International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **14(1),** 1-7, February **(2025)** *Int. Res. J. Biological Sci.*

The in -vitro activity of an extract derived from selected Endophytic fungal species isolated from *Psidium guajava* against Trypanosoma Brucei Brucei

Hussaina Sani Baba[1*], Faruk Sani Nas[1], Ahmad Kabir Maigari[1] and Salisu Abubakar[1,2]
[1]Department of Biological Science, College of Natural and Pharmaceutical Sciences, Bayero University, P.M.B. 3011, Kano, Kano State
[2]Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, P.M.B. 186, Garki, Abuja, Nigeria
hussainasanibaba@gmail.com

Available online at : www.isca.in, www.isca.me
Received 20th January 2024, revised 13th June 2024, accepted 12th September 2024

Abstract

The research focused on the isolation and identification of endophytic fungi, as well as the assessment of the potency of secondary metabolites produced by the isolates from Psidium guajava L. (leaves and stems) against Trypanosoma brucei brucei. A range of endophytic fungal species were isolated using modified surface sterilisation techniques. A random selection of three isolates was made in order to analyse their produced secondary metabolites. Phytochemical screening was carried out using standard procedures on ethylacetate and methanolic extract (50:50v/v). The extracts were also subjected to in vitro anti-trypanosomal activity against Trypanosoma brucei brucei at different doses ranging from 10 mg/ml to 0.15625 mg/ml.In addition the brine shrimp fatality assay was used to assess the extracts' toxicity tests. The presence of steroids, tannins, saponins, flavonoids, phenols, terpenoid, and alkaloids was shown by the phytochemical data. The results of the in vitro antitrypanosomal activity showed that the extracts significantly reduced the concentrations of surviving trypanosomes six hours after incubation when compared to the number in the negative control wells. The Aspergillus nidulan extract had the maximum activity among the studied extracts, with no surviving parasites at 10 mg/ml, 5 mg/ml, 2.5, and 1.25 mg/ml. In comparison to the reference standard, potassium dichromate (LC50 0.80µg/ml), the Brine Shrimp lethality assay showed that extracts of A. nidulan, Fusarium moniliforme, and Rhizoctonia sp. were less toxic to brine shrimp (LC_{50} 206µg/mL, 173.5µg/mL, and 110µg/mL). The statistical package for social science (SPSS) version 2.0 was used to analyse the mean survival rate using two-way Analysis of Variance ($p<0.05$). This discovery offered empirical evidence that endophytic fungus of Psidium guajava generate a profusion of non-toxic medicinal chemicals, much like their host plants.

Keywords: Brine shrimp, Endophytic fungi, *Trypanosoma brucei brucei*, Phytochemical, *Psidium guajava.*

Introduction

Trypanosomiasis is an African important unicellular blood protozoan parasitic disease of both humans and animals[1]. The disease in animal is commonly called *Nagana* in Zulu land which means powerless, useless, or depressed spirits[2]. It is mainly transmitted cyclically by tsetse fly of genus *Glossina* and mechanically by other biting flies[3]. Different species of trypanosomes infects cattle and other ruminants. These include *Trypanosoma brucei brucei, Trypanosoma congolense and Trypanosoma vivax.* The two species infective to humans are *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* in humans World Health Organization[4]. Trypanosomiasis is one of the most prevalent and economically significant diseases of domestic animals in the family Bovidae Food and Agricultural Organization[5]. The control of Human African Trypanosomiasis (HAT) continues to rely principally on old expensive medicines such as pentamidine, nitrofurans and arsenicals[6,7]. In recent times, drug development has led to the production of eflornithine (DMFO) as the only new trypanocide in the last fifteen years and it is only effective in the last stage of Gambian sleeping sickness and its regimen is complex and difficult to apply[8].

After continued effort especially in the control of the fly vector, there has been decline in number of new cases. The number of Human African Trypanosomiasis (HAT) case reported in 2012 is about 6,314 new cases[4].

