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Original Research Article

In-vitro Evaluation of the Trypanocidal Activity of *Andrographis paniculata* Against *Trypanosoma brucei brucei*

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ABSTRACT

Andrographis paniculata belongs to the Family Acanthaceae and is used for medicinal purposes due to its broad-spectrum activities against cancer, hepatitis, HIV, anti-inflammatory, management of cardio-vascular diseases and boosting the immune system. The purpose of this research is to establish the *in-vitro* trypanocidal activity of *Andrographis paniculata* against *Trypanosoma brucei brucei.*

The dried leaf portion was pulverized in a mortar to semi-fine powder and macerated with 70% ethanol. The ethanolic extract was fractionated with hexane, dichloromethane, ethyl acetate and methanol to afford their various crudes which were subjected to *in-vitro* tests against *Trypanosoma brucei brucei* using Diminazene diaceturate as a standard control.

Apoptosis was observed at higher concentrations (10, 5, and 2.5 mg/mL) but trypano-static at lower concentration (1.25 mg/mL). Trypanocidal activity of the various extracts showed comparable inhibitory effect (100%) on *Trypanosoma brucei brucei* with the standard drug. From this study, *T. b. brucei* (Federe strain) was visible in bloodstream within *Trypanosoma brucei brucei* 7 days post infection (dpi) and reached cerebrospinal fluid within 11 dpi. Data on acute toxicity showed the plant to be non-toxic on the experimental animals.

A. paniculata has demonstrated medicinal properties used in traditional medicine in some countries to treat various diseases. The potentials of the extracts used from this study showed the extract to possess trypanocidal activity in the management of *T. b. brucei*. Further purification of the ethyl acetate fraction for structural and *in-vivo* studies against *Trypanosoma brucei brucei* is recommended.

Keywords: *Trypanosoma brucei brucei, Andrographis paniculata,* Trypanocidal activity, Fingerprinting, Phytochemical screening.

Introduction

The Human African Trypanosomiasis (HAT) research is well documented in the literature and affects mostly countries in the African continent. The trypanosomes and other parasites use many immune evasion pathways to sustain their survival and finalize their life cycles within their hosts.[1] The complexity of constituents from natural sources having anti-protozoal activities have been reported in the literature which covers compounds having trypanocidal activity on the trypanosomes responsible for Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT).[1-2] Several plant constituents have been screened for their *in-vitro* activity on bloodstream trypomastigotes of African trypanosomes while little *in-vivo* test conducted with no report on clinical examination.

Therefore, screening of natural products against trypanosomes should be encouraged for the discovery of new trypanocidal lead compounds. Current anti-trypanosomal remedies are synthetic drugs and those available such as pentamidine, suramin, melarsoprol, and eflornithine pose a serious set-back ranging from little activity, the emergence of drug resistance over prolong use, the complexity of the drug administration, and high cost of production, unavailability and numerous undesirable side effects.[3] These trends have driven scientific efforts geared towards the research of plants and marine sources as cheaper, available and non-toxic trypanocides. With the availability of this plant in Nigeria, there is no reported anti-trypanosomal activity carried out despite its numerous medicinal uses, it is therefore, justified to evaluate the extracts of this plant for the discovery of trypanocidal compounds that can be used for the management of trypanosomiasis. The *Andrographis paniculata* (Acanthaceae) plant is native to South Asia such as China, India and Taiwan and now can be found in Africa. It is a medicinal herb with an extremely bitter taste and can be used in the management of liver disorder, common cold and upper respiratory tract infections.[4] In Chinese traditional medicine, *A. paniculata* is used to relieve internal heat, inflammation and pain and also for detoxification. The plant has been reported to possess antimicrobial, antioxidant and anti-inflammatory activities,

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antihyperglycemic and hypoglycemic activities.[5] Also, prophylactic activity of *A. paniculata* against *Trypanosoma b. brucei* has been reported.[6] It is against this backdrop that this research is focused at evaluating the *in vitro* trypanocidal activity of *Andrographis paniculata* against *Trypanosoma brucei brucei*.

