Impacts of ethanolic extracts of *Thysanophora penicilloides* and *Aureobasdium pullulans* and *Sporangia spp* on infectivity of brine shrimp larvae, as shown in Table 2, the ethanolic extracts of *Thysanophora penicilloides, Aureobasdium pullulans* and *Sporangia spp* had ceased motility of the brine shrimp larvae within 48 hours at 1000, 100 and 10 µL. However, the positive control caffeine immobilised the motility of brine shrimp larvae within 24 hours, respectively, whereas in the untreated control (seawater) survival continues for more than 72 hours.

All extracts of fungi tested were observed to be highly toxic based on Clarkson's criteria, as seen in Table 3.

In vitro activity of ethanolic extract of *Thysanophora penicilloides* on *T. brucei brucei*

The result on the effects of ethanolic extract of *Thysanophora penicilloides* on *T. brucei brucei* is shown in Table 4 In second, fourth, sixth and eight hours, the highest mortality of 3.75±1.50, 4.25±3, 6.25±1.50 and 7.00±1.41 were all observed in positive control (DA), while the lowest mortality of 0.50±1.00, 0.00±0.00, 1.00±1.15 and 1.75±2.36 were observed in the negative control, over the same time intervals. However, in the second, fourth and sixth hours, there were statistically significant differences ($p>0.05$) in mortality counts between the positive control and all levels of the concentrations of the extract. But at the time of the eighth hour, there were no statistically significant differences ($p>0.05$) between the positive control and concentrations of the extract due to the high mortality values of 5.50±1.73 observed, even though only at a high level of concentration, which is 1.0 ml.

Table 4. In *vitro* activity of *Thysanophora penicilloides* extract on *T. brucei brucei*

Concentration (mg/ml)	Times (hours)				
	1st	2nd	4th	6th	8th
1.0	0.00±0.00	0.00±0.00^{a}	0.25±0.50^{a}	4.00±0.82^{b}	5.50±1.73^{bc}
0.75	0.00±0.00	0.00±0.00^{a}	0.75±1.50^{a}	1.25±1.50^{a}	2.25±2.52^{a}
0.50	0.00±0.00	0.00±0.00^{a}	0.75±1.50^{a}	2.25±0.50^{ab}	1.75±2.36^{a}
0.25	0.00±0.00	0.25±0.50^{a}	0.00±0.00^{a}	2.25±0.96^{ab}	2.00±1.63^{a}
0.10	0.00±0.00	0.00±0.00^{a}	0.75±1.50^{a}	2.25±0.06^{ab}	2.50±1.00^{a}
+ve Cont. (DA)	0.00±0.00	3.75±1.50^{b}	4.25±3.10^{b}	6.25±1.50^{c}	7.00±1.41^{c}
-ve Cont.	0.00±0.00	0.50±1.00^{a}	0.75±0.50^{a}	1.00±1.15^{a}	2.75±2.06^{ab}

n = 3. Values in each column having the same superscript are not significantly different (p > 0.05). abc = superscript.

Table 5. *In vitro* activity of *Aureobasdium pullulans* extract on *T. brucei brucei*

Concentration (mg/ml)	Times (hours)				
	1st	2nd	4th	6th	8th
1.0	0.00±0.00	0.00±0.00^{a}	0.00±0.00^{a}	2.50±1.91^{a}	6.00±2.94^{b}
0.75	0.00±0.00	4.00±3.74^{a}	1.75±2.36^{ab}	6.25±2.23^{b}	9.00±1.63^{c}
0.50	0.00±0.00	3.25±3.99^{a}	0.00±0.00^{a}	7.50±1.29^{a}	9.50±1.73^{c}
0.25	0.00±0.00	3.75±1.71^{a}	3.50±4.51^{bc}	7.00±0.82^{b}	10.07±0.96^{c}
0.10	0.00±0.00	3.75±3.86^{a}	3.50±1.29^{bc}	8.25±2.75^{b}	8.50±2.08^{bc}
+ve Cont. (DA)	0.00±0.00	4.00±1.83^{a}	6.00±0.83^{c}	9.00±0.82^{b}	9.75±1.71^{c}
-ve Cont.	0.00±0.00	2.50±1.73^{a}	0.75±0.96^{ab}	3.00±1.41^{a}	3.00±0.82^{a}

n = 3. Values in each column having the same superscript are not significantly different (p > 0.05). abc = superscript.

