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TRYPANOCIDAL ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *ANDROGRAPHIS PANICULATA*

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ABSTRACT
Andrographis paniculata used in this study belongs to Acanthacae family and is commonly known as king of bitters. This study aimed at evaluating effect of *A. paniculata* leaf extract on experimental rats infected with *Trypanosoma brucei brucei*. Cold maceration was used for extraction and the biomass was fractionated in open column chromatography. One of the ethanolic fractions obtained from *A. paniculata* was evaluated for *in vitro* and *in vivo* activities using RPMI 1640 cell culture media and experimental wistar rats respectively. The fraction exhibited trypanocidal activity by completely clearing trypanosomes within 18h incubation when compared with standard control (diminazene diaceturate). Our findings from *in vivo* assay of the isolate on *T. brucei brucei* showed that trypanocidal activity is dose-dependent. Parasitic replication was faster among the experimental rats treated with lower concentrations in comparison with higher concentration. Moderate average packed cell volume (PCV) was recorded among the treated animals compared with the positive control. Three major absorption bands of interest in the fraction examined were 3268cm^{-1}, 2926 cm^{-1} - 2855 cm^{-1} and 1748 cm^{-1} revealing the presence of O-H$_{str.}$, C-H$_{str.}$ and C=O$_{str.}$ stretching vibrations. *A. paniculata* can be explored for development of new trypanocidal agent for the management of trypanosomiasis.

Keywords: *Andrographis paniculata*, *Trypanosoma brucei brucei*, diminazene diaceturate, packed cell volume

1. INTRODUCTION

African trypanosomiasis is a deadly disease associated to poverty, disaster, war, internal crisis in a particular region. The disease is transmitted through biting of infected tsetse flies (*Glossina* sp.). *Trypanosoma brucei gambiense* affects Central and West Africa region while *Trypanosoma brucei rhodesiense* affects East Africa region. Efforts to control the disease rely on policy implementation that targets transmission, diagnosis, treatment and management of both human and animal trypanosomiasis. For human African trypanosomiasis (sleeping sickness), World Health Organization (WHO) in conjunction with other stakeholders such as Tropical Disease Research (TDR) set the goal of elimination of sleeping sickness; gambiense form of the disease by 2030 (Franco *et al*., 2018). However, toxicity and burdensome mode of administration of most of the available drugs might influence rate of achieving this goal. African animal Trypanosomiasis impact on food security and revenue generation in sub-Saharan African stands at US$4 billion (FAO, 2018). The loss is on increase due to development of resistance to the available drugs for treatment and management of animal Trypanosomiasis (Achenef and Bekele, 2013; Giordani *et al*., 2016). Various industries are now on the search for sources of alternative which include synthetic and natural products. Medicinal plants remain a major source of alternative medicine and scientific investigations have shown that various plants indicate promising results for the development of cheaper less toxic drugs for treatment and management of trypanosomiasis (Simoben *et al*., 2018). *Andrographis paniculata* evaluated in this study belong to Acanthacae family of the plant kingdom and commonly known as king of bitters. The plant has exhibited multifunctional medicinal properties with activity against fever, annular ulcer, cancer, diabetes, snakebite, inflammation, microbial and parasitic infections amongst others (Verma *et al*., 2012; Agbonlahor *et al*., 2014). This study was aimed at evaluating effect of *A. paniculata* leaf extract on experimental rats infected with *Trypanosoma brucei brucei*.

2. MATERIALS AND METHODS

2.1 Plant preparation and identification

The plant material was collected from the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria in May 2018 and authenticated by Malam U. S. Gallah of the Department of Biological Science, Kaduna State University with voucher number (1895) which was deposited at the herbarium section of the University.

2.2 Chemicals/Solvents

RPMI 1640 cell culture media (L-gutamine, 25Mm HEPES) and Diminazene diaceturate (DD) 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 and were used without further purification.

2.3 Extraction and Phytochemical analysis

Dry leaves of *A. paniculata* were crushed to powder form using a pestle and mortar in the laboratory. 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 rotary evaporator and further concentrated on a water bath set at 40^{o}C to obtain the crude extract. The maceration process was repeated with the dried marc using 70% ethanol for 48 h; filtered and concentrated using rotary evaporator to afford a dark brown solid mass. The two crude samples were kept in refrigerator until required for analysis. The plant part was evaluated for presence of secondary metabolite using method described by Edeoga *et al.* (2005).

