© 2018 IJSRSET | Volume 4 | Issue 10 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
Themed Section : Engineering and Technology

Bulk Screening of Medicinal Plants and their Antitrypanosomal Activity

Gana James[1*], Adekojo Solomon Adeolu[1], Danwake Jibrin Garba[1], Abubakar Baman Mohammed[1], Shaba Peter[2]

[1]Federal College of Education, School of Vocational Education Department of agricultural education, P.M.B 39, Kontagora Niger state, Nigeria

[2]Niger state College of Agriculture, Department of Animal Health and Production Technology. P.M.B. 109, Mokwa, Nigeria

*Corresponding email : jamesgana38@gmail.com

ABSTRACT

The recent resistance to available trypanocids and by trypanosomes had hampered effective treatment and control. Herbal medicine has attracted much attention as potential therapeutic agent in the prevention and management of parasitic and infectious diseases, as they can yield potential leads to address emerging infections and resistance, indeed, the use of medicinal plant in treatment and management of human and animal diseases has been practiced before the advent of chemotherapy. The studied were carried by harvested fresh different parts of medicinal plants (leaves, stem, fruit, bark and root) and identified appropriately and processed accordingly. Nigerian medicinal plants were screened for possible anti-trypanosomal activity that could be further work on for isolation of trypanocidal compounds that may hold the key for development of new trypanocides and screen bulk of medicinal plants via in vitro media. In this current research on antitrypanosomal, from medicinal plants, out of the medicinal plants, Annona senegalensis (leaves, stem and roots), Terminalia avorensis and vitexdoriana displayed varied moderate to higher antitrypanosomal activity than other medicinal plant screened. The varied antitrypanosomal activity in this research report which ranged from immobilization to complete killing of the trypanosomes are comparable to trypanocidal activityof comparative invitrotrypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian savannah plants. The finding indicates that few of the medicinal plants screened contained trypanocidal compound(s), if isolated in further research could be a promising candidate for a future trypanocide.

Keywords : Medicinal Plants, Antitrypanosomal , Trypanosomal, Trypanocidal.

I. INTRODUCTION

Trypanosomosis, a disease caused by blood protozoan parasites of genus *Trypanosoma*, is on the increase in different parts of the world, (e.g. Africa and Latin America), where millions of population and cattle are affected with considerable morbidity and mortality (1,2, 3 and 4). It is important parasitic disease of veterinary and medical (3,5). The disease is caused by different pathogens such as *Trypanosomaevansi* in animals as to *Trypanosomabruceirhodesiense* and in humans (3). Human African trypanosomosis, for instance, affects more than 6o million people with 300-500, 000 new cases per annum (6 and 2). 3 billion pounds or more are lost annually in Africa from animal trypanosomosis (4)

Reports of resistance to available trypanocides (7) and by trypanosomes (8) had hampered effective treatment and control. Reports of naturally active

extras/fractions/compounds from medicinal plants against trypanosomes have been reported (9,7,8,10,11and12)

Trypanosomosis has been a problem to livestock and humans over a century (4).The current trypanocides have been in use for over half a century, and rare bedeviled with lots of problems such as toxicity, unavailable limited classes, costly, cumbersome in its administration in humans, toxicity and developments of resistant strains of trypanosomes (7,4).

Medicinal plants have been sources of some natural products-classes of drugs developed and in used in humans (e.g. arteminisin, quinine) . On this premise, medicinal plants will be screened against pathogenic trypanosomes for possible its anti-trypanosomal activity for further bioassay-guided purification for the isolation of trypanocidal compound(s).

Research has been on going on distinct aspects of trypanosomes for more than a century (9).Of all these, trypanosomes, a blood flagellate protozoan parasite that affect both animals and humans had defiled the processes of developing a vaccine for it (6,9). No new trypanocide has been introduce into the market for more than 60 years on, and current drugs in used are beset with lots of problems such as toxicity, high cost and in certain circumstances, limited availability just to mention few (7). For more than three decades, a new approach was conceived to search and develop a new trypanocide via medicinal plants, which has been the bane of medicines prior to the First World War. For this, the cumbersome processes entails extractions, purifications, isolationsand structural elucidations of trypanocidal compounds,*in vitro* and *in vivo*antitrypanosomal activity couple with cytotoxicity on mammalian cell lines and many others (13). On this note, the under listed points underscored the initial stages of ongoing distinct research on the medicinal plants globally for possible identification and isolation of trypanocidal extracts/compounds that could pave way for further research into development of a new trypanocide.

The documented West Africa plants that inhibited *Trypanosoma brucei* motility in less than thirty (30) minute at 4 mg/ml. Twenty three (23) West Africa plants inhibited the parasite motility within thirty one to sixty (31-60) minutes at 4 mg/ml. In this study, the highest activity of the plants was observed at 4 mg/ml where complete cessation of the parasites motility were observed in this following manner: in five (5) minutes (*Waltheria indica*), ten (10) minutes (*Vernonia amygdalina*), fifteen (15) minutes (*Albizia ferruginea* and *Camellias inensis*) and twenty five (25) minutes (*Chamaecrist amimosoides* and *Hyptis suaveolens*). The *in vitro* antitrypanosomal effect demonstrated by the plants investigated in this study, especially *Waltheria indica* and *Vernonia amygdalina* was comparably higher than activities reported for most of other Africa plants.(14) reported that parasitic diseases, especially those defined as neglected diseases by the WHO, remain a major public health predicament, which affects hundreds of millions of people especially in the developing countries. Furthermore, infections such as HIV, malaria, tuberculosis, diarrhea diseases, pneumonia, leishmania, and human African trypanosomosis are responsible for one in two deaths in developing countries where poverty, limited access to health care, drug resistance and a changing environment make population particularly vulnerable. As a consequence, herbal medicine has attacked much attention as potential therapeutic agent in the prevention and management of parasitic and infectious disease. As they can yield potential leads to address emerging infections and resistance indeed, the use of medicinal plant in the treatment and management of human and animal diseases has been practiced before the advent of chemotherapy. The present review has endeavored to provide an overview of the potential of medicinal plants, particularly, those from the Africa biodiversity to target three common parasitic and infectious diseases, namely malaria, leishmania and

human African trypanosomosis that have plagued humans since time immemorial.

