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IN VITRO ANTITRYPANOSOMAL ACTIVITY OF METHANOL FUNGAL EXTRACTS OF *ASPERGILLUS FUMIGATUS* AND *ASPERGILLUS NIGER* AGAINST *TRYPANOSOMA BRUCEI BRUCEI,* (FEDERE STRAIN)

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ABSTRACT

The anti-trypanosomal activity of fungal extracts against *Trypanosoma spp*. is reported. *Aspergillus niger* and *Aspergillus fumigatus* isolated from rice bran were cultured on potatoes dextrose agar. Each fungal moss was separately harvested, and extracted in methanol. The respective fungal extracts were evaluated for *in vitro* anti-trypanosomal activity against *T. b. brucei*, Federe strain, at test concentrations ranging from 15.625µg/ml to 1000 µg/ml. The results of the study showed that methanolic fungal extracts exhibited significant in vitro anti-trypanosomal activity comparable to Berenil™ (Diminazene aceturate). The minimum trypanocidal concentrations (MTC) for *A. fumigatus*, *A. niger* extracts and Berenil were 125 µg/ml, 250 µg/ml, and 500 µg/ml, respectively, six-hour post-incubation; the respective median inhibitory concentrations (IC_{50}) were 44.79 ± 5.32 µg/ml, 49.86 ± 0.038 µg/ml, and 124.49 ± 29.3 µg/ml. It may be inferred that the fungal extracts contain chemical moieties with cidal effects against African trypanosomes, and could be potential sources of drug for the treatment of trypanosome infection.

Keywords: *In Vitro, Aspergillus niger, Aspergillus fumigatus, Trypanosoma brucei brucei, African trypanosomiasis*

INTRODUCTION

African trypanosomiasis is still considered a cause of underdevelopment in the sub-Saharan African. Treatment options for trypanosome infections in both human and livestock is limited, relying on a handful of drugs. While efforts at developing more effective drugs are ongoing, they have largely been directed at screening of chemical libraries and plant secondary metabolites. The chemotherapy of human and animal African trypanosomiasis has largely relied on a few repertoires of drugs, some of which are associated with several clinical limitations (Giodani *et al*., 2016; Solokova *et al.*, 2010; Vincent *et al*., 2010; Wenzler*et al*., 2013). These shortcomings have prompted research into newer and more effective anti-trypanosomal agents, usually involving the screening of large libraries of chemically synthesized compounds for potential lead moieties (Gong *et al*., 2017; Jamshidi-Kia et al., 2018; Medina-Franco, 2012; Prakash and Devangi, 2010). Plants have been known to synthesize diverse groups of chemical compounds with proven medicinal properties (Guerriero*et al*., 2014; Kabera*et al*., 2014). In fact, some therapeutically useful drugs are of plant origin (Baluna and Kinghorn, 2005; Rates, 2001; Yuan *et al*., 2016). As such, secondary metabolites of plant origin have also been screened for their antitrypanosomal effects (Ibrahim *et al*., 2014; Mann and Ogbadoyi, 2012). Besides plants, fungi are an excellent source of secondary metabolites. Some of the interesting compounds produced by endophytic microbes are cryptocin, cryptocandin, jesterone, oocydin, isopestacin, the psuedomycins and ambuic acid (Lakshmi and Selvi, 2013). The anticancer drug Taxol has be found to be expressed by certain species of fungi *Taxomycesandreanae* (Boruta, 2018). Our survey of literature indicated that there has been only very few investigations into the antitrypanosomal activity of secondary metabolites of fungal origin. It is therefore the object of this study to evaluate the antitrypanosomal activity of the methanol extracts of two endophytic fungi, *Aspergillus niger* and *A. fumigatus* isolated from rice bran.

MATERIALS AND METHOD

Rice bran sourced from the rice milling factory located in Otukpo, (Benue State of Middle Belt Nigeria) and packaged in dry, air- tight containers was transferred to the Parasitology laboratory of Nigerian Institute for Trypanosomiasis, Kaduna.

Isolation and identification of fungi: Two endophytic fungi were isolated from rice bran in accordance with two previous studies of Rajan and Nair (2011) who isolated *A. fumigatus* from aged rice bran, Anupama and Ravindra (2001) and Stephen *et al*., (2018) who also isolated *A. niger* from dry wood and rice husks respectively. The fungal species were isolated from rice bran as follows. To surface-sterilize the bran, 10 grams of rice bran was rinsed in sterile water, and then soaked in 70% ethanol for 30 s, 2.4% sodium hypochlorite solution for 4 min and then 70% ethanol for 30s. Finally, the bran was washed (3x) in sterile distilled water for 1 min (Kaanichee*t al*., 2019;Edor*et al*., 2018).

After surface sterilization, the rice bran was aseptically placed in petri dishes containing potato dextrose agar (PDA), supplemented with chloramphenicol (250 mg/L) to inhibit bacterial growth. The plate was incubated at room temperature (RT, 25 °C) for five days. The lacto phenol cotton blue staining method was used to identify the resulting fungal colonies. To obtain pure cultures, mycelium from each fungal colony was transferred into separate PDA slants (Sudhakar*et al.*, 2017).

Fermentation and Extraction: Inoculums of *A. niger* and *A. fumigatus* were separately introduced into ten 1-L flasks containing sterilized PDA broth, five flasks per species. The flasks were incubated at RT for 21 days, after which the respective fungal mycelia were recovered from the broth, air dried and later macerated in methanol for 24 hr. The resultant filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator to obtain the crude methanol extracts (Hastuti*et al*., 2008).