Some of the major phytochemical constituents of the plant include diterpenoids, flavonoids and polyphenols with potent health benefits. The derivatives of the isolated compound such as Andrographolide exhibit anti-inflammatory, anti-cancer and anti-viral activities which suggest its potential use in drug development.[7] The main objective of this research is to carry out extraction, fractionation and screening of phytoconstituents of the leaf portion and to characterize the fractions for trypanosomal activity *in-vitro*.

Materials and Methods

Sample collection and identification

Plant material was collected from the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria in May 2019. Authentication of the plant was carried out by Malam U. S. Gallah of the Department of Biological Science, Kaduna State University with voucher number (1895) deposited at the herbarium section of the University.

Chemicals/solvents

Diminazene diaceturate (DD), RPMI 1640 cell culture media (L-gutamine, 25Mm HEPES) were purchased from a local scientific store and the products were of high standard (Sigma Chemical Co.). All solvents used were of analytical grades from sigma-Aldrich and Fluka respectively and were used without further purification.

Extraction and isolation

The *Andrographis paniculata* dry leaves were powdered using a pestle and mortar. Exactly 350 g was macerated using n-hexane (2.3 L) in an aspirator with occasional agitation for 24 h. This was filtered and concentrated using a rotary evaporator and further concentrated on a water bath set at 40^{o}C to afford the crude extracts **(**5 g, 1.43%). The maceration process was repeated, with the dried marc using 70% ethanol for 48 h and was filtered and concentrated using **a** rotary evaporator to afford a dark brown solid mass (15 g, 4.20%). The two crude samples were refrigerated until required for analysis.

Qualitative screening for phytochemicals

Qualitative screening for phytochemical of the extracts was carried out using standard methods.[8-9] The phytochemical constituents screened were terpenoids/steroids, cardiac glycosides, anthraquinones, saponins, alkaloids, carbohydrates, tannins, and flavonoids.

Fractionation and chromatographic purification

The crude 70% ethanol extract (15 g, 4.20%) was subjected to fractionation using dichloromethane and ethyl acetate which yielded (6.78 g, 44.47%) and (8 g, 53.33%), respectively. These fractions were refrigerated until required for further analysis. Thin-layer chromatography (TLC) was carried out on the fractions using pre-coated thin-layer chromatographic plate. The precoated plate was activated for 10-15min at 110^{o}C and allowed to cool before use. Crude extracts of dichloromethane and ethyl acetate were examined on the TLC plate using hexane and ethyl acetate (1:1v/v) as mobile phase. The various R_f – values were calculated for the well resolved components.

Exactly 7 g of the ethyl acetate fraction was dissolved in 10 mL of ethyl acetate and 5 g of silica-gel (60-200 mesh) was added and triturated using pestle and mortar to make fine homogenous powder. The powdered material was chromatographed on silica-gel (237 g) in an open glass column (5 cm x 100 cm) using a combination of hexane: ethyl acetate; 350 mL (95:5, 90:10, 85:15, 80:20 up to 5:95) and ethyl acetate: methanol in a gradient mobile phase system yielding twenty-five fractions. The fractions collected were pooled together based on their similar R_f-values to give a total of eight fractions coded as A, B, C, D, E, F, G and H. The Liquid chromatography profiling and infrared fingerprinting of one of the fractions (G: m.pt = 288-290^{o}C) was further obtained based on its high yield and relative purity (m.pt.288-290^{o}C). The following HPLC analytical conditions were used: Column, ODS C_{18}, modifier; isocratic elution with methanol and water (65:35) while the flow rate used (1.5 mL/min) and wavelength of detection (223 nm) respectively. The Infrared fingerprinting was obtained on KBr disc.