(15) reported activity of the methanolic extracts of *Quercus borealis* leaves and *Zingiber officinale* roots at concentrations (250-100 µg/ml) were screened for their antitrypanosomal activity against *Trypanosoma evansi* maintained *in vitro* in culture medium seeded with Vero cells. *In vitro* cytotoxicity of the chloroform extract at concentrations (100 – 1.56 µg/ml) was carried out on Vero cells grown in Dulbecco's Modified Eagle Medium (DMEM) at appropriate conditions. Antitrypanosomal activity of methanolic plant extracts (MPES) varied from immobilization (clumped trypanosomes in the bottom of the wells) to the killing of the trypanosomes depending on the extract concentrations and time of exposure. MPE of *Quercus borealis* exhibited a significant trypanocidal activity by reduction of average mean trypanosomes counts from initial concentration (40.00 ± 0.00) at 250 µg/ml and complete killing of trypanosomes counts from 40.00 ± 0.00 to 0.58 ± 0.02 at all concentrations. Both MPE of *Quercus borealis* and *Zingiber officinale* and diminazine aceturate were cytotoxic to the vero cells in all concentration except at 1.56, 6.25 – 1.56 and 6.25 – 1.56 µg/ml, respectively.

The documented that new cases of vector borne *Trypanosoma cruzi* infection usually occur in persons who live in primitive houses in an area where the sylvatic cycle is active. The living quarters are invaded by infected triatomines, which becomes domiciliary. Infected insect takes blood meals from humans and their domestic animals and deposit parasite-laden feces. The parasites are then transmitted via contact with breaks in the skin, mucosal surfaces, or the conjunctivas. Transmission can also occur congenitally or via blood transfusion or organ transplantation. (16) documented that there seemed to be little or no literature about the toxicity of *Lannea schimperi* leaves, which makes it important to investigate the toxicity of the plant leaves so as to establish its safety or other wise to human handlers and animal consumers. Toxicological effects of its methanolic leaves extracts were investigated in mice using Lorke's method of 1983. This was conducted in two phases. In the initial phase, mice were divided into three (3) groups of three mice each and treated at doses of 10, 100 and 1000 mg/kg respectively with methonolic leaves extract of *Lannea schimperi*, intraperitoneally (I.P). This was then obtained for 24 hours for signs of toxicity, including death. In the final phase, mice were divided into four (4) groups of one mouse each and treated with the extract at doses of 140, 225, 370 and 600 mg/kg, respectively. The acute toxicity (LD_{50}) of the extract was determined to be 288.53 mg/kg. Also the anti-trypanosomal activity of the extract was evaluated against *Trypanosoma brucei brucei in vitro* at concentration of 3, 6, 12 and 24 mg/ml. Susceptibility of the organism was determined in culture medium containing 5% dextrose and 0.9% saline solution alone as control 3, 6, 12 and 24 mg/ml of these plants extracts in the same solution. Complete mortality of the organism was observed at the concentration of 24, 12, 6 and 3 mg/kg within 30, 60, 180 and 330 minutes respectively, in a dose dependent manner; the organism however survived for six (6) hours in the control test tube. All the tested incubated samples were assayed for infectivity in mice. The infectivity to mice of all trypanosomes incubated at various extract concentrations (24, 12, 6 and 3 mg/ml) was negative four days post inoculation, but the infectivity of the control incubated for 360 minutes was positive with parasitic load of 2.5×10^6 four days post inoculation. The result suggests that methanolic leaves extract of *Lanneas chimperi* possess some trypanocidal principles which may require further scientific elucidations. (18) documented that phytochemical analysis and *in vitro* antitrypanosomal screening of crude methanol extracts of the leaves of *Waltheria indica, Vernonia amygdalina, Albizia ferruginea, Camellia sinensis, Chamaecrist amimosoides* and *Hyptis Suaveolens* were carried out using standard methods. Highly parasitized blood from infected

donor rats was diluted with glucose phosphate buffered Saline solution and incubated with varying concentrations (1 mg/ml, 2 mg/ml and 4 mg/ml) of the extracts in Eppendorf tubes for sixty (60) minutes. Aliquots from the mixtures were removed and observed under microscope for parasite motility at five (5) minutes interval. Phytochemical screening revealed the presence of tannins, alkaloids, flavonoids, phenols and glycosides in all the plants extracts. Saponins was absent in *Hyptis Suaveolens* while, steroids and phlobatannins are absent in *Chamaecrist amimosoides* and *Hyptis suaveolens*. All the plants' extracts showed significant Cessation of parasite motility with increase in incubation time and concentration of the extract. Complete Cessation of the parasite motility was observed for all the extracts within sixty (60) minutes of the study. The most active extract was *Waltheria indica* at 4 mg/ml which caused complete cessation of the parasite motility within 5 minutes whereas the least active plants was *Chamaecrist amimosoides* and *Hyptis suaveolens*. Both caused complete cessation of the parasite motility within 25 minutes. Berenil, the standard drug, however, caused cessation of trypanosomal motility within 5 minutes even at 1 mg/ml. These results showed that methanolic extracts of the plants leaves screened especially *Waltheria indica* and *Vernonia amygdalin*a have significant *in vitro* antitrypanosomal activity and may be potential source for the *in vivo* treatment of trypanosomosis. (19) documented that African trypanosomosis is a parasitic disease that affects both human and livestock across the sub – Saharan Africa. Chemotherapy of the disease remains far from being satisfactory. The aim of the study is to explore an alternative source of antitrypanosomal agent from the leaves of *Corymbia torelliana*. The dried leaves were pulverized and extracted with methanol by maceration. Phytochemical investigation was carried out and subsequent *in vitro* studies of the extract on the survival of *Trypanosoma congolense*. Motility assessment of trypanosomes maintained in the phosphate Ringer glucose saline was carried out after exposure to the extract over a period of 105 minutes. Observations were made at 0 minutes and subsequently at intervals of 15 minutes. The phytochemical assay revealed the presence of saponins, tannins, flavonoids, cardiac glycosides, steroids and terpenes. The extract showed *in vitro* anti-trypanosomal effect against the parasite concentrations at 100 mg/kg, 0.2 mg/ml, 0.03mg/ml and at 0.00005 mg/ml. The highest effective dose was at 100 mg/ml and the lowest at 0.00005 mg/ml. This study showed that the methanolic leaf extract of *corymbia torelliana* was rich in phytochemical components and has great potentials for the treatment of trypanosomosis .The presence of these secondary metabolites in this plant might be responsible for the anti-trypanosomal activity exhibited by its extracts.