However, the projected population at risk is 65 million, although the anticipated number of actual human cases is only about 20,000[4]. Trypanocidal medications, which are costly and cause resistance, have formed the foundation of control tactics, whereas vector control techniques have shown to be environmentally unsustainable[9]. At the moment, trypanocidal medications remain the mainstay of trypanosomiasis control. However, these programs are limited by factors such as toxicity, low clinical efficacy, and drug resistance[10].

This highlights how important it is to find novel therapeutic and preventative medicines in order to manage the illness. The need for strong medications with antitrypanosomal action derived from plants has arisen in order to tackle resistance to chemotherapy treatments. In vitro activity of certain isolated endophytic fungal crude extracts from *Psidium guajava* on *Trypanasoma brucei brucei* were the main focus of this research.

Materials and Methods

Equipments, Sterilization, and Routine Laboratory Operations: Fungal growth media, plates and polyethene bags were sterilized by autoclaving at 121ºC for 20 minutes. Glass wares were sterilized by dry heat using oven (Memmert Electric Co., Ltd. Japan) at 180ºC for one hour. Wire loops, forceps were sterilized by flaming. Elution was sterilized by filtration through 0.22µm pore size membrane filter. In a sterile glass filtration unit using negative pressure generated by a vacuum pump. Mycological media were purchased from (Sigma–Aldrich) and prepared in accordance with manufacturer's instructions. All aseptic operations were carried out in a safety cabinet (Labconco, Missouri, USA) that had been left running with the ultraviolet rays for at least ten (10) to fifteen (15) minutes beforeuse, and analytical weighing balance (OHAUS Cop., USA) which is routinely calibrated.

Assortment of Sample and Processing: Mature healthy Plants organs, without any visible signs of disease, were collected from Old Campus, Botanical Garden, Bayero University Kano. From various sections of the tree plant, leaves, stems, and roots were collected. The samples were delivered to the laboratory on the same day, enclosed in a sterile polyethene bag, for the isolation work, fresh plant materials were employed to lower the risk of contamination. Assertion No. 36 was issued by Dr. Yusuf Nuhu of the Herbarium Unit of the Department of Plant Biology at Bayero University Kano, Nigeria, to identify and authenticate the plant.

Isolation Procedure: Fungal endophytes isolation was done under extremely sterile conditions. All the work was carried out under biosafety cabinet. The leaves stem and roots were cut into 0.5-1cm length. With slight modification, the isolation approach was employed by previous researchers[11]. The surface sterilization was carried out using sodium hypochlorite (3% NaOCl v/v) and (70% v/v) ethanol. Each set of plant materials were treated with 70% ethanol for 1 minute followed by immersion in sodium hypochlorite for 3 minutes and rinsed with sterile water. This was followed by immersion in 70% ethanol for 30 seconds and finally, rinsed with sterile water three times. The treated samples were later dried on a sterile tissue paper. The efficiency of the surface sterilization procedure was ascertained by culturing the final rinsed water followed by subsequent observation. In each Petri dish, 3-7 segments of the treated samples were placed on a solid medium PDA supplemented with Chloramphenicol (Laborate phamac.inc.), in order to retard the growth of bacteria. The plates were incubated at 25°C ± 2 for 1-4 weeks.

Sub-culturing of Endophytic Fungi: In order to obtain monoclonal culture, Sub-culturing was done by transferring hyphal tips from the master plates to Potato Dextrose Agar (PDA) plates without addition of antibiotics for proper propagation, and also to obtain monoclonal (pure) cultures for identifications[12].

Identification of selected fungal Species: The endophytic fungi isolates were identified macroscopically on the basis of their morphological and cultural characteristics on PDA media, while microscopically using staining techniques[13,12].

Fungal Macroscopic and Microscopic Identifications: The morphology of the isolates from both upper and reverse side of the cultured was identified based on the following features, such as colour (Reverse and Upper) texture, and colonial topography[12].

The microscopic morphological features were examined by one step staining technique using methylene blue. The definitive microscopic identification is based on the morphology of the spores and hyphae. Spores such as (sprongiospore, chlamydiospores microconidia and macro-conidia), characteristic shape such as (fusiform, ovoid, cylindrical, porospore and oblong), arrangement of the spores on the hyphae such as (singly, in chains, cluster, terminal, and flower-like).Hyphae: The size and shape of the hyphae are also very relevant which showed the characteristics such as; spiral and pectinate[12].