In vitro activity of ethanolic extract of *Aureobasidium pullulans* on *T. brucei brucei*

The result of the *in vitro* effects of *Aureobasidium pullulans* extract on *T. brucei brucei* is shown in Table 5. After one hour of observation, no mortality count in all the concentrations of the extract, including the negative and positive controls. Although the high mortality values of 4.00±1.83, 6.00±0.83, 9.00±0.75 and 10.07±0.96 were observed in extracts at both hours, there are no significant differences ($p<0.05$) between the positive controls (DA). While the lowest mortality of 0.00±0.00, 0.00±0.00, 2.50±1.91 and 3.00±0.82 were observed in 1.0 mg/ml concentration at both hours, except in eight hours, which is in the negative control. According to statistical analysis, the *Aureobasidium pullulans* extract has some effects on trypanosomes.

In vitro activity of ethanolic extract of *Sporangia spp* on *T. brucei brucei*

The result of the effects of the extract of *Sporangia spp* on *Trypanosoma brucei brucei* is shown in Table 6. The observation of the mortality started one hour after inoculation. The highest mortality values of 6.25±1.50, 6.25±0.95, 8.00±1.63 and 8.50±1.29 were all observed in the concentration of the extracts, particularly at 1.0 mg/ml concentrations, while the lowest mortality value of 0.00±0.00, 0.50±0.57, 0.75±0.95 and 2.75±2.06 was observed in the negative control. Thus, according to the statistical data analysis, there were significant differences ($p<0.05$) between the positive control and both levels of concentration of the extracts, especially at fourth, sixth and eighth hours, respectively. Even at a lower level of the extract, which is 0.25 mg/ml, concentrations of the extract show higher mortality than the positive control, which is DA. This shows that the *Sporangia spp* extract has the effect on *Trypanosoma brucei brucei*.

DISCUSSION

It is exciting that the extracts have effects like the standard drugs; both the extract and standard drugs cured the experimental infection. Despite the fact that the standard drugs have a high percentage of trypanocidal activity when compared to both the extracts of *Thysanophora penicilloide* and *Aureobasidium pullulans*, the extracts show effectiveness on trypanosomiasis, especially at high concentrations of 1.0, 0.75 and 0.25, respectively, though only at the maximum time of sixth and eighth hours,

Table 6. *In vitro* activity of *Sporangia spp* extract on *T. brucei brucei*.

Concentration (mg/ml)	Times (hours)				
	1st	2nd	4th	6th	8th
1.0	0.00±0.00	6.25±1.50^{b}	6.25±0.95^{b}	8.00±1.63^{c}	8.50±1.29^{c}
0.75	0.00±0.00	2.00±1.83^{a}	6.25±2.75^{b}	7.75±2.06^{c}	7.50±2.08^{c}
0.50	0.00±0.00	2.25±1.26^{a}	6.00±0.81^{b}	6.25±0.95^{bc}	7.75±2.06^{c}
0.25	0.00±0.00	1.25±2.50^{a}	2.25±2.06^{a}	6.25±2.50^{bc}	5.75±2.50^{bc}
0.10	0.00±0.00	0.00±0.00^{a}	0.50±1.00^{a}	0.75±0.95^{a}	4.25±0.95^{ab}
+ve Cont. (DA)	0.00±0.00	0.75±0.96^{a}	2.50±1.29^{a}	4.00±1.41^{b}	5.75±0.95^{bc}
-ve Cont.	0.00±0.00	0.50±1.00^{a}	0.50±0.57^{a}	1.00±1.15^{a}	2.75±2.06^{a}

n = 3. Values in each column having the same superscript are not significantly different (p>0.05). abc = superscript.