II. MATERIALS AND METHODS

PLANT MATERIALS.

Fresh different parts of medicinal plants (e.g. leaves, fruits, stem, barks and roots) were harvested and subsequently identify by appropriate department and process accordingly. The medicinal plants were obtained from different part of Nigeria, and especially from traditional healers that have been using those medicinal plants for treatment of human and animal trypanosomosis in their localities.

EXTRACTION.

Both cold and hot extraction (e.g. Soxlet extraction) methods was carried out.Example of cold extraction: Twenty grammes of cured dried parts of medicinal plants was pounded into powder with pestle and mortar. The sample was cold extracted twice with 200 ml of methanol (analytical grade) according to (20). The filtrates was dried at 37°C and stored at 4 °C until used. Example of hot extraction: In this method, soxlet apparatus was used for the extraction.

THIN LAYER CHROMATOGRAPHY (TLC) PLATES

Aliquots (0.2ml) of methanolic plant extract (MPE) and other solvent extracts was applied on TLC plates,

which was dried under room temperature and immersed inside the solvent systems in glass jar listed in the next subsection. This was done to detect, if any, the presence of bioactive constituents in applied solvent extracts. After full development of plates in solvent systems, plates were dried at room temperature. Then, one set of TLC plates was immersed in iodine vapours in a glass jar. Second set of TLC plates was sprayed with Vanillin-sulphuric acid spray. Both media used facilitated the detection of bioactive constituents. This was carried out according to the method of (20).

SOLVENT SYSTEMS APPLIED.

The following examples of solvent systems were tested to develop the TLC plates to obtain a more suitable system for both extract and fractions according to the method of Stahl (20).
Chloroform/hexane/acetic acid (50:50:1)
Chloroform/ethyl acetate/acetic acid (50:50:1)
Methanol and chloroform (20: 80)

TEST ORGANISM.

Trypanosoma spp (e.g. Trypanosoma evansi, T. brucei) was obtained from Nigeria Institute of Trypanonosomiais and Onchocerchiasis. It was maintained in the laboratory by serial sub-passage in Swiss albino mice. The strain was routinely tested for virulence following the method of (21)

IN VITROTRYPONOCIDAL ACTIVITY.

(A). Motility check method

In vitro antitrypanosomal assay was conducted according to (22) with some modifications. Stock solutions of the respective extracts were prepared by dissolving 10 mg of each extract in 1ml phosphate buffered (pH 7.4) containing 1% (w/v) glucose (PBS-G), or 2% DMSO. A 5mg/ml test solution of each extract was prepared from the 10mg/ml stock solution by the addition of PBS-G. 100 µl of the 5 mg/ml of the extracts were respectively dispensed in duplicates into the wells of a 96-multi well plate. 30 µl of blood containing *T. brucei brucei* was added to the solution of extract so that the trypanosome count was between 20-25 trypanosomes per field. Wet smears from each blood-extract mixture were prepared from each well and observations on trypanosome motility were made at a five minute interval for a total duration of sixty minutes.

(B) *In vitro* trypanocidal activity was carried out on two media: (I) on modified method of (23). In this method, a Vero cell line was grown in Dulbecco's Modified Eagle Medium (DMEM) (Sigma) in 96-well flat bottom micro culture plates (Nunc, Denmark). Each well received 100 µµ of DMEM containing 5x105 cells/ml. The plates were incubated at 37C under 5% CO2 for 48h to complete development of monolayer. After the formation of confluent monolayer, the medium (DMEM) was discarded and replaced with a fresh DMEM. And the medium was supplemented with 20-40% fetal calf serum (FCS), Gibco USA and antibiotics (100 units penicillin, 100 µg streptomycin and 40 µg gentamycin). A high parasitemic blood from mouse was diluted with DMEM to obtain a final parasite of 1x106 parasites/ml. The suspension (100 ml of medium with trypanosomes was added at rate of 1:1 to methanolic plant extract (MPE) of a medicinal plantat concentrations (250-1000 µg/ml). The suspension (100 ml of medium with trypanosomes) was added at rate of 1:1 to test extract and the plates were incubated at 37^{0C}C under 5% CO_2. The mixture was incubated for 9 h. The test was repeated at least thrice .and the plate was incubated under the same conditions mentioned above. The test was repeated at least thrice. Stock of test MPE was solubilized in 1% dimethylsuphoxide (DMSO).

(II) On Alsever's medium: Trypanosomes were suspended in Alsever's solution with inactivated bovine serum at 58ºC for 1 h. Trypanosomes concentration was $1x10^6$ parasites/ml. 180 µl of the medium was added to the test extract of *E.*

officinalis(20 µl)andincubated at 37ºC with 5% carbon dioxide for 5 h. On hourly basis, drops of the incubated mixture were observed under inverted microscope for antitrypanosomal activity (24,11).
The concentration of DMSO in the experiment had no deleterious effect by itself on host cells or parasites.1% DMSO in distilled water was used as control.

IN VIVO INFECTIVITY ASSESSMENT

In vivo infectivity of MPE and other portions of purified dried parts of medicinal plants were carried out after successful completion of anti-trypanosomal activity. Contents of microculture plate wells that contained reduced and apparently killed trypanosomes with MPE of medicinal plants (e.g. *E. officinalis)*dried fruits was inoculated (0.1ml mouse-1) into two groups of mice (six group-1) via intra-peritoneal, and observed for more than 60 days for parasitaemia (25,11).