Fungal Cultivation: The Cultivation of selected fungal species was done on Potato Dextrose Broth; the actively growing pure culture blocks were placed in 250ml Erlenmeyer flask containing 100ml of the medium. The flask was incubated inside incubator shaker (annova 44 UK) at 25±2°C for 1 week with periodical shaking at 150 rpm. After the incubation period, the cultured was taken out and filtered through sterile cheese cloth to remove the mycelia mats[13,12].

Secondary Metabolites Extraction: The fungal metabolites from the identified isolate *A. nidulan*, *F. moniliforme* and *Rhizoctonia* sp was extracted using ethyl acetate/methanol as a solvent of extraction. Equal volume of the filtrate and solvents (50:50v/v) was taken and shaken vigorously for 10 minutes. Using separating funnel the mixtures was separated. The solvents of extraction were evaporated and the resultant component was dried in a water bath (Buchi waterbath B-480) at 400C to yield the crude extract[13,14.]

Phytochemical Screening of Fungal Metabolites Produced by Endophytic Fungi: The crude ethyl acetate/methanolic extract was used for phytochemical screening to analyse the presence of various secondary metabolites such as alkaloids, phenols, flavonoids, steroids, saponins and tannins[12].

Test organisms: Trypansoma brucei brucei: Cryopreserved stabilate of *Trypansoma brucei brucei* was obtained from the cryobank maintained at the Vector and Parasitology Study Department of the Nigerian Institute for Trypanosomiasis Research (NITR) Surame Road, Unguwar Rimi, Kaduna State, Nigeria. After thawing the stabilate at 37°C, trypanosomes were screened for viability by examining wet smears prepared from the stabilate in the light microscope at x400 magnification.

The presence of motile trypanosomes was taken as indication of trypanosome viability[15].

The Donor Animals: Two albino Wistar rats were utilized as donor animals. Each rat was intraperitoneally inoculated with blood suspension (inoculum) prepared from the thawed stabilize. The inoculum was prepared by the addition of normal saline to a small amount of the blood from the stabilate until the trypanosome count was 2 per microscopic field. The rats were inoculated with 0.2ml of the prepared blood suspension. Parasitemia in the inoculated rats was monitored three days after inoculation to determine the establishment of active infection. Subsequently, the level of parasitemia was determined daily until parasite count was about was 10^9 per milliliter of blood. Following infection with the trypanosomes, at which point the donor rats were sacrificed[15].

Determination of Parasitemia: The trypanosome count in the infected rats was estimated by the rapid matching method[16]. 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 placed over it; the blood spread into a thin circular film. The slide was then placed in the light microscope and examined at x400 magnification. The distribution of trypanosomes among the red blood cells (RBCs) was matched against the Lumsden's chart and the approximate number of trypanosomes per milliliter of blood estimated[15].

Collection of Parasitized Blood: Blood containing trypanosomes was collected into a 5 ml syringe from the donor rats by the cardiac puncture technique after chloroform anesthesia. The blood was dispensed into ethylene diamine tetra acetate (EDTA) sample container, and gently mixed together to prevent clotting of the blood[15].

Medium and Supplementation of Medium: RPMI 1640 medium (Caisson Laboratory, USA) containing L-glutamine and sodium carbonate was used for the *in vitro* assay. The media was further supplemented with gentamycin (40µg/L), 10% (v/v) heat inactivated goat serum and 1% (w/v) glucose[15].

Reference Drug and Reconstitution of the Plant Extracts: Solutions of the extracts were reconstituted in the supplemented medium. Initially, 100 mg/ml stock solutions of the respective extracts subsequently, the respective stock solutions were serially diluted to yield extracts with concentrations ranging from 10 mg/ml to 0.3125 mg/ml[15].