Characterization of trypanosome

Trypanosoma brucei brucei (Federe strain) was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria and inoculated intraperitoneally into two albino rats which serve as donor animals. They were properly monitored for 72 h to ascertain the presence of trypanosome. One of the animals was humanized (according to the animal management guidelines of the Nigerian Institute for Trypanosomiasis Research) to get infected blood into **a** sample bottle containing EDTA. The blood was diluted with phosphate buffer saline-glucose (PBSG) to have the desired parasites per mL (10^4 in 0.2 mL). Parasite count was estimated using the rapid matching method.[10] Albino rats were randomly selected into three (3) groups with three animals, negative control, positive control and standard control (Diminazene diaceturate; Trypadim® Merial, France).

In-vitro infectivity test of Andrographis paniculata leaf extract against Trypanosoma brucei brucei

Exactly 50 mg each of 70% ethanol crude leaf extract, dichloromethane and ethyl acetate fractions of *A. paniculate* were weighed separately and dissolved in 250 µL of dimethyl sulfoxide (DMSO) to give a concentration of 200 mg/mL extract solution in DMSO (stock solution). RPMI 1640 cell culture media (L-gutamine, 25 mM HEPES) was supplemented with 10% (v/v) heat-activated horse serum, 1% (w/v) glucose-D and 40 mg/mL gentamycin sulphate. The supplemented media was used to dilute the stock solution of the extract to obtained desired concentrations. 950 µL of the supplemented media was added to 50 µL of the 200 mg/mL extract solution to give 10, 5, 2.5, 1.25 and 0.75 mg/mL. These concentrations were prepared by serial dilution (2-fold dilution) of 10 mg/mL for each extract. 100 µL of the different concentrations was dispensed in triplicate into wells of a 96 well micro-titre plate. An equal volume of standard control (Diminazene diaceturate; Trypadim® Merial, France) at the same concentrations was also dispensed in triplicate. A rat infected with trypanosome (*Trypanosoma brucei brucei*) was humanly anaesthetized using chloroform; blood was collected by cardiac puncture into a sample bottle containing EDTA. The whole blood was mixed with the supplemented media at ratio 1:1 (v/v). 30 µL of the diluted infected blood was added to each of the well of micro-titre plate containing either extract or Trypadim®. The resulting trypanosome count was approximately 10 per field (3.7×10^7 parasite/mL). Control wells contain 100 µL of the supplemented media and 30 µL of the diluted infected blood. The micro-titre plate was placed in a candle jar containing about 5% CO_2 at 34^{o}C. Trypanosome count in each well was observed 2 h post- incubation using **a** microscope at x40 magnification. In order to ascertain the trypanostatic observed at 0.75 mg/mL, mixture in micro-titre plate wells for each group (crude extracts and Trypadim®) was pulled together and made up to 0.5 mL with 5% D-glucose. Two active albino rats were inoculated intraperitoneally (0.2 mL) with the residual solution for the extract and standard control. These animals were monitored for parasitemia for 21 days.

Acute toxicity test

The method of Organization for Economic Co-operation and Development (OECD) guideline 42310 and the principles of good practice and animal handling (National Institute of Health Guide for Laboratory Animals) were used for the acute toxicity test. The animal care ethical clearance committee no. ARAF/08/18 was obtained from Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna, Nigeria. Twelve matured female albino Wistar rats with an average weight of 196 g were randomly selected into four (4) groups of three (3) animals each (group A-D); group A was administered 300 mg/kg

body weight 70% crude ethanol extract, 2000 and 5000 mg/kg body weight for groups B and C while group D was used as control. The extract was administered orally using oral gavage. All the animals were examined periodically during the first 24 h (0.5, 1, 2, 4 and 24 h) for gross behavioral changes and signs of toxicity such as motor coordination, compulsion, salivation, loss of reflex, changes in skin and furs, eye discharge, respiratory disorder and death.
The histopathological test was carried out on different organs (liver, spleen and kidney) collected from the experimental rats to determine the level of toxicity. Mortality if any was determined for two weeks.[10]

Statistical analysis
The group means ± SEM was calculated for each extract using the analysis of variance (ANOVA) while post-test analysis was carried out using Duncan comparisons. All statistical analysis was performed using GraphPad Prism software package (version 5).

