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Anti Trypanosomal Activity of Crude Extracts Of Endophytic Fungi (Acremonium Sp.) Isolated From Ocimum Gratisimum Leaf.

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Abstract*: Trypanosomiasis is a parasitic disease that affects both humans and animals. It is caused by the parasitic protozoan, trypanosome,. The development of a vaccine for the treatment of the disease is becoming increasingly complicated due to the problem of antigenic variation, thus the reliance on chemotherapeutic agents to control the disease.Drugs derived from natural sources play an important role in the avoidance and treatment of human diseases.Recent studies have shown a variety of health properties of endophytic fungal extract. The ethylaceted and the methanolic extraction were carried out. The bioactive fungal extract was tested invitro on Trypanosoma brucei brucei (federe strain). The in vitro assessment of Acremonium sp extract show graded trypanocidal activity at different concentrations with LC_{50}of0.204, as indicated in the table of percentile. It also shows that as the concentration Increase the mortality also increases.*
Key words*Trypanosome Endophytic Brucei Acremonium*

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I. Introduction

Agriculture is the mainstay of many economics and it is fundermental to the socioeconomic development of a nation, because it is a major element and factor in national development (Ahmed 1993). The majority of the disease burden faced is from infection with endemic diseases, in particular African Animal Trypanosomiasis (AAT), tick borne diseases and helminthiases, all of which decrease production and increase morbidity and mortality.

Trypanosomiasisis one of the major problems of animal health, and the cause of about 3million deaths each year with 20% less in calving, 25% reduction in milk yields and 50% reduction in livestock numbers which leads to the reduction in work efficiency of the animals, thereby causing hindrance in crop production (Swallow, 2000). African trypanosomiasis is a parasitic disease that affects both humans and animals. It is caused by the parasitic protozoan, trypanosome, a single cell flagellate which is transmitted by arthropod vectors of the genus Glossina and survives in the blood and tissues of the host. Trypanosomas rhodesiense and T. gambiense are the causative agents of sleeping sickness in humans and associated diseases in animals (Warren, 1988; Kuzoe, 1993).

The development of a vaccine for the treatment of the disease is becoming increasingly complicated due to the problem of antigenic variation (Jackson et al., 2012)Recently, researchers have shown an increased interest to discover novel anti-trypanosomal agents from plants, particularly with information obtained from ethno medicinal data, whereby a plant is selected based on prior knowledge of its use in folk medicine (Wurochekke et al., 2012) Nevertheless, recent studies have also indicated antiparasitic activity of endophytic fungi, such as Bioactive endophytic fungi isolated from Caesalpinia echinata Lam. (Brazilwood) and identification of beauvericin as a trypanocidal metabolite from Fusarium sp (Campos et al., 2015)There is a large volume of published studies describing the role of endophytic fungi in the area of research. Studies based on estimation of microbial populations have revealed that only about 1% of bacteria and 5% of fungi have been characterized and the rest remain unexplored for their contribution to the human welfare (Staley et al., 1997). The potential of microorganisms is further limited by the presence of orphan biosynthetic pathways that remain unexpressed under general laboratory conditions (De Bok et al., 2006). However, the vast choice of techniques pertaining to the growth and manipulation of microorganisms like media engineering, co-culture, chemical induction, epigenetic modulation and metabolite remodeling, coupled with the fermentation technology for scale up, make them suitable for production of useful natural products, both known and novel (De Bok et al., 2006).

Several studies have shown a variety of health properties of endophytic fungal extract such as antifungal, antiproliferative (Ibrahim et al., 2017), antimicrobial, anticancer (Jinfeng, et al., 2017) and acetylcholinesterase inhibitory activity (Wang et al., 2016). Moreover, recent studies have also indicated

antiparasitic activity, antimalarial activity (Baba et al., 2015; Campos et al., 2015; Martinez et al., 2012; Moreno et al., 2011) to mention a few. Nevertheless, the potentials of Acremonium sp and Fusarium monoliforme have not been explored, hence the relevance of this study.

II. Materials And Method

Extraction of the Fungal Isolates

The culture broth and the mycelial mass of the fungal culture were separated in different conical flask. Equal volumes of Ethylacetate were used to extract the culture broth in 500 ml separating funnel. While the mycelial mass was extracted in 200 ml of methanol in separate flasks and kept in a dark environment at room temperature for 3days.