Drug incubation survival assay: The Drug Incubation Survival Test (DIST) was used for this assay. 100µl of the reconstituted solutions of each of the extracts, as well as the reference drug, were separately dispensed in triplicate into wells of a 96-well microtitre plate. 30µl of the blood suspension containing *T. brucei brucei* was added to each of these wells, and gently mixed together. Control wells containing only 100µl supplemented medium and 30µl 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 of these wells, six (6) hours post-incubation. Each smear was examined in the 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, trypanosome counts were also taken for smears prepared from the control wells. Reduction in the number of motile trypanosomes compared to the control wells was taken as index for *in vitro* activity[15].

Brine Shrimp Lethality Bioassay: To estimate toxicity activity, the lethality bioassay of brine shrimp was employed, utilising a technique developed[17].

Brine Shrimp Hatching: *Artemia salina* leach (brine shrimp eggs) collected from pet shops was used as the test organism. One teaspoon of shrimp eggs was gently poured in a 300ml of conical flasks full of sea water. It was then allow shaking for 48 while illuminated using an electric bulb, which help attracting the hatched shrimps, using 100µl pipette, 10 hatched shrimp larvae were selected and transferred in to different test tube.

Preparation of test solutions with samples of experimental plants: For each test sample 32 mg were taken and dissolved in 200µl of pure dimethyl sulfoxide (DMSO) and finally the volume was made to 20 ml with sea water. Thus the concentration of the stock solution was prepared 1600µg/ml. Then the solution was serial diluted to 800, 400, 200, 100, 50, 25 µg/ml with sea water. Then 2.5 ml of the extract solution was added to 2.5 ml of sea water containing 10 nauplii.

Preparation of the positive control group: Positive control in a toxicity study is a widely accepted toxic agent and the result of the test agent is compared with the result obtained for the positive control. In the present study Potassium Dichromate (Positive control) was used at concentration 800, 400, 200, 100, 50, 25µg/ml.

Preparation of the negative control group: For the negative control 50 µl of DMSO was added to each of three premarked test tubes containing 4.95 ml of simulated sea water and10 shrimp nauplii to use as control groups.

Counting of nauplii: After 24 hours, the test tube were inspected using a magnifying glass against a black background and the number of survived nauplii in each test tube was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. For each tube, count the number of dead and number of live nauplii, and determine the % death, Percentage of Death (%M) = X100.

Data analysis: The results were analyzed using the Statistical Package for Social Science (SPSS) version 2.0 software. All 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 two-way analysis of variance (ANOVA) at significance level of $p = <0.05$.

Results and Discussion

Isolation and Identification of Endophytic Fungi: The healthy leaf, stem and root of the plant *Psidium guajava* were subjected to surface sterilization and different species of endophytic fungi were isolated and identified based on colony morphology and microscopicstudies.

Phytochemical Screening of Fungal Extracts: The results of qualitative fungal metabolites analysed revealed that the fungal ethylacetate / mehanolic crude extracts contain steroid, tannins, saponins, flavonoids, alkaloids, phenols and terpenoids (Table-1). These chemicalconstituents are responsible for different medicinal properties of extracts.

Trypanosomes Survival Rate in Endophytic Fungal Extract: Table-2 shows the effects on *Trypanosoma brucei brucei* 6 hours after incubation of graded doses of crude ethylacetate/ metabolites extracts of *A. nidulan*, *F. moniliforme*, and *Rhizoctonia sp*. When compared to the number of surviving trypanosomes in the negative control wells, the results showed that the crude extracts considerably ($p<0.05$) decreased the number of surviving trypanosomes in the extract concentrations. In addition, it was found that at higher concentrations, *A. nidulan* extract exhibited the highest anti-trypanosomal activity, followed by *F. moniliforme* and extracts of *Rhizoctonia sp*. The former showed 0% survival and 100% mortality of trypanosomes between 1.25 and 10 mg/ml concentrations, while the latter showed some survival at concentrations of 10 mg/ml. *Rhizoctonia sp*, however, showed notable efficacy against trypanosome survival at lower concentrations. The extracts' reported effects on the quantity of trypanosomes that survived were likewise concentration dependent, with a decrease in trypanosome survival observed at higher concentrations.