***In vitro* anti-trypanosomal screening**: screening of the fungal extracts for *in vitro* anti-trypanosomal activity was conducted in 96 well micro plate according to the method of Bulus *et al.* (2012). RPMI 1640 medium (Caisson Laboratory, USA), supplemented with 10% (v/v) heat inactivated horse serum, gentamycin (40µg/mL, and 1% (w/v) glucose was used to reconstitute the extract to yield concentrations of the extracts ranging from 1000 µg/ml and 15.625 µg/ml. 100µL of the reconstituted solutions of each extract was separately dispensed in triplicate into wells of the titre plate. 30µLof the blood suspension containing *T. b. brucei* was added to each of these wells and gently mixed together. The trypanosome density after addition of the blood to medium was adjusted to 20 trypanosomes per field. Control wells containing only 100µLsupplemented medium and 30µL of the blood suspension were also included. The micro plate was place in a desiccator containing about 5% carbon dioxide and maintained at 37°C in an incubator. Wet smears were prepared from each well 6 hours post-incubation. Each smear was examined in a light microscope (x400 magnification) and the counts of motile trypanosomes were taken over three fields of view, a total of nine observations per concentration of extract. Similarly, counts sof motile trypanosomes were also taken for smears prepared from the control wells.

Data analysis:

The results were analyzed using the Statistical Package for Social Science (SPSS) version 2.0 software. Descriptive data are presented as mean ± standard error of mean of trypanosome counts. Values are given as mean ± SEM (standard error of mean). In each column, values with different superscripts have statistically significant difference .The mean trypanosome count per concentration of extracts were compared using one-way analysis of variance (ANOVA) and student paired *t* test at significance level of $p = 0.05$.

Results

Table 1 shows the effects of graded concentrations of the methanol extracts of *A. niger* and *A. fumigatus* against *Trypanosoma brucei brucei* (Federe strain), 6-hour post-incubation. The results indicated that the methanol extracts significantly ($p < 0.05$) reduced the number of surviving trypanosomes in a concentration dependent manner when compared to number of surviving trypanosomes in the negative control wells. Furthermore, it was observed that at higher concentrations, *A. fumigatus* extract (AFE) demonstrated higher anti-trypanosomal activity than the *A. niger* extract (ANE), with 0% survival 100% mortality) recorded between 125 and 1000 µg/ml concentrations of AFE; for ANE, 0% survival of trypanosomes were seen between 250 and 1000 µg/ml concentration. At lower concentrations, however, ANE demonstrated significant activity against trypanosome survival compared to AFE. The reference drug Diminazene aceturate (DM) also demonstrated significant anti-trypanosomal activity, *in vitro*. The observed effects of the drug on the number of surviving trypanosomes was also in a concentration dependent pattern with increasing concentrations resulting in decreasing number of survival. A survival rate of 0% (100% mortality) was achieved at 1000 and 500 µg/mL of DM. Thus the minimum trypanocidal concentrations (MTC) of DM, ANE and AFE were 500, 250 and 125 µg/ml, respectively.

Table 1: Showing mean number of surviving trypanosomes six hour post incubation in methanol extracts of *A. niger* and *A. fumigatus*

	Mean trypanosome count/field		
Concentration (µg/ml)	*A. niger*	*A. fumigatus*	Diminazene aceturate
1000	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
500	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	**0.00 ± 0.00^{a}**
250	**0.00 ± 0.00^{a}**	**0.00 ± 0.00^{a}**	1.00 ± 0.447^{a}
125	0.17 ± 017^{a}	0.00 ± 0.00^{a}	7.17 ± 0.980^{b}
62.5	0.83 ± 0.167^{b}	1.17 ± 0.307^{a}	8.50 ± 0.671^{b}
31.25	0.83 ± 0.167^{b}	6.17 ± 0.401^{b}	9.83 ± 0.401^{b}
15.625	3.17 ± 0.477^{c}	11.5 ± 0.719^{c}	15.5 ± 0.619^{c}
Control	19.3 ± 0.715^{d}	19.3 ± 0.715^{d}	19.3 ± 0.715^{a}
p value	< 0.05	< 0.05	< 0.05

Values are given as mean ± SEM (standard error of mean). In each column, values with different superscripts have statistically significant difference ($p< 0.05$)

DISCUSSION

Results of the *invitro* assay showed that the methanol fungal extracts exhibited significant ($p < 0.05$) anti-trypanosomal activity which is collaborated by Rajan and Nair (2011) who isolated *A. fumigatus* from aged rice bran, Anupama and Ravindra (2001) and Stephen *et al*., (2018) who also isolated *A. niger* from dry wood and rice husks respectively. The observed *in vitro* anti-trypanosomal effects of the methanol extracts of *A. niger* and *A. fumigatus* could be attributed to the presence of bioactive secondary metabolites. Endophytic fungi have been reported to expresses a diverse array of medically important secondary metabolites (Hao*et al*., 2016). The distribution of metabolites in organic materials may differ both qualitatively and quantitatively. This may account for the higher anti-trypanosomal activity seen with the *A. fumigatus* extract. However, the reduced activity observed with lower concentrations of the *A. fumigatus* methanol extract when compared with the *A. niger* extract is indicative of a qualitative rather than a quantitative difference in the secondary metabolites. The results of this study, therefore, indicate that the methanol extracts of *Aspergillus niger* and *Aspergillus fumigatus* possess significant anti-trypanosomal activity comparable with the trypanocide Diminazene aceturate, *in vitro.*

CONCLUSION

The results of the present study indicate that methanol extracts of endophytic fungi (*A.fumigatus* and *A. niger*) demonstrated significant activity against *T. b. brucei*, *in vitro*, with lower minimum trypanocidal concentrations when compared with the trypanocide, Diminazene aceturate. Thus the tested fungi extracts can be considered as possible replacements for the more expensive, less available and synthethic trypanocides presently in use,

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