Results and Discussion

Phytochemical constituents of Andrographis paniculata
The results of the qualitative phytochemical constituents of the leaf extract is shown in Table 1, which revealed the presence of carbohydrates, saponins, flavonoids, tannins, terpenoids/steroids, alkaloids, cardiac glycosides and anthraquinones in the extracts. Alkaloids were present in the ethanol and ethyl acetate extracts but absent in dichloromethane fraction. Alkaloids are defensive secondary metabolites on herbivores and pathogens. They are generally exploited as pharmaceuticals, stimulants, narcotics and poisons due to their potent biological activities.[11] Tannins were present in all the extracts analyzed (Table 1) and are widely used in herbal medicine to treat wounds and to arrest bleeding.[12,13] The growth of many fungi, yeasts, viruses and bacteria can be inhibited by tannins.[13]
Flavonoid was present in ethyl acetate fraction and absent in dichloromethane fraction and ethanol leaf extract (Table 1) respectively. Flavonoid compounds are well known for their antioxidant activity and their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radicals. Flavonoids have also been reported as having hepatoprotective, antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic and vasodilatory activities.[14] Saponin was present in all the three extracts analyzed and are well known for their antioxidant properties. They have a property of precipitating and coagulating red blood cells as well as having hepatoprotective, antibacterial, antifungal and antiviral activities.[15]
Cardiac glycoside was present in the ethyl acetate fraction but absent in ethanol and dichloromethane extracts. *A. paniculata* is very bitter, and the bitterness in the plant may probably be due to the presence of these secondary metabolites (glycosides). Some cardioactive glycosides have been shown to exhibit activity in cough suppression (anti-tussive) and are very toxic thereby limiting their wide spread use.[16] Terpenoids/sterols were present in the ethanol and dichloromethane extracts but absent in the ethyl acetate fraction. The bioactive constituents of the *A. paniculata* can be attributed to its terpenoids with higher concentration and potent activity. The presence of these secondary metabolites in the extracts analyzed could be responsible for the anti-typanosomal activity observed in this study and also validate the claims of the medicinal and clinical uses of *A. paniculata* in the management of **a** variety of diseases especially the neglected tropical diseases (NTD's).
The dichloromethane and ethyl acetate fractions of *A. paniculata* leaf gave four spots on TLC profiling (Figure 1) with a solvent system of hexane: ethyl acetate (1:1). The R_f value of dichloromethane and ethyl acetate fractions were calculated as: 0.27, 0.73, 0.89, 0.95 and 0.24, 0.71, 0.898, 0.95, respectively. These profiles may serve as a characteristic fingerprint of *A. paniculata* leaf and therefore suitable for monitoring the identity and purity of the resolved constituents.

Thin-layer chromatography
The dichloromethane and ethyl acetate fractions of *A. paniculata* leaf gave four spots on TLC profiling (Figure 1) with solvent system of hexane/ethyl acetate (1:1; v/v). The R_f values of dichloromethane and ethyl acetate fractions were calculated as: 0.27, 0.73, 0.89, 0.95 and 0.24, 0.71, 0.898, 0.95, respectively. These profiles may serve as a characteristic fingerprint of *A. paniculata* leaf and therefore suitable for monitoring the identity and purity of the resolved constituents. One of the fractions obtained from open column chromatography (Fraction G) a pale-yellow powder with a melting point of 288-290^{o}C was confirmed by TLC in different solvent systems viz: hexane/chloroform (4:1 v/v), petroleum ether/ethyl acetate (7:3 v/v) and hexane/acetone (7:3 v/v) gave one spot each with different R_f-values. The sample was submitted for High-Performance Liquid Chromatography Profiling (HPLCP) and Fourier Transformed Infrared (FTIR) analysis.