Table-1: Preliminary Phytochemical Screening of Endophytic Fungal Extracts.

Phyto-chemical	*A. nidulan*	*F. moniliforme*	*Rhizoctonia sp.*
Steroid	+	+	-
Phenols	+	-	-
Tannin	+	+	+
Saponin	+	+	+
Alkaloid	+	+	+
Flavonoid	+	+	+
Terpenoid	-	-	-

+ Indicates presence of constituents, - Indicates absence of constitute.

Toxicity Test: The result of toxicity test using brine shrimp lethality of the crude extracts revealed that, the percentage mortality and the LC_{50} values obtained for extracts and positive control (Potassium dichromate) were showed in (Table-3). From the results obtained, the ethylacetate / methanolic extracts showed non-toxic potential with LC_{50} values of 110µg/ml, 173.5µg/ml and 206µg/ml respectively, whereas the LC_{50} of the positive control (potassium dichromate) was 0.80µg/ml (Table-3).

Table-2: Mean± SE Survival Rate of Trypanosomes Incubated in Fungal Extracts.

Conc.	*A. nidulan*	*F. moniliforme*	*Rhizoctonia* sp	Control
10	0.0 ± 0.0^{Aa}	0.0 ± 0.0^{Aa}	7 ± 0.2^{Bb}	29 ± 0.1^{Dd}
5	0.00 ± 0.0^{Aa}	0.0 ± 0.0^{Aa}	12 ± 0.2^{Bc}	30 ± 0.1^{Dd}
2.5	0.00 ± 0.0^{Aa}	0.0 ± 0.0^{Aa}	13 ± 0.1^{Bc}	28.3 ± 0.1^{Dd}
1.25	0.0 ± 0.0^{Aa}	1.7 ± 0.1^{Aa}	15 ± 0.1^{Bc}	20.33 ± 0.4^{Dd}
0.625	0.7 ± 0.1^{Aa}	4 ± 0.1^{Aa}	14.3 ± 0.2^{Bc}	29.7 ± 0.1^{Dd}
0.3125	4.3 ± 0.12^{Aa}	8.3 ± 0.15^{Aab}	16 ± 0.1^{Bc}	28 ± 0.1^{Cd}
0.1625	5.3 ± 0.1^{Aab}	12.3 ± 0.1^{Bab}	20 ± 0.1^{Ccd}	30 ± 0.0^{Dd}

All values are given as mean ± SEM (standard error of mean). In each column, and rows values with different superscripts have statistically significant difference ($P<0.05$). Conc. = Concentrations.

Table-3: Brine shrimp lethality test of endophytic fungal extracts of *A. nidulan, F. moniliforme and Rhizoctonia* sp.

Extracts	Concentrations (µg/ml)	Mortality after 4 hours	% Mortality	LC_{50} (µg/ml)
A. nidulan	800	6	60	206
	400	5	50	
	200	4	40	
	100	3	30	
	50	2	20	
	25	2	20	
F. Moniliforme	800	7	70	173.5
	400	6	60	
	200	5	50	
	100	4	40	
	50	2	20	
	25	1	10	
Rhizoctonia sp.	800	7	70	110
	400	7	70	
	200	5	50	
	100	4	40	
	50	2	20	
	25	1	10	
Positive Control (Potassium dichromate)	800	10	100	0.80
	400	10	100	
	200	10	100	
	100	9	90	
	50	7	70	
	25	6	60	

In general, plants provide endophytic fungus with a special habitat. Several previous studies discovered distinct and bioactive natural chemicals in endophytic fungus isolated from several therapeutic plants. These fungi have been isolated from various plant organs such as leaves, stems, or inner bark[18]. Endophytes, which generate secondary metabolites with pharmacological characteristics analogous to those of the host plants, are found in most therapeutic plants[19]. *Psidium guajava* L. is a genus that contains a significant amount of naturally occurring pharmacologically active chemicals, including flavonoids, tannins, phenolic compounds, essential oils, sesquiterpene alcohols, triterpenoid acids, and other compounds[20].