IN VITRO CYTOTOXICITY TEST

Cytotoxic effects of the MPE and other portions of purified pooled of medicinal plants (e.g. *E. officinalis)* dried fruits was determined according to the method described by (26). Vero cell line was grown in Dulbecco's Modified Eagle Medium (DMEM)(Sigma) Gibco, USA antibiotics (100 units penicillin, 100 µg streptomycin and 40 µg gentamycin) in 96-well flat bottom microculture plates (Nunc, Denmark). Each well received 100 µl of DMEM containing 5x105 cells/ml. The plates were incubated at 37 °C under 5% CO_2 for 48 h. After the formation of confluent monolayer, the medium was discarded and replaced with a fresh one. A high parasitaemic blood from mouse was diluted with DMEM to obtain a final parasite of $1x10^6$ parasites/m. Confluent monolayer of Vero cell was treated with serial dilutions of MPE of medicinal plants and pooled urified fractions (PPFs) of medicinal plants (e.g. of *E. officinalis*)(1.56-100 µg/ml) in triplicate and incubated under the same conditions described previously. After 24 h of incubation, the culture plate was observed for evidence of cytotoxic effects. The plate was incubated for 72 h and observed daily. It was repeated thrice. In each case, after the 72 h of incubation, the culture media of the incubated Vero cells were discarded. The adhered cells were stained with a drop of crystal violet in phosphate buffered solution. The plate was incubated for/ 24 hours at 37□C in an ordinary incubator. After 24 h of incubation, the culture plate was observed for evidence of cytotoxic effects.

PHYTOCHEMICAL SCREENING

This was done according to the methods of (27)

STATISTICAL ANALYSIS

Results of trypanocidal activity was expressed as mean ± SEM. Statistical significance was determined by Sigma Stat (Jandel), USA.

III. RESULTS

Results of *in vitro* antitrypanosomal activities of different medicinal plants cum parts via distinct solvents are as presented in the Tables below.

In *in vitro* motility test. there was variant displaying of different degrees of antitrypanosomal activity in all the extracts tested from different parts of the medicinal plants screened. But notably, medicinal plants such as *Annona senegalensis* (leaves, stems and roots), *Terminalia avorensis,* and *Vitexdoriana* displayed varied moderate to higher antitrypanosomal activity than other medicinal plants screened.

In both methods used in detecting antitrypanosomal activity of medicinal plants screened, the results indicated different degrees of antitrypanosomal activity of medicinal plants/parts, which ranged from immobilization and the killing of trypanosomes in respective solvent extract

Table 1. *In vitro* antirtypanosomal activity of methanolc extract of *Afromesia laxiflora* leaves against *Trypanosoma brucei*.

Conc. (mg/ml)	Hours post incubation							
	1	2	3	4	5	6	7	8
5	18.6±0.0^{a}	17.15±0.0^{a}	16.17±0.0^{a}	15.23±0.0^{a}	14.13±0.0^{a}	12.24±0.0^{a}	10.12±0.0^{a}	7.17±0.20^{a}
2.5	20.12±0.7^{b}	19.35±0.47^{b}	18.17±0.40^{b}	17.25±0.0^{a}	17.16±0.0^{a}	16.15±0.0^{a}	15.15±0.0^{a}	14.71±0.40^{a}
1.25	24.5±0.58^{c}	24.12±0.60^{c}	22.50±0.43^{c}	21.53±0.48^{a}	20.33±0.31^{a}	19.18±1.67^{a}	18.45±0.0^{a}	17.35±4.10^{a}
0.625	26.3±1.16^{d}	25.2±0.61^{d}	24.7±3.40^{d}	23.3±0.68^{b}	22.6±0.80^{b}	21.7±3.80^{b}	20.3±1.88^{b}	19.17±5.70^{b}
0.3125	28.3±0.72^{e}	27.3±0.56^{e}	27.3±0.84^{e}	27.0±0.89^{d}	26.8±1.22^{c}	26.3±0.88^{b}	25.0±0.68^{b}	24.0±0.68^{c}
0.15625	29.7±1.12^{de}	28.6±0.49^{e}	27.7±0.88^{de}	27.8±0.95^{c}	26.5±0.76^{c}	25.2±0.31^{c}	23.7±0.49^{c}	21.5±0.76^{d}
Diminazine	5.3±0.33^{b}	4.0±0.52^{b}	1.5±0.50^{ab}	0.17±0.16^{a}	0.0±0.0^{a}	0.17±0.16^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Control	25.5±0.99^{e}	25.7±0.33^{e}	25.5±1.1^{f}	25.6±1.23^{cd}	23.0±0.97^{c}	22.7±1.09^{d}	21.5±0.99^{d}	20.7±0.68^{e}

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

Table 2.*In vitro* antirtypanosomal activity of methanolic extract of Lawmsonia *inemia* leaves against *Trypanosoma brucei*

Conc. (mg/ml)	Hours post incubation							
	1	2	3	4	5	6	7	8
5	28.6±0.0^{a}	28.15±0.0^{a}	27.17±0.0^{a}	26.23±0.0^{a}	25.12±2.3^{a}	24.20±4.4^{a}	23.5±6.7^{a}	22.6±5.5^{a}
2.5	29.5±0.76^{b}	29.83±0.47^{b}	28.17±0.40^{b}	28.35±0.0^{a}	26.13±0.0^{a}	25.25±0.0^{a}	24.17±0.0^{a}	24.21±5.7^{a}
1.25	31.0±0.58^{c}	31.17±0.60^{c}	30.50±0.43^{c}	29.83±0.48^{a}	28.33±0.21^{a}	28.17±1.67^{a}	27.34±5.6^{a}	26.15±7.8^{a}
0.625	33.0±1.16^{d}	32.1±0.61^{d}	32.7±0.40^{d}	31.0±0.68^{b}	30.7±0.80^{b}	30.7±0.80^{b}	29.3±0.88^{b}	29.17±0.70^{b}
0.3125	36.3±0.72^{e}	36.3±0.56^{e}	35.3±0.84^{e}	35.0±0.89^{d}	34.8±1.22^{c}	33.3±0.88^{b}	33.0±0.68^{b}	32.0±0.68^{c}
0.15625	39.7±1.12^{de}	38.6±0.49^{e}	38.7±0.88^{de}	37.8±0.95^{c}	37.5±0.76^{c}	25.2±0.31^{c}	35.7±0.49^{c}	34.5±0.76^{d}
Diminazine	5.3±0.33^{b}	4.0±0.52^{b}	1.5±0.50^{ab}	0.17±0.16^{a}	0.0±0.0^{a}	0.17±0.16^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Control	25.5±0.99^{e}	25.7±0.33^{e}	25.5±1.1^{f}	25.6±1.23^{cd}	23.0±0.97^{c}	22.7±1.09^{d}	21.5±0.99^{d}	20.7±0.68^{e}

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

Table 3.*In vitro* antirtypanosomal activity of aqueous extract of *Prosopis africana* root against *Trypanosoma brucei*