Liquid chromatography profiling
A relatively pure (solid with melting point = 288-290^{o}C) fraction of *Andrographis paniculata* isolated from the leaves was analyzed with High-Performance Liquid Chromatography (HPLC) using the following analytical conditions; Column, ODS C_{18}, modifier, isocratic elution with methanol and water (65:35). The flow rate used (1.5 mL/min) and wavelength of detection (223 nm). Chromatogram of the fraction (Figure 2), showed one sharp and intense peak with retention time (R_t = 0.840 min) which differs from the retention time of standard andrographolide (2.871 min) earlier reported.[17] The sharp and intense peak eluting at 0.840 min may probably be an adrographolide which may be attributed to the variation of the active principles due to climatic and soil conditions. The HPLC profiling showed the relative purity of one of the fractions isolated and is an important tool in chromatographic purification of organic compounds. This isolate was further analyzed using the Fourier Transform Infrared spectrometer to determine the functional groups present.
The FT-IR analysis of isolate A3 (Figure 3) showed four important functional groups namely: O-H_{str} (3350 cm^{-1}), C-H_{str} (2929 cm^{-1} and 2855 cm^{-1}), C=O_{str} (1736 cm^{-1}) and C=C_{str} (1640 cm^{-1}). These functional groups can be useful in the structure elucidation of the fraction. However, data obtained from HPLC and FT-IR is insufficient to ascertain the compound structure.

Acute toxicity test
Within the study period of 14 days, no signs of acute toxicity such as motor in-coordination, compulsion, salivation, loss of reflex, eye discharge, and respiratory disorder in all the experimental animals. However, rough furs were observed in the group administered with a dose of 5000 mg/kg body weight (BW). In addition, no loss of weight and mortality was observed in all the animals suggesting the relative safety of *A. paniculata*. The histopathological results (data not shown) indicate mild lesions in the livers of the animals administered with 5000 mg/kg BW. Our findings from this study supported earlier report that a single oral administration of *A. paniculata* with an upper fixed dose of 5000 mg/kg BW has no significant acute toxicological effects.[18]

Parasite characterisation
Trypanosoma brucei brucei is known to be a pathogenic specie that possesses the ability to cross blood-brain membrane within the shortest period. In this study, we observed that *T. b. brucei* (Federe strain) isolate from Nigeria was visible in the bloodstream 7 days post infection (dpi) and reached cerebrospinal fluid (CSF) within 11 days post infection (Table 3). Diminazene diaceturate; Trypadim® possesses trypanocidal effect on *T. b. brucei* at hemolytic (early) and encephalitic (late) stages. This implies that *in vivo* therapeutic investigation of any natural product or synthetic product should be administered at 7 dpi for hemolytic stage and 11 dpi for the encephalitic stage of trypanosomal infection. One of the symptoms of parasitic infection is the unstable temperature which in turn leads to weight loss because of a reduction in food intake.
However, stability in weight was observed in the experimental animal used for characterization of *T. b. brucei* (Table 4); there was a significant difference ($P < 0.05$) in temperature after 10 dpi when compared with the negative control. The present study supports the earlier reports on the trypanosome effect on temperature.[6]