This investigation also revealed the existence of various phytochemical compounds with antitrypanosomal activity in the roots, stems, and leaves of endophytic fungi that were isolated from *Psidium guajava*. As shown in Table-1, the extracts' phytochemical screening results indicated the presence of terpenoids, alkaloids, flavonoids, phenols, tannins, and saponins. It has been observed that the majority of these substances exhibit antioxidant, anticancer, and anti-trypanosomal properties[21-23].

According to the in vitro experiment, the methanolic fungal extracts had strong anti-trypanosomal properties, which is consistent with a study that found that several endophytic fungal extracts have antimicrobial properties[24]. *Aspergillus* strains have been reported to exhibit mild antileishmanial activity, according to another investigation[25]. The existence of bioactive secondary metabolites may account for the observed in vitro anti-trypanosomal activities of the ethylacetate/methanolic extracts of *A. nidulan*, *Rhizoctonia sp*. and *F. moniliforme*. Furthermore, a physiological process-related chemical compound's synergistic or addictive action may be the cause of the strong anti-trypanosomal activities detected in the extracts[26].

Researchers have found that alkaloids can prevent the production of proteins, intercalate DNA, disrupt membrane fluidity, prevent the creation of microtubules, or cause trypanosomes in the circulation to undergo programmed cell death[27]. Tannins are well recognized for their anticancer, antiviral, antibacterial, and antiparasitic properties[28]. It is well known that flavonoids have strong anti-trypanposomal properties[29,30]. Additionally, it is known that saponins interact with the cholesterol on the parasite cell membrane to prevent protozoa from developing, ultimately resulting in the death of the parasite. Numerous investigations using saponins have exhibited their beneficial impact on enhancing nutritional absorption through the process of membrane depolarization, which increases intestinal permeability[31].

As a result, the combined effects of the phyto-constituents present in the extracts may be responsible for the action of fungal extracts in lowering parasite mortality.

When compared to the reference standard potassium dichromate (LC_{50} 0.80µg/mL), see Table 3 shows that all extracts were less hazardous to brine shrimps in the toxicity assay (LC_{50} 206µg/mL, 173.5µg/mL, and 110µg/mL). Consequently, it is harmless and non-toxic. Research indicates that an extract is considered highly toxic if its LC_{50} is less than 1.0 µg/mL, mildly toxic if it is less than 100µg/mL, and non-toxic if it is more than 100 µg/mL, as explained in detail[32]. It was observed that the concentration dependence of the brine shrimp mortality rate in this investigation was correlated with a rise in extract concentration.

Conclusion

In conclusion, extract of *A. nidulan* shows the highest *in vitro* activity against *Trypanosoma brucei brucei* at 10, 5, 2.5 and 1.25 mg/ml, whereas, extract of *F.moniliform* shows activity against *T. brucei brucei* at 10, 5 and 2.5 mg/ml, while *Rhizoctonia* sp. shows the least activity against *T.brucei brucei in vitro.* The outcomes of this study demonstrated that *A. nidulan* produced active metabolites such as steroids, phenols, tannins, saponins, alkaloids, and flavonoids in its ethylacetate and methanolic extracts, while *F. moniliforme* produced all of the listed metabolites except phenols. However, all of the metabolites mentioned above aside from steroids and phenols are present in *Rhizoctonia* sp. The extracts are less toxic to brine shrimp, suggesting that the extracts of endophytes from *Psidium guajava* may be safer as potential antitrypanosomal candidates for the effective treatment of Trypanosomiasis which proved the importance of medicinal plants in the treatment of disease-afflicting animals. When compared to potassium dicromate, the fungal endophytes extract from *Psidium guajava* similarly showed minimal cytotoxicity in the brine shrimp mortality test.

References

1. Cayla, M., Rojas, F., Silvester, E., Venter, F. and Matthews, R. K. (2019).African trypanosomes. *Journal of parasites and vector*, (12), 190.

2. Oyda, S. (2017). Review on traditional ethno-veterinary medicine and medicinal plants used by indigenous people in Ethiopia: practice and application system. *Int J Res*, *5*, 109-19.