The methanolic fraction was filtered and concentrated in rotary evaporator at 40^0C. The ethyl acetate fraction was transferred into a separating funnel and mixed vigorously. The mixture was allowed to stand until two clear immiscible layers were formed, the bottom layer containing the broth media, while the upper layer contained the Ethyl acetate. The bottom layer was discarded while the concentrated methanolic fraction was then added to the ethylacetate in a separating funnel and mixed vigorously. The content was allowed to sediment forming two layers. The bottom layer was decanted the extract was washed with a solution of the sodium chloride. This process was done five (5) times until the ethyl aceted fraction becames clear. Ten grams (10g) of Sodium sulphate (anhydrous) was mixed with the ethylacetate fraction to remove water. The extract was then filtered through a watsman filter paper (1mm) cycles and concentrated at 40 ^{0}C in a rotary evaporator. The concentrated extract was then transferred to a 250 ml beaker and covered with a perforated foil paper for the absolute evaporation of the solvent.

Serum Preparation for In vitro Assessment

Blood from a healthy male goat was obtained from the abattoir at the point of slaughter. The blood was kept overnight at 4 ^{0}C in a refrigerator. After clot retraction, the un-clarified serum was collected and centrifuged at 4000 rpm for five minutes. The clarified serum was carefully collected into a sterile container using a micropipette. The collected serum was placed in a water bath at 56 ^{0}C for 30 minutes to inactivate the serum complement proteins and later stored at -20 ^{0}C until required.

Supplementation of the RPMI medium:

The RPMI 1640 medium was supplemented with 40 µg/ml gentamycin sulphate 10 % inactivated goat serum; and 10 % glucose. The pH of the medium was adjusted to7.7. The media was freshly constituted before use.

3.7.2 Sample Preparation

Stock solutions of the fungal extracts were prepared within the range of 5 mg/ml to 0.078 mg/ml in the freshly supplemented medium and Diminazene aceturate were also prepared with the same range. Exactly 5 mg of each extract was weighed and dissolved in 1ml of culture medium; the stock solution of each extract was serially diluted in two folds to give a range of solutions of strengths ranging from 5 mg/ml to 0.078 mg/ml.

Collection of Parasitized Blood

Blood from a mouse infected with Trypanosoma brucei brucei (Federe strain) at about 10^8 trypanosomesper millilitre of blood was collected by cardiac puncture after chloroform anaesthesia. The collected blood was immediately dispensed into an EDTA container and gently mixed together to avoid coagulation. The blood was diluted with the supplemented medium (2:1).

Anti-Trypanosoma Assay

A new technique by (Herbart and Lumsden, 1976) for estimating the absolute level of parasitemias in trypanosome infections was used. At higher levels of infection this was achieved by matching microscopic fields of a wet blood film against charts and, where fewer organisms were present, by counting the number of trypanosomes in 5, 10, or 20 such microscope fields. Good estimates of the number of organisms per milliliter of blood was made rapidly over the whole range of microscopically patent parasitemia, i.e., above antilog 5.4 (250,000) organisms/ml.

One hundred microliters (100 µl) of each concentration was separately dispensed into wells of microtitre plate in triplicates. Fifty microliters (50 µl) of blood suspension containing viabletrypanosomes of about 30 was added to each well containing the fungal extract and wells containing prepared Diminazene aceturate as positive control (+control), Whereas the negative control (-control) wells contained only trypanosomes and the supplemented medium.

Microscopic assessment of in vitro activity of the fungal extract

Assessment of invitro anti-trypanosomal activity of each extract was performed in triplicate wells of microtitre plates. After six hours post-incubation, wet smears of the blood mixture from each well was prepared and examined under the light microscope at x400 magnification. Trypanosome count was taken for each smear

over three fields of view; motility of the parasites was also assessed relative to the trypanosome motility in the control well.

The percentage mortality of trypanosomes in each of the treatment well was calculated relative to trypanosome count in the control wells.

III. Result And Discussion

Generally, the results of the in vitro assessment of Acremonium sp. xtracts on Trypanosoma brucei brucei (Federe strain) show graded trypanocidal activity at different concentrations. Total elimination of parasites was observed at 5.0 mg/ml, 2.5 mg/ml, and 1.25 mg/ml of Acremonium sp extract as compared to positive control (Diminazene) which showed total elimination only at 5.0 mg/ml (Table 3). Percentage mortality increased with increase in concentration of the extract. The remaining concentrations 0.3125, 0.15625, 0.078125 showed the parasites were active (Table 3). The reverse was observed in Diminazene with presence of parasites in all concentrations except at 5.0 mg/ml were no parasite was observed. All parasites were active for negative control at 5.0 mg/ml- 0.07 mg/ml concentrations. The result also indicates that at lowest concentration of 0.625 mg/ml, a signicant difference was observed between the fungal extract and Diminazine. thus indicating higher trypanocidal activity six hours post incubation.The single most striking observation to emerge from the results was that the trypanocidal activity of Acremonium sp extract at 2.5 mg/ml concentration shows a complete elimination of the parasite, while at the same concentration in diminazene aceturate (A standard drug for treatment of trypanosomiasis) some parasite were seen. The lethal concentration (LC_{50}) of Acremonium sp. was 0.022 and for diminazine aceturatewas 1.097 mg/ml. it also shows that as the concentration Increase the mortality also increases.