Conc. (mg/ml)	Hours post incubation							
	1	2	3	4	5	6	7	8
5	13.3±0.84^{b}	7.5±1.03^{a}	9.17±1.08^{b}	6.67±0.96^{b}	2.5±0.76^{a}	2.5±0.76^{a}	1.33±0.42^{a}	0.53±0.48^{a}
2.5	18.8±0.95^{cd}	11.5±1.31^{b}	12.2±0.48^{c}	13.2±1.30^{cd}	16.8±1.09^{bc}	10.5±1.41^{b}	12.7±1.35^{c}	13.2±0.79^{b}
1.25	15.5±1.38^{b}	14.3±2.23^{b}	14.7±1.12^{c}	14.8±1.14^{de}	16.3±1.65^{bc}	14.7±1.05^{c}	15.3±1.15^{c}	17.8±0.65^{cd}
0.625	17.0±1.29^{b}	12.0±1.06^{b}	12.2±1.08^{c}	14.7±1.48^{de}	16.5±1.59^{bc}	17.2±1.66^{cd}	13.7±1.28^{c}	14.2±1.05^{bc}
0.3125	16.7±2.45^{b}	7.33±0.49^{a}	10.8±0.95^{bc}	12.8±0.65^{cd}	16.2±1.25^{bc}	15.8±0.94^{c}	12.3±0.92^{c}	16.2±1.30^{c}
0.15625	9.0±0.33^{a}	5.5±72^{a}	10.3±0.13^{bc}	11.0±0.45^{c}	19.2±1.62^{c}	14.3±1.26^{c}	8.5±0.85^{b}	16.2±1.01^{c}
Diminazine	5.3±0.33^{a}	4.0±0.52^{a}	1.5±0.50^{a}	0.17±0.16^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Control	22.1±3.31^{e}	22.2±1.18^{c}	17.7±0.56^{d}	17.3±1.91^{e}	15.2±0.65^{b}	19.7±1.17^{d}	19.3±1.	19.7±0.92^{d}

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

Table 4.*In vitro* antirtypanosomal activity of methanolic extract of *Cassia sierberina* stem against *Trypanosoma brucei*

Conc. (mg/ml)	Hours post incubation							
	1	2	3	4	5	6	7	8
5	20.3±0.84^{b}	19.5±1.03^{a}	18.17±1.08^{b}	17.67±0.96^{b}	16.5±0.76^{a}	14.5±0.76^{a}	13.33±0.42^{a}	12.83±0.48^{a}
2.5	24.8±0.95^{cd}	23.5±1.31^{b}	22.2±0.48^{c}	22.2±1.30^{cd}	21.8±1.09^{bc}	20.5±1.41^{b}	21.7±1.35^{c}	19.2±0.79^{b}
1.25	26.5±1.38^{b}	26.3±2.23^{b}	25.7±1.12^{c}	24.8±1.14^{de}	23.3±1.65^{bc}	22.7±1.05^{c}	22.3±1.15^{c}	20.8±0.75^{cd}
0.625	29.0±1.29^{b}	29.0±1.06^{b}	28.2±1.08^{c}	27.7±1.48^{de}	26.5±1.59^{bc}	25.2±1.66^{cd}	24.7±1.28^{c}	24.3±1.09^{bc}
0.3125	33.7±2.45^{b}	33.33±0.49^{a}	32.8±0.95^{bc}	32.8±0.65^{cd}	31.2±1.25^{bc}	32.8±0.94^{c}	32.3±0.92^{c}	31.2±1.30^{c}
0.15625	39.0±0.33^{a}	39.5±72^{a}	38.3±0.13^{bc}	38.0±0.45^{c}	37.2±1.62^{c}	36.3±1.26^{c}	35.5±0.85^{b}	35.2±1.01^{c}
Diminazine	5.3±0.33^{a}	4.0±0.52^{a}	1.5±0.50^{a}	0.17±0.16^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Control	22.1±3.31^{e}	22.2±1.18^{c}	17.7±0.56^{d}	17.3±1.91^{e}	15.2±0.65^{b}	19.7±1.17^{d}	19.3±1.	19.7±0.92^{d}

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

Table 5. *In vitro* antirtypanosomal activity of methanolic extract of *Terminadus indica* leaves gainst *Trypanosoma brucei*

Conc. (mg/ml)	Hours post incubation							
	1	2	3	4	5	6	7	8
5	23.3±0.84^{b}	22.5±1.03^{a}	22.17±1.08^{b}	21.67±0.96^{b}	20.5±0.76^{a}	19.5±0.76^{a}	18.63±0.42^{a}	17.43±0.48^{a}
2.5	24.8±0.95^{cd}	24.5±1.31^{b}	23.2±0.48^{c}	23.2±1.30^{cd}	22.8±1.09^{bc}	22.5±1.41^{b}	22.7±1.35^{c}	21.2±0.49^{b}
1.25	26.5±1.38^{b}	26.3±2.23^{b}	24.7±1.12^{c}	24.8±1.14^{de}	23.3±1.65^{bc}	23.7±1.05^{c}	22.3±1.15^{c}	22.8±0.35^{cd}
0.625	29.0±1.29^{b}	29.0±1.06^{b}	28.2±1.08^{c}	27.7±1.48^{de}	26.5±1.59^{bc}	26.2±1.66^{cd}	25.7±1.28^{c}	24.3±1.19^{bc}
0.3125	32.7±2.45^{b}	31.33±0.49^{a}	31.8±0.95^{bc}	31.8±0.65^{cd}	31.2±1.25^{bc}	30.8±0.94^{c}	30.3±0.92^{c}	29.2±1.30^{c}
0.15625	34.0±0.33^{a}	34.5±72^{a}	33.3±0.13^{bc}	33.0±0.45^{c}	33.2±1.62^{c}	32.3±1.26^{c}	32.5±0.85^{b}	31.2±1.01^{c}
Diminazine	5.3±0.33^{a}	4.0±0.52^{a}	1.5±0.50^{a}	0.17±0.16^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Control	22.1±3.31^{e}	22.2±1.18^{c}	17.7±0.56^{d}	17.3±1.91^{e}	15.2±0.65^{b}	19.7±1.17^{d}	19.3±1.	19.7±0.92^{d}