3. Dabo, N. T. and Maigari, A. K. (2017). Soft Options for Effective Diagnosis of African Animal Trypanosomosis: A Review. *International Journal of Medical Evaluation and Physical Report*, 1(2), 1 - 9.

4. World Health Organization (2020). Applied microbiology: Rhodesian human African trypanosomiasis (rHAT) in Kafue Nation Park, Zambia-David square Zambia Hokkaido University. *Journal of food and microbiology*, (4),1-12.

5. Food and Agricultural Organization (2020). Tsetsefly and Trypanosomiasis Control. https://www.researchgate.net. *Retrieved June*, 2020.

6. Delespaux, V., Vitouley, H. S., Marcotty, T., Speybroeck, N., Berkvens, D., Roy, K., ... & Van den Bossche, P. (2010). Chemosensitization of Trypanosoma congolense strains resistant to isometamidium chloride by tetracyclines and enrofloxacin. *plos neglected tropical diseases*, 4(9), e828.

7. Vitouley, H. S., Mungube, E. O., Allegye-Cudjoe, E., Diall, O. and Bocoum, Z. (2011). Improved PCR-RFLP for the Detection of Diminazene Resistance in Trypanosoma congolense under Field Conditions using Filter Papers for Sample Storage. PloS *Neglected Tropical Diseases*, 5, 30-42.

8. Harry, P. and Koning, D. (2020). The drugs of sleeping sickness: Their mechanism of action and resistance and a brief history. *Journal of tropical medicine and infectious diseases*, 514- 23.

9. Shaba, P., Pandey, N. N., Sharma, O. P., Rao, J. R. and Singh, R. K. (2012). Therapeutic effects of *Zanthoxylumalatum* leave and *Eugenia caryophyllata buds* (fruits) against *Trypanosoma evansi*. *Journal of Veterinary Advances*, 2(2), 91-97.

10. Henrietta, A. O., Folasade, F. T. and Funmilayo, .A. I. D. (2015). Antitrypanosomal activity of *khaya senegalensis* and *Anogeissusleiocarpus stem bark* on *trypanosome brucei brucei* infected rats. *African journal of Biotechnology*, 14(6):525-529.

11. Oses, R., Valenzuela, S., Freer, J., Sanfuentes, E., & Rodriguez, J. (2008). Fungal endophytes in xylem of healthy Chilean trees and their possible role in early wood decay. *Fungal Divers*, 33(7), 77-86.

12. Salisu, A., R.W., Ndana, A. and Samuel, A. (2017). Bioprospective potentials of endophytic fungi (*penicillium SPP*) isolated from leaves of *Azadirachta indica* (A.JUSS). *International Journal of Biological Research*, 5(1), 15-21.

13. Sadananda, T. S., Nirupama, R., Chaithra, K., Govindappa. M., Chandrappa, C. P., Vinay R. B. (2011). Antimicrobial and antioxidant activities of endophytes from *Tabebuiaargentea* and identification of anti-cancer agent (lapachol). *Journal of Medicinal Plants Research*, 5(16), 3643 – 3652.

14. Madki, M. A., Manzoor, A. S., Powar, P. V. and Patil. K. S. (2010). Isolation and biological activity of endophytic fungi from *Withania Somnifera*. *International Journal of Pharmaceutical Sci.*, 2(3), 848-858.

15. Bulus, T. and Addau, F. T. (2013). Comparative Anti-trypanosomal Screening of Methanolic of *K. senegalensis* and *M. oleifera*. *Science World Journal*, 8(2), 1597 – 6343.

16. Herbert, W. J. and Lumsden, W. H. R. (1976). Trypanosoma brucei brucei, a rapid "matching" method for estimation of host's parasitemia. *Experimental Parasitology*, (40), 427 – 431.

17. Magadula, Joseph J., Joseph N. Otieno, Ramadhani S. Nondo, Claude Kirimuhuzya, E. Kadukuli, John A. Orodho and Paul Okemo. (2012). Anti-Mycobacterial and Toxicity Activities of Some Priority Medicinal Plants from Lake Victoria Basin, Tanzania. *European Journal of Medicinal Plants*, 2(2), 125-131.