Table 7. Phytochemicals constituent of *Thysanophora penicilloides* extract.

Secondary metabolites	*Thysanophora penicilloides*
Saponin	+
Steroid	+
Alkaloid	+
Flavonoid	-
Phenol	-

respectively. However, this is what captivates the conducting of the Blood Incubation Infectivity Test (BIIT) to assess and validate the accuracy and efficacy of the extracts. In this study, although the doses of 1 mg/kg, 0.75 mg/kg and 0.50 mg/kg of the prepared extract produced poor efficacy, the life span of the rats was prolonged, but they died on days 14 and 15 post-infection, respectively. The study revealed that the extracts have less effect due to the low quantity of the extract when compared to the work of Abakpa *et al.* (2013), who reported that the doses of 100 mg/kg, 300 mg/kg and 500 mg/kg of fungal extract from *Moringa oleifera* produced poor efficacy. Although the life spans of the rats were prolonged, they died on days 15, 20 and 19 post-infection, respectively. Thus, the lives of the rats that were prolonged in this study could have probably been due to the effect of saponin, alkaloid and steroid, which are scavengers of free fatty acids, which are the initiators of the pathological effects of trypanosomiasis. However, it appears reasonable to speculate that this extract may belong to the group that acts by static action affecting the growth and multiplication of trypanosomes rather than eliminating them altogether (Atawodi *et al,* 2010).

The *in vitro* antitrypanosomal activity with the ethanol extract of *Sporangium spp,* which immobilised the motility of trypanosomes within 2-8 hours at 1.0 mg/ml concentration, is comparable with *Diminazine aceturate,* which had shown a similar effect within 6 and 8 hours of incubation, respectively. In addition, the activity of ethanol extract of *Sporangium spp* at 0.75 and 0.50 mg/ml concentration reduced motility within 6 and 8 hours, respectively. This shows that the result of this study indicates the ethanol extract of *Sporangium spp* has efficacy and cures trypanosomiasis when compared to the work of Ogbadoyi *et al.* (2011), who used the fungal extract of *A. nilotica* at a dose of 50 mg/kg body weight and acted faster, clearing parasites from circulation within two days. And he suggested the reduction of the quantity of the extract due to high toxicity detected while conducting his research work, and finally concluded that the methanol fungal extract of the stem bark of *A. nilotica* cures experimental African trypanosomiasis in mice, and it is potentially useful as an anti-sleeping sickness agent. It can also be compared with the work of Margia (2015), while conducting *in vitro* antitrypansomal activity of methanolic extract of *Clutia absyssinica,* which at effective concentration of 4 mg/ml inhibited motility of *T. brucei brucei* within 50 minutes of incubation.

However, the *in vitro* anti-trypanosomal activities of *Sporangium spp. were* not confirmed by the blood incubation infectivity test due to the high efficacy shown. It has been suggested that complete immobility of the parasite *in vitro* may not necessarily indicate that the parasites were dead, but rather that the parasites may have lost their infectivity (Yusuf *et al.,* 2012). The parasite might have recovered and become infective at a time in contact with suitable physiological conditions. Prolongation of the period of animal inoculated with the *in vitro* mixtures containing a lower concentration of ethanolic extract of *Thysanophora penicilloides* and *Aureobasidium pullulans* is in agreement with the findings of Feyera *et al.* (2014).