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

Table 6. *In vitro* antirtypanosomal activity of methanolic extract of *Xeminia americana* leaves against *Trypanosoma brucei*

Conc. (mg/ml)	Hours post incubation							
	1	2	3	4	5	6	7	8
5	16.3±0.84^{b}	14.5±1.03^{a}	10.17±1.08^{b}	8.67±0.96^{b}	6.5±0.76^{a}	3.5±0.76^{a}	2.43±0.42^{a}	0.93±0.45^{a}
2.5	18.8±0.95^{cd}	16.5±1.31^{b}	14.2±0.48^{c}	14.2±1.30^{cd}	13.8±1.09^{bc}	12.5±1.41^{b}	12.7±1.35^{c}	11.2±0.59^{b}
1.25	19.5±1.38^{b}	18.3±2.23^{b}	18.7±1.12^{c}	18.8±1.14^{de}	17.3±1.65^{bc}	17.7±1.05^{c}	16.3±1.15^{c}	16.8±0.45^{cd}
0.625	21.0±1.29^{b}	21.0±1.06^{b}	20.2±1.08^{c}	20.7±1.48^{de}	19.5±1.59^{bc}	19.2±1.66^{cd}	18.7±1.28^{c}	18.3±1.09^{bc}
0.3125	23.7±2.45^{b}	23.33±0.49^{a}	22.8±0.95^{bc}	22.8±0.65^{cd}	21.2±1.25^{bc}	21.8±0.94^{c}	20.3±0.92^{c}	20.2±1.30^{c}
0.15625	25.0±0.33^{a}	25.5±72^{a}	24.3±0.13^{bc}	24.0±0.45^{c}	23.2±1.62^{c}	23.3±1.26^{c}	22.5±0.85^{b}	22.2±1.01^{c}
Diminazine	5.3±0.33^{a}	4.0±0.52^{a}	1.5±0.50^{a}	0.17±0.16^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Control	22.1±3.31^{e}	22.2±1.18^{c}	17.7±0.56^{d}	17.3±1.91^{e}	15.2±0.65^{b}	19.7±1.17^{d}	19.3±1.	19.7±0.92^{d}

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

Table. 7. *Invitro* antirtypanosomal activity of methanolic extract of *Terminalia avorensis* leaves against *Trypanosoma brucei.*

Conc. (mg/ml)	Hours post incubation							
	1	2	3	4	5	6	7	8
5	15.3±0.84^{b}	12.5±1.03^{a}	10.17±1.08^{b}	7.67±0.96^{b}	5.5±0.76^{a}	3.5±0.76^{a}	0.53±0.42^{a}	0.13±0.48^{a}
2.5	18.8±0.95^{cd}	15.5±1.31^{b}	12.2±0.48^{c}	10.2±1.30^{cd}	10.8±1.09^{bc}	10.5±1.41^{b}	8.7±1.35^{c}	6.2±0.79^{b}
1.25	19.5±1.38^{b}	16.3±2.23^{b}	14.7±1.12^{c}	14.8±1.14^{de}	13.3±1.65^{bc}	13.7±1.05^{c}	12.3±1.15^{c}	12.8±0.75^{cd}
0.625	20.0±1.29^{b}	18.0±1.06^{b}	18.2±1.08^{c}	16.7±1.48^{de}	16.5±1.59^{bc}	15.2±1.66^{cd}	14.7±1.28^{c}	13.3±1.09^{bc}
0.3125	21.7±2.45^{b}	20.33±0.49^{a}	20.8±0.95^{bc}	21.8±0.65^{cd}	21.2±1.25^{bc}	20.8±0.94^{c}	20.3±0.92^{c}	19.2±1.30^{c}
0.15625	23.0±0.33^{a}	22.5±72^{a}	22.3±0.13^{bc}	22.0±0.45^{c}	21.2±1.62^{c}	21.3±1.26^{c}	20.5±0.85^{b}	20.2±1.01^{c}
Diminazine	5.3±0.33^{a}	4.0±0.52^{a}	1.5±0.50^{a}	0.17±0.16^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Control	22.1±3.31^{e}	22.2±1.18^{c}	17.7±0.56^{d}	17.3±1.91^{e}	15.2±0.65^{b}	19.7±1.17^{d}	19.3±1.	19.7±0.92^{d}

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

Table 8. *In vitro* antirtypanosomal activity of methanolic extract of *Vitexdoniana root* against *Trypanosoma brucei.*

Conc. (mg/ml)	Hours post incubation							
	1	2	3	4	5	6	7	8
5	14.3±0.84^{b}	12.5±1.03^{a}	10.17±1.08^{b}	6.67±0.96^{b}	2.5±0.76^{a}	0.3±0.76^{a}	0.73±0.42^{a}	0.93±0.48^{a}
2.5	16.8±0.95^{cd}	.145±1.31^{b}	12.2±0.48^{c}	11.2±1.30^{cd}	10.8±1.09^{bc}	10.5±1.41^{b}	9.7±1.35^{c}	8.2±0.79^{b}
1.25	17.5±1.38^{b}	15.3±2.23^{b}	14.7±1.12^{c}	14.8±1.14^{de}	13.3±1.65^{bc}	13.7±1.05^{c}	12.3±1.15^{c}	12.8±0.75^{cd}
0.625	19.0±1.29^{b}	16.0±1.06^{b}	16.2±1.08^{c}	15.7±1.48^{de}	15.5±1.59^{bc}	14.2±1.66^{cd}	14.7±1.28^{c}	13.3±1.09^{bc}
0.3125	22.7±2.45^{b}	22.33±0.49^{a}	21.8±0.95^{bc}	21.8±0.65^{cd}	20.2±1.25^{bc}	20.8±0.94^{c}	19.3±0.92^{c}	19.2±1.30^{c}
0.15625	24.0±0.33^{a}	23.5±72^{a}	23.3±0.13^{bc}	22.0±0.45^{c}	22.2±1.62^{c}	21.3±1.26^{c}	21.5±0.85^{b}	20.2±1.01^{c}
Diminazine	5.3±0.33^{a}	4.0±0.52^{a}	1.5±0.50^{a}	0.17±0.16^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Control	22.1±3.31^{e}	22.2±1.18^{c}	17.7±0.56^{d}	17.3±1.91^{e}	15.2±0.65^{b}	19.7±1.17^{d}	19.3±1.	19.7±0.92^{d}