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In-vitro infectivity test

The efficacy of *A. paniculata* leaf extract against *T. b. brucei* for its trypanocidal activity was observed at higher concentrations (10, 5, and 2.5 mg/mL) but trypanostatic effect occurred at lower concentrations (1.25 and 0.75 mg/mL). This was comparable to the conventional trypanocidal drug (Trypadim®) used (Table 4). The trypanocidal activity of the extracts could be attributed to the synergistic effect generated from secondary metabolites present in the extract. Parasite motility can be used as an indicator of viability of zoo flagellate parasites[19] and complete hemolysis of trypanosomes may serve as a measure of anti-trypanosomal potential of crude extract when compared to viable trypanosomes observed in the control group. The result obtained is similar to other investigations that some plants had promising activity against trypanosomes.[1,6,18-20] Besides, it is reported[21] that methanol extract of *A. paniculata* significantly inhibited *Plasmodium falciparum* at lower concentration of 7.2 µg/mL with 50-percent inhibitory concentration (IC_{50}). This implies that *A. paniculata* possesses phytochemicals which are capable of exerting an inhibitory effect on protozoan. Protozoan parasite of the genus Trypanosoma has been reported[22,23] to be responsible for the Neglected Tropical Diseases (NTD). The disease is transmitted by vectors, the bites of tsetse flies *(Glossina spp*.) which facilitate entry of the parasite into the human host. The parasite further invades the central nervous system upon multiplication and subsequent crossing of the blood -brain barrier, it is at this stage that the more obvious symptoms will manifest.[24] The mechanism of action of the extracts is unknown at this stage of the study. However, natural products are known to possess phytochemical constituents with antioxidant activity capable of mopping radicals that may impose peroxidative damage to a very sensitive enzyme which is important in redox balance of trypanosome. Moreover, saponins are reported to interact with parasite membrane, protein and phospholipids thereby causing parasite apoptosis.[14] It has been established[25] that a flavonol; quercetin which most plants possess directly induces death of *Trypanosoma brucei gambiense* without affecting normal cell viability. In this study, it was shown that the leaf extract of *A. paniculata* with trypanocidal activity has no effect on the morphology of red blood cells. This implies that *A. paniculata* toxicity level is minimal as was observed in the toxicity study. Anti-malarial activity of *A. paniculata* on *Plasmodium falciparum* without toxic side effects on haematological parameter and some organs like liver and kidney has been reported.[19] In addition, xanthones isolated from *A. paniculata* roots was found to exhibited substantial anti-protozoal activity against *T. b. brucei*, *T. cruzi* and *L. infantum*.[24] Several investigations on the plant have established that it possesses various biological activities such as anti-microbial, anti-inflammatory, anti-oxidant, cytotoxicity, immunostimulant, anti-diabetic, anti-angiogenic, sex hormone modulatory, hepato-renal protective, liver enzymes modulatory and insecticidal activities.[4,5,8,25] Result obtained after 21 days post inoculation of extract/trypanosome mixture (Table 4) suggested that *A. paniculata* has no potency against *T. b. brucei* at lower concentrations. Therefore, mobility index alone is not sufficient to determine anti-trypanosomal efficacy of any substance.

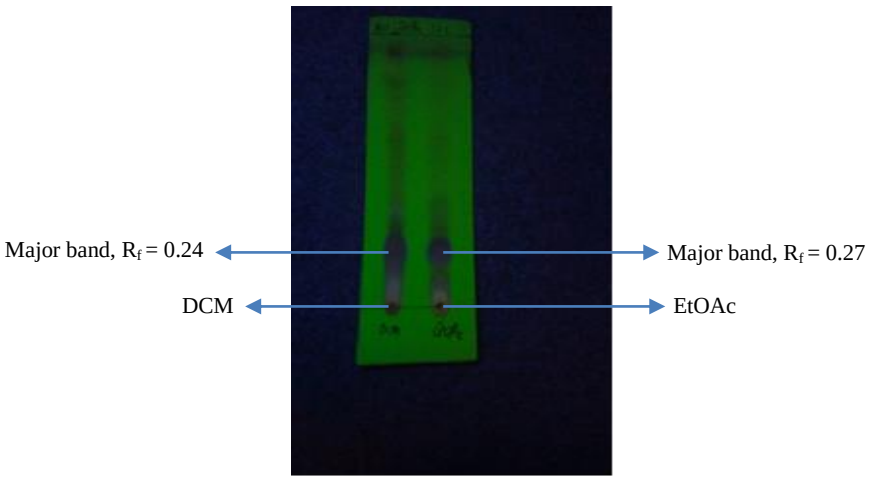

Figure 1: TLC profile of dichloromethane (DCM) and ethyl acetate (EtOAc) fractions viewed under **a** UV lamp at 365nm.