Conclusion

This study evaluated in vitro antitrypanosomal activity of *Thysanophora penicilloides, Aureobasidium pullulans* and *Sporangia spp* extract on *T. brucei brucei*. The high concentration of 1.0 mg/ml of the ethanolic extract of *Sporangium spp.* showed potential *in vitro* activity than *Thysanophora penicilloides* and *Aureobasidium pullulans*

by immobilised motility of trypanosomes within 2, 4, 6 and 8 hours, respectively, and abrogated infectivity of trypanosomes to rats, which remained aparasitaemic for several days after the inoculation of the *in vitro* mixture. Generally, the current study established that *Sporangium spp* have potential antitrypanosomal activity, which can be considered as a potential source for new drugs in chemotherapy of Humana African trypanosomiasis. However, based on this study, fungal extracts of *Thysanophora penicilloides and Aureobasidium pullulans* have no trypanocidal effect but has the potential to prolong the life span of rats due to its antioxidant activities.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Abakpa, S. A. V., Daramola, O. O., Takeet, M. I., Akande, A. F., & Oyewusi, J. A. (2013). Anti-trypanosomal effect of the aqueous extract of moringa oleifera leaf and associated haematological changes in rats infected with *Trypanosoma brucei*. *International Journal for Agro Veterinary and Medical Sciences*, *7*(4), 135-141.

Araújo, I. F., Marinho, V. H. D. S., Sena, I. D. S., Curti, J. M., Ramos, R. D. S., Ferreira, R. M., Souto, R. N., & Ferreira, I. M. (2022). Larvicidal activity against Aedes aegypti and molecular docking studies of compounds extracted from the endophytic fungus Aspergillus sp. isolated from Bertholletia excelsa Humn. & Bonpl. *Biotechnology Letters*, *44*(3), 439-459.

Assefa, S. (2017). Potential anti-trypanosomal plants against African animal trypanosmiasis. *Journal of Pharmacognosy and Phytochemistry*, *6*(5), 77-85.

Atawodi, S. E., & Shehu, H. (2010). Antitrypanosomal property of some extracts of different parts of Moringa oleifera, Lam. *Electronic Journal of Biology*, *6*(1), 19-23.

Feyera, T., Terefe, G., & Shibeshi, W. (2014). Evaluation of in vivo antitrypanosomal activity of crude extracts of Artemisia abyssinica against a Trypanosoma congolense isolate. *BMC Complementary and Alternative Medicine*, *14*, 117.

Kar, A., Choudhary, B. K., & Bandyopadhyay, N. G. (2003). Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *Journal of ethnopharmacology*, *84*(1), 105-108.

Ogbadoyi, E., Garba, M., Kabiru, A., Mann, A., & Okogun, J. (2011). Therapeutic evaluation of *Acacia nilotica* (Linn) stem bark extract in experimental African trypanosomiasis. *International Journal of Applied Research in Natural Products*, *4*(2), 11-18.

Peiris, S., Fernando, D. T. K., Senadeera, S. P. N. N., & Ranaweera, C. B. (2023). Phytochemical screening for medicinal plants: guide for extraction method. *Asian Plant Research Journal, 11*(4), 13-34.

Petrini, O. (1986). Taxonomy of endophytic fungi of aerial plant tissues. In: Fokkema, N. J., van den Huevel, J. (eds). Microbiology of the phyllosphere (pp. 175-187). Cambridge, UK: Cambridge University Press.

Saldanha, I., Betson, M., Vrettou, C., Paxton, E., Nixon, J., Tennant, P., Ritchie, A., Matthews, K. R., Morrison, L. J., Torr, S. J., & Cunningham, L. J. (2024). Consistent detection of Trypanosoma brucei but not T. congolense DNA in faeces of experimentally infected cattle. *Scientific Reports*, *14*, 4158.

World Health Organisation (WHO). WHO interim guidelines for the treatment of gambiense human African trypanosomiasis. World Health Organisation. Retrieved from https://www.who.int/publications/i/item/9789241550567.

Yusuf, A. B., Umar, I. A., Musa, U. B., & Nok, A. J. (2012). Screening of Vernonia amygdalina and Hymenocardia acida extracts and 1, 3-diaminopropane for their antitrypanosomal activities: in vitro model. *Journal of Medicinal Plants Research*, *6*(19), 3573-3578.