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

Table 9. *In vitro* antirtypanosomal activity of extract of petroleum ether extract *Annona senegalensis* root against *Trypanosoma brucei.*

Conc. (mg/ml)	Hours post incubation							
	1	2	3	4	5	6	7	8
5	10.3±0.84^{b}	7.5±1.03^{a}	5.17±1.08^{b}	1.67±0.96^{b}	0.5±0.76^{a}	0.5±0.76^{a}	0.23±0.42^{a}	0.43±0.48^{a}
2.5	15.8±0.95^{cd}	11.5±1.31^{b}	9.2±0.48^{c}	6.2±1.30^{cd}	1.8±1.09^{bc}	0.5±1.41^{b}	0.7±1.35^{c}	0.0±0.0^{b}
1.25	19.5±1.38^{b}	14.3±2.23^{b}	10.7±1.12^{c}	8.8±1.14^{de}	6.3±1.65^{bc}	3.7±1.05^{c}	1.3±1.15^{c}	0.8±0.75^{cd}
0.625	21.0±1.29^{b}	19.0±1.06^{b}	15.2±1.08^{c}	12.7±1.48^{de}	10.5±1.59^{bc}	9.2±1.66^{cd}	7.7±1.28^{c}	5.3±1.09^{bc}
0.3125	24.7±2.45^{b}	22.33±0.49^{a}	22.8±0.95^{bc}	21.8±0.65^{cd}	21.2±1.25^{bc}	17.8±0.94^{c}	15.3±0.92^{c}	10.2±1.30^{c}
0.15625	27.0±0.33^{a}	25.5±72^{a}	25.3±0.13^{bc}	23.0±0.45^{c}	23.2±1.62^{c}	22.3±1.26^{c}	22.5±0.85^{b}	19.2±1.01^{c}
Diminazine	5.3±0.33^{a}	4.0±0.52^{a}	1.5±0.50^{a}	0.17±0.16^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Control	22.1±3.31^{e}	22.2±1.18^{c}	17.7±0.56^{d}	17.3±1.91^{e}	15.2±0.65^{b}	19.7±1.17^{d}	19.3±1.	19.7±0.92^{d}

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

Table 10. *In vitro* antirtypanosomal activity of aqueous extract of *Annona senegalensis* stem against *Trypanosoma brucei.*

Conc. (mg/ml)	Hours post incubation							
	1	2	3	4	5	6	7	8
5	11.3±0.84^{b}	8.5±1.03^{a}	5.17±1.08^{b}	2.67±0.96^{b}	0.5±0.76^{a}	0.5±0.76^{a}	0.43±0.42^{a}	0.63±0.48^{a}
2.5	13.8±0.95^{cd}	11.5±1.31^{b}	8.2±0.48^{c}	6.2±1.30^{cd}	4.8±1.09^{bc}	1.5±1.41^{b}	0.7±1.35^{c}	012±0.49^{b}
1.25	17.5±1.38^{b}	15.3±2.23^{b}	12.7±1.12^{c}	8.8±1.14^{de}	6.3±1.65^{bc}	4.7±1.05^{c}	2.3±1.15^{c}	0.8±0.75^{cd}
0.625	22.0±1.29^{b}	20.0±1.06^{b}	17.2±1.08^{c}	14.7±1.48^{de}	12.5±1.59^{bc}	9.2±1.66^{cd}	6.7±1.28^{c}	4.3±1.09^{bc}
0.3125	27.7±2.45^{b}	26.33±0.49^{a}	25.8±0.95^{bc}	23.8±0.65^{cd}	20.2±1.25^{bc}	18.8±0.94^{c}	17.3±0.92^{c}	16.2±1.30^{c}
0.15625	30.0±0.33^{a}	28.5±72^{a}	27.3±0.13^{bc}	25.0±0.45^{c}	22.2±1.62^{c}	20.3±1.26^{c}	19.5±0.85^{b}	18.2±1.01^{c}
Diminazine	5.3±0.33^{a}	4.0±0.52^{a}	1.5±0.50^{a}	0.17±0.16^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Control	22.1±3.31^{e}	22.2±1.18^{c}	17.7±0.56^{d}	17.3±1.91^{e}	15.2±0.65^{b}	19.7±1.17^{d}	19.3±1.	19.7±0.92^{d}

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

IV. DISCUSSION

In this current research report on antitrypanosomal from medicinal plants, *Xeminia americana, Terminadus indica, Prosopis africana, Afromosia laxiflora, Cassia sierber, Terminalia catappa, Vitexdoniana, Terminalia ivorensis, Terminalia superb, Lawsonia inemis, Securidacalonge pedunoulata* and *Terminalia indica* were screened against *Trypanosoma brucei.* Out of these medicinal plants, *Annona senegalensis* (leaves, stems and roots), *Terminalia avorensis,* and *Vitexdoriana* displayed varied moderate to higher antitrypanosomal activity than other medicinal plants screened.

The varied antitrypanosomal activity in this research report which ranged from immobilization to complete killing of the trypanosomes are comparable to trypanocidal activity of comparative *in vitro*trypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian savannah plants Atawodi S.E (2005); antitrypanosomal activity of some medicinal plants from Nigerian (Abiodun et al., 2012); .*in vitro* screening of American plants extracts on *Trypanosoma cruzi* and *Trichomonas vaginalis* (Muella-Serrano et al., 2000); antitrypanosomal and cytotoxicity of methanolic *Plumbagozeylanica* root back against *Trypanosoma evansi* (Shaba et al., 2006); Transferin coupled azanthraquinone enhances the killing effects on trypanosomes (Nok AJ Nock C..2002).

This findings indicate that few of the medicinal plants screened do contain trypanocidal compound(s) if isolated in further research could be a promising candidate for a future trypanocide.

V. CONCLUSION

Different medicinal plants/parts screened for this initial stage of detecting antitrypanoaomal activity from the different solvent extracts in the current research indicate different levels of antitrypanosmal activity. This indicates the presence of antitrypanosomal compound(s)in them. But extracts of *Annona senegalensis* (leaves, stems and roots), *Terminalia avorensis,* and *Vitexdoriana* medicinal plants shown more activity than others, which probably indicate a promisingly trypanocidal compound(s) to be purified and isolated in the near future.