18. Gurupavithra, S. and Jayachitra, A. (2013). Isolation and identification of endophytic fungi from *Ocimum sanctum* and analyse its antioxidant properties. *International Journal Research of Pharmaceutical and Biomedical Sciences*, 4(4), 1120-1125.

19. Yadav, M., Yadav, A. and Yadav, J. P. (2014). In vitro antioxidant activity and Total phenolic content of endophytic fungi isolated from *Eugenia jambolana* L. *Asian Pacific Journal of Tropical Medicine*, 7S1: S256 – S261.

20. Haida, K. S., Baron, A, and Haida, K.S. (2011). Phenolic compounds and Antioxidant activity of two varieties of *Psidium guajava* and rue. *Revive Brazilian Ciênc Saúde*, (28): 11-19.

21. Camacho, M. R., Mata, R., Castaneda, P., Kirby, G. C., Warhurst, D. C., Croft, S. L. and Phillipson, J. D. (2000). Bioactive compounds from *Celaeno Dendron Mexicanum*. *Planta Medica*, (66), 463- 468.

22. Elfita, E., Muharni, M., Latief, M., Darwati, D., Widiyantoro, A Supriyatna, S., Bahti, H. H., Dachriyanus, D., Cos, P. and Maes, L. (2009). Antiplasmodial and other Constituents from four Indonesian *Garcinia spp*. *Phytochemistry*, (70), 907-912.

23. Aisha, A. F., Abu-Salah, K. M., Ismail, Z. and Majid, A. M. (2012). In vitro and in vivo anti-colon cancer effects of Garcinia *mangostanaxanthones* extract. *BMC Complementary and Alternative Medicine*, (12), 104-109.

24. Utami, S. H., Dwi, R., Rai, Y.S. Sugi, H., Chomisatut, T., Fitri M. And Faiza, N. I. N. (2020). Antimicrobial activity of endophytic fungi isolated from *physalisangulata* L plant. *Journal of Energy and Natural Resources*, 9 (1): 10-13.

25. Sergio, M., Lilia C., Sarah H., Elizabeth A., Carmenza S., Alicia I., William H., and Luis C. (2021). Screening and evaluation of anti-parasitic and In vitro anticancer activity of Panamanian and entophytic fungi. *Journal of Marine Drugs*, 11(10), 4058-4082.

26. Feyera, T., Terefe, G. and Shibeshi, W. (2014). Evaluation of In vivo anti trypanosomal activity of crude extracts of Artemisia abyssinica against *Trypanosoma congolense* isolate. *BMC Complementary and Alternative Medicine*, (14), 117-120.

27. Omar, M. and Khan, F. (2007). Trypanothionereductase: a viable chemotherapeutic target for anti-trypanosomal and antileishmanial drug design. *Drug Target Insights,* (2), 129-146. 29.

28. Pelegrini, P. B., Murad, A. M., Silva, L. P., Dos Santos, R. C. and Costa, F. T. (2008). Identification of a novel storage glycine-rich peptide from guava (*Psidium guajava*) seeds with activity against Gram-negative bacteria. *Peptides*, (29), 1271-1279.

29. Johnson, T. O. and Omaniwa, B. P. (2014). In vivo trypanosidal activity of ethanoliccrude extract and phytochemical fraction of *Garcina kola* seeds. *Biology*, (4):212 – 222.

30. Nwodo, N. J., Brun, R. and Osadebe, P. O. (2015). In vitro and in vivo evaluation of the Anti-trypanosomal activity of fractions of Holarrhenaafricana. *Journal Ethnopharmacology*, (113), 556-559.

31. Efterpi, C., Eleftherios, B., Ilias, G. and Panagiota, F. (2012). Aromatic Plants as Sources of Bioactive Compounds. *Agriculture*, (2), 228-243.

32. Sosovele, M. E., Hosea, K. M. and Lyimo T. J. (2012). In vitro antimicrobial activity of crude extracts from marine Streptomyces isolated from mangrove sediments of Tanzania. *Journal of Biochemistry and Technology*, (3). 431-435