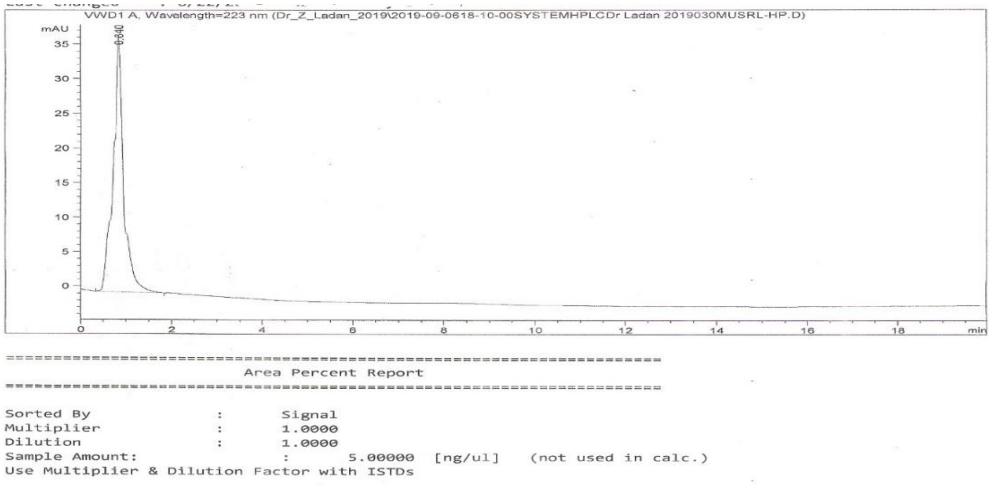

Figure 2: High Performance Liquid Chromatography profiling of isolated major band

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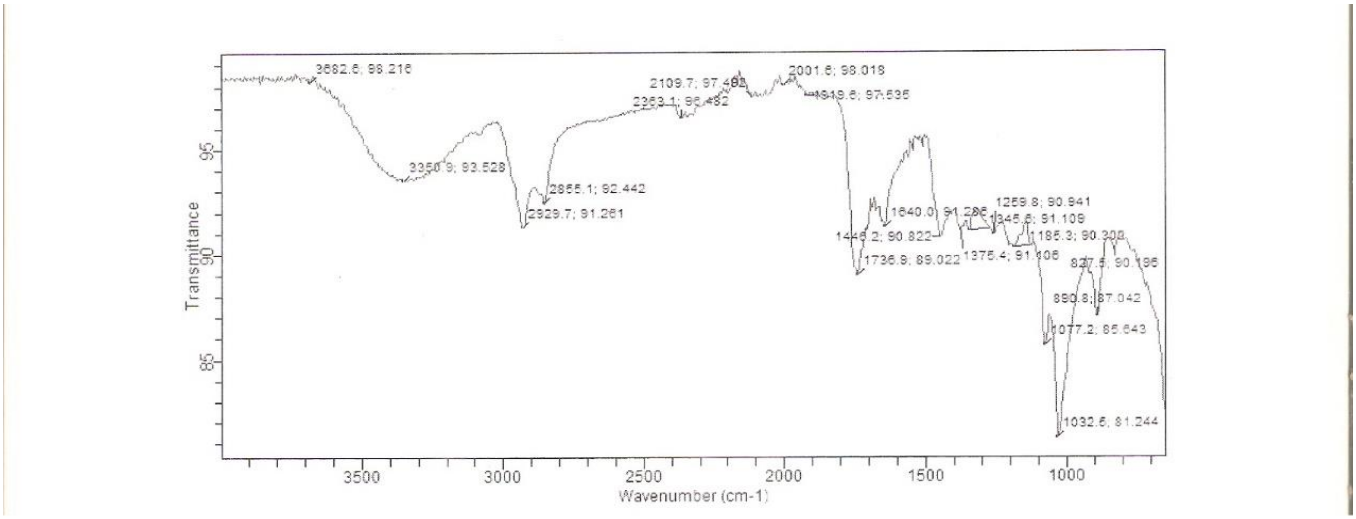

Figure 3: FTIR spectrum of isolated HPLC major peak

Table 1: Phytochemical Constituents of *Andrographis paniculata* leaf extract

Phytochemical constituents	Observation		
	70% EtOH extract	DCM extract	EtOAc extract
Saponins	+	+	+
Terpenoids/steroids	+	+	-
Cardiac glycosides	-	-	+
Anthraquinone	+	-	+
Carbohydrates	+	-	-
Tannins	+	+	+
Flavonoids	-	-	+
Alkaloid	+	-	+