VI. REFERENCES

[1]. Simarro PP, Franco JR, Cecchi G, Paone M, Diarra A.(2010). Human African trypanosomiasis in non-endemic countries. J Travel Med 2012. 19: 44-53. doi: 10.1111/j.1708 8305.2011.00576.x

[2]. World Health Organization (2010).Working to overcome the global impact of neglected tropical diseases: First WHO report on neglected tropical diseases. No. 1.2010, WHO, Geneva.

[3]. World Health Organization (2012). Accelerating Work to Overcome Neglected Tropical Diseases: a Roadmap for Lmplementation. Geneva: World Health Organization. Accessed January, 1 2012.

[4]. World Health Organisation. (2016).Therapeutic activity of partially purified fractions of Emblicaofficinalis (Syn. Phyllanthusemblica) dried fruits against TrypanosomaevansiJ. Pharma and Pharmacol, 4: 546c-558.

[5]. Abiodun O.O., Gbotosho GO, Ajaiyeoba EO., Brun R, Oduola AM.(2012).Antitrypanosomal

[6]. activity of some medicinal plants from Nigerian ethnomedicine.Parasitol Res. 2:521-526.

[7]. World Health Organization (2004) .Anon; communicable Disease Surveillance and Rsponse.WHO, Geneva.

[8]. Nok AJ Nock C.(.2002).Transferin coupled azanthraquinone enhances the killing effects on

trypanosomes. The role of lysosomalmannosidase, J Patasites; 9: 375-379

[9]. Kubuta B.K. Kisaburo N. Nobutoshi M. Patrick M. Zakayi K. Samuel K. Martinf. Taba M. Kalulu. Haq M. Mitsuru Y. Mayumi O. Taroh K. Michael D. Yoshihiro U.(2005). Kola acuminataproanthocyanidins: a class of anti-trypanosomal compounds effective against Trypanosomabrucei. Intl J. Parasitol: 35, 91-103.

[10]. Freiburghaus F, Steck A, Ptander H, Brun R. (1998). Bioassay Guided lsolation of a Diastereoisomer of Kolavenol from Entadaabsyssinicaactive on Trypanosoma bruceirdesiense. J Ethnopharmacol 61:179-183.

[11]. Shaba P., Sahab D., Singh R K, Chaudary P. (2006).Trypanocidal Activity ofArsenicum album (C-30) against Trypanosomaevansi. Intl J. Pharrmaceutsciinvnt, 5: 40-46

[12]. Shaba P, Pandey NN, Sharma OP, Rao J R, Singh RK. (2006). Antitrypanosomal and cytotoxicity of methanolic Plumbagozeylanicaroot back against Trypanosomaevensi. JVet Pub Hlth4: 31-6.

[13]. Shaba P, Dey S, Kurade NP, Singh RK. (2016) Antitripanosomal Activity of Picrorrhizakurroa Rhizomes against Trypanosomaevansi. Advances in Pharmacog. and Phytomed., 1(1)49-5, 4.

[14]. Ameenah, G. and Mohammed, F. M.(2013). African flora as potential sources of medicinal plants: Towards the chemotherapy of major parasitic and other infection diseases - A review. Jordan Journal of Biological Sciences. 6:77 - 84.

[15]. Shaba P, Pandey N.N, Sharma O P, Rao J R., Singh RK.(2011). Antitrypanosomal Activity of piper nigrumL (Black pepper) against trypernosomaevansi. JVet Adv 2:161-167

[16]. Mikail, H. G. (2015). Acute toxicity and in vitrotrypanocidal activity of the methanolic leaves extract of Lanneaschimperi. International journal of Advanced Research in Biological sciences, 2 (11): 52-57.

[17]. Madaki, F. M., Kabiru, A.Y., Mann, A., Abdulkadir, A., Agadi, J.N. and Akinyode, A.O. (2016). Phytochemical Analysis and In-vitro Antitrypanosomal Activity of Selected Medicinal Plants in Niger State, Nigeria. International Journal of Biochemistry Research and Review,11 (3): 1 - 7.

[18]. Ogbole, E., Dashak, D. A., Nvau, J. B., Daben, M.R., Abongaby, G., Obaloto, O.B., Oladipo, O.O. and Igweh, A.C. (2017). Phytochemical screening and in vitro evaluation of the antitrypanosomal action of the methanolic leaf extract of Corymbiatorelliana International Journal of Ethno medicine and Pharmacognosy, 3(1): 20 - 29.

[19]. Stahl E. Thin layer chromatography. A Laboratory Handbook Springer, New York, 1969

[20]. Williamson J, March JC, Scott-Finning JJ. (1982).Drug synergy in experimental African trypanosomiasis. Tropennmedizin und Parasitologie 1982; 33: 76-82

[21]. Atawodi S.E (2005): Comparative in vitrotrypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian savannah plants. African Journal of Biotechnology; 4(2): 177-182.

[22]. Oliveira BA, Pereira DG, Fernandes AMAP, De Castro SL, Souza ARM, Brito AO.(1990) DeSouza Philipson J D. Plants as a Source of Valuable Products. In: Secondary Products from Plants Tissue Culture, Charlwood, B.V. and M.J. Rhodes (Eds.). Clarendon Press, Oxford, pp: 1-22,

[23]. Talakal, T.S., Dwivedi, S.K. and Sharma, P. (1995).In vitro and in vivoantitrypanosomal activity of Xanthium strumariumleaves. Journal of Ethnpharmacology, 49, 141-145.

[24]. Igweh AC, Aguiyi JC, Okwuaasaba F.K. (2002).Antitrypanosomal Effect of the Aqueous Extract of Brassiscaoleracea.JFitotera; 71: 17-21.

[25]. Sidwell, RW, Huffman J.H. (1997). Antiviral drug resistance. Res Virol 1997; 148: 353-365.

[26]. Kumar KJ.(2006).Effect of geographical variation on contents of tannic acid, gallic acid, chebulinic acid and ethyl gallate in Terminalia Chebulafruits. NatProd 2: 170-175.

[27]. Muella-Serrano, S; Nogal,J .J; Martinez-Diaz, R. AEscario J. A; and Gomez Barrio, A. (2000).In vitro screening of American plants extracts on Trypanosomacruzi and Trichomonasvaginalis. J. Ethnopharmacol., Limerick, v. 71, p. 101-107