Table 1 Trypanocidal activity of Acremonium sp and Fusarium monoliforme on Trypanosoma brucei brucei (federe strain) 6 hr. post- incubation

Concentration (mg/ml)	FUNGAL EXTRACTS Acremonium sp	DIMINAZENE +ve control	CONTROL -ve
5.0 W1			
W2	0, 0, 0	0, 0, 0	29, 30,28
W3	0, 0, 0	0, 0, 0	25,27,28
	0, 0, 0	0, 0, 0	30,30,27
2.5			
W1	0, 0, 0	1, 1, 1	26,27,23
W2	0, 0, 0	3, 1, 1	24,27,24
W3	0, 0, 0	0, 1, 0	27,26,22
1.25			
W1	0, 0, 0	3, 4, 2	24,25,27
W2	0, 0, 0	4, 3, 5	28,29,27
W3	0, 0, 0	2, 3, 2	28,29,25
0.625			
W1	1, 0, 0	5, 6, 2	25,26,24
W2	0, 0, 0	6, 8, 2	26,24,25
W3	0, 0, 0	3, 4, 3	24,26,24
0.3125			
W1	1, 1, 1	8, 9, 7	26,24,27
W2	1, 0, 1	8, 9, 9	24,26,27
W3	1, 1, 1	7, 6, 7	30,18,28
0.15625			
W1	4, 5, 4	11, 13, 9	28,22,19
W2	4, 6, 3	12, 12, 8	25,27,28
W3	3, 4, 4	9, 11, 9	24,25,29
0.078125			
W1	3, 5, 4	11, 12, 11	26,28,27
W2	3, 4, 5	11,10, 7	28,25,29
W3	3, 2, 3	8, 8, 9	26,29,30

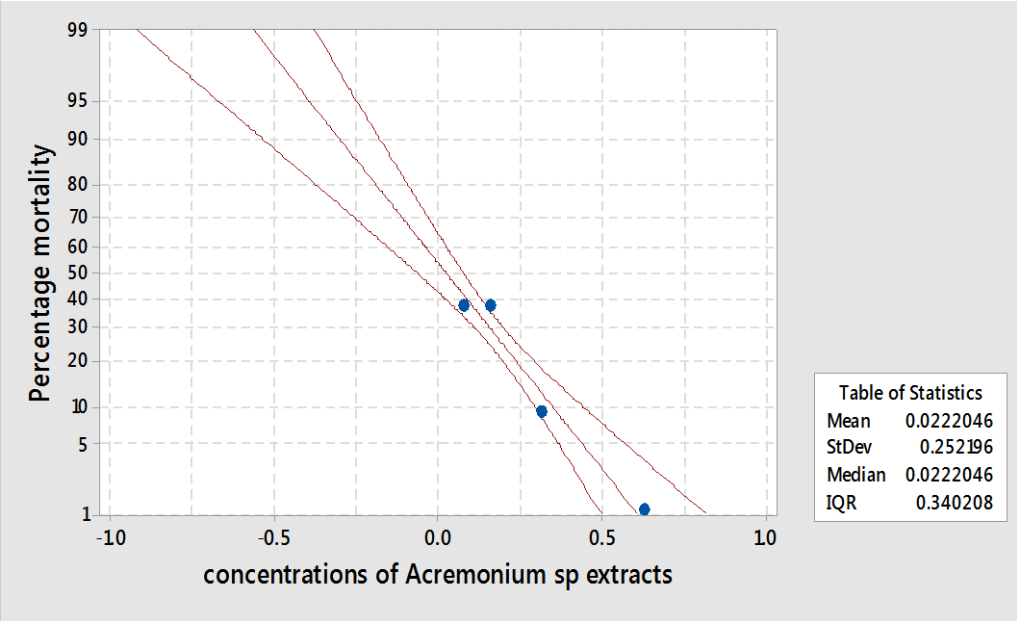

Figure 1 PROBABILITY PLOT FOR NUMBER OF MORTALITY OF T. BRUCEI BRUCEI IN ACREMONIUM SP EXTRACT

Figure 5 shows the probability plot for percentage mortality while exposure to different concentrations of Acremonium sp extract. The Lc_{50} for Acremonium sp was found to be at 0.02 mg/ml. the upper and the lower limits as presented in the graph show that the risk estimates for exposure at 0.02 mg/ml for Acremonium sp are precise and lie between 0-0.02 quarters. When the IQR value are between 0- 100 are precise.

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