Note: (+) present; (-) absent; EtOH = Ethanol, DCM = Dichloromethane, EtOAc = Ethyl acetate

Table 2: Post Infection Parasitemia

	No. of rats	Av. Parasitemia/field	No. of rats	Av. Parasitemia/field	No. of rat	Av. Parasitemia/field	No. of rats	Av. Parasitemia/field	No. of rats	Av. Parasitemia/field	No. of rats	Av. Parasitemia/field
Positive control	3	0.04	3	6.7	2	16	2	50	1	60	1	massive
Trypadim®	3	0.06	3	18.7	3	-	3	-	3	-	3	-

Note: Trypadim® was administered after 9 days post infection. No animal survive infection without treatment beyond 21 days

Table 3: Mean Weight (g) and Mean Temperature (^{0}C) of experimental rats

	3 days post Infection	7 days post Infection	9 days post Infection	12 days post Infection	16 days post Infection
			Weight (g)		
Negative Control	80.40 ± 3.59^a	94.00 ± 3.90^a	102.00 ± 2.70^a	106.00 ± 2.90^a	109.00 ± 2.70^a
Positive Control	109.00 ± 9.12^a	121.00 ± 8.40^a	125.00 ± 8.80^a	124.00 ± 8.40^a	123.00 ± 8.30^a
Trypadim®	130.00 ± 12.10^b	136.00 ± 22.0^a	138.00 ± 22.00^a	139.00 ± 22.00^a	136.00 ± 22.00^a
			Temperature (^{0}C)		
Negative Control	35.90 ± 0.47^a	36.00 ± 0.71^a	35.00 ± 0.24^a	35.00 ± 0.53^a	35.00 ± 0.38^a
Positive Control	36.10 ± 0.98^a	37.00 ± 0.20^a	35.00 ± 0.03^a	37.00 ± 0.10^b	36.00 ± 0.34^a
Trypadim®	35.90 ± 0.21^a	35.00 ± 0.29^a	35.00 ± 0.03^a	37.00 ± 0.40^b	36.00 ± 0.64^a

Values are mean ± SEM, n = 3. Values with different superscripts along a column are significantly different at P < *0.05*

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Table 4: *In-vitro* infectivity test (parasite/mL) of *Andrographis paniculata* leaf against *T. brucei brucei*

Concentration (mg/mL)	70% Ethanol Crude extract	Dichloromethane	Ethylacetate	Trypadim
10	-	-	-	-
5	-	-	-	-
2.5	-	-	-	-
1.25	$1.0 \times 10^{6*}$	3.0×10^{5}	-	-
0.75	$9.5 \times 10^{6*}$	9.5×10^{6}	$3.0 \times 10^{5*}$	$3.0 \times 10^{5*}$
Control	3.7×10^{7}	3.7×10^{7}	3.7×10^{7}	3.7×10^{7}

Post Inoculation of extract/trypanosome mixture

	6 Days Post Inoculation		9 Days Post Inoculation		13 Days Post Inoculation		15 Days Post Inoculation		21 Days Post Inoculation	
	No. of rat	Average Parasitemia per field	No. of rat	Average Parasitemia per field	No. of rat	Average Parasitemia per field	No. of rat	Average Parasitemia per field	No. of rat	Average Parasitemia per field
Leaf extract	2	Massive	1	Massive	0	-	0	-	0	-
Trypadim®	2	-	2	-	2	4	1	10	0	-

Note: * represents stagnation of trypanosomes

Conclusion

Andrographis paniculata remains one of the traditionally used herbs for the treatment of many diseases. In this study we have reported the presence of some secondary metabolites in the plant leaves and it potency against *T. b. brucei*. We also profiled chromatographic data of some of the components of *A. paniculata* leaves and reported the plant as a relatively safe herb. However, we recommend that further work should be carried out to isolate, purify and characterize the active constituents responsible for the trypanocidal activity observed, also, the mechanism of interaction of the active principles with the trypanosome at the molecular level is required.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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