2.4 Chromatography

The concentrated 70% ethanol crude extract (15 g, 4.20%) was subjected to sequential fractionation using dichloromethane and ethylacetate to obtain 6.78 g (44.47%) and 8 g (53.33%) respectively. Thin layer chromatography (TLC) was carried out on the fractions using precoated thin layer chromatography plate. The plate was activated for 10 -15 min at 100° C -110°C and allowed to cool before use. The crude extracts (dichloromethane and ethylacetate) were examined on the TLC plate with hexane and ethylacetate (1:1; vol/vol) mobile phase. The crude ethylacetate fraction (7 g) was dissolved in 10 mL of ethylacetate and 5g of silica-gel (60-200 mesh) and macerated very well using pestle and mortar to make fine homogenous powder. The powdered material was chromatographed on the silica-gel (237 g) in open column chromatography (5cm x 100cm) using the combination of hexane: ethylacetate; 350 mL (95:5, 90:10, 85:15, 80:20,75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95) and ethylacetate: 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 sub fractions (A, B, C, D, E, F, G and H). One of the fractions (sub fraction D) was further analyzed due to its high yield using Fourier Transform Infrared (FT-IR) spectrometer.

2.5 Bioassay test of sub fraction D (isolate) obtained from *A. paniculata* leaves against *Trypanosoma brucei brucei*

Exactly 10 mg of the sub fraction D (isolate) was weighed and dissolved in 500 µL (0.5 mL) of dimethylsulfoxide (DMSO) to give a concentration of 20mg/mL solution of isolate (fraction D) in DMSO (stock solution). RPMI 1640 cell culture media (L-gutamine, 25mM 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 to obtained desired concentration. 950 µL of the supplemented media was added to 50 µL from 20 mg/mL of isolate solution to have 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.625 µg/mL and 7.813 µg/mL solutions; preparation was done by serial dilution (2-fold dilution) of 1000 µg/mL. 100µL of different concentrations (500, 250, 125, 62.5, 31.25, 15.625, and 7.813 µg/mL) from the isolate were dispensed into wells of a 96 well micro-titre plate in triplicate. Equal volume of standard control (Diminazene diaceturate; Nozomil®, Holland) at 500, 250, 125, 62.5, 31.25, 15.625, and 7.813 µg/mL was also dispensed in triplicate. A rat infected with trypanosome (*Trypanosoma brucei brucei*) was humanly anaesthetised 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). 30µL of the diluted infected blood was added to each of the well of micro-titre plate containing either isolate or Nozomil®. The resulting trypanosome count was approximately 20 per field (9.5 x 10^7 parasite/mL). Control wells contain 100µL of the supplemented media and 30µL of the diluted infected blood. The micro-titre plate was placed in a candle jar containing about 5% CO_2 at 34^0C. Trypanosome count in each well was observed 18h post incubation using microscope at X40 magnification. Parasite per mL was estimated using Herbert and Lumdsen (1976) rapid matching method. To ascertain the inhibitory effect of *A. paniculata* leaf (sub fraction D) against *T. b. brucei*, Twenty Four matured albino wistar rats of an average weight 120 g were randomly selected into six (6) groups of four (4) animals each; negative control, positive control, diminazene diaceturate, 5 mg, 10 mg and 20 mg of the isolate. The animals were obtained from Animal Unit, Vector and Parasitological Studies Research Department, Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna, Nigeria. Animal ethical approval and trypanosome (*T. b. brucei*) were also obtained from the same institute. Parasite was inoculated intraperiteonially (2.5 x 10^4 parasites) into animals in five groups (positive control, diminazene diaceturate, 5 mg, 10 mg and 20 mg of the isolate); temperature, weight, packed cell volume and parasitemia were monitored throughout the experiment. Trypanosome count was observed using microscope at X40 magnification. Administration of standard drug (diminizene diaceturate) and isolation was done intraperiteonially after 7 days post infection (dpi) for five consecutive days. The bioassay was terminated after 35 days observation.

2.6 Statistical Analysis

The group means ±SEM was calculated for each analyte by analysis of variance (ANOVA), post-test analysis using Turkey's comparisons. All statistical analysis was performed using GraphPad Prism software package (version 5).

3. RESULTS

3.1 Phyto-chemicals and Chromatography

Presence of secondary metabolites in *A. paniculata* leaves has been adequately reported in our previous study (Olanrewaju *et al.* 2017). Some of the metabolites include; saponins, tannins, flavonoids, alkaloids, anthraquinones, triterpenes amongst others. Ethylacetate fraction derived from 70% ethanol extract of *A. paniculata* was subjected to open column chromatography using gradient mobile phase to various sub fractions. Eight sub fractions were obtained; A, B, C, D, E, F and H. Sub fraction D was further purified using Hexane : Ethylacetate; 4:6 vol/vol and a relatively pure pale yellow isolate was obtained with R_f value of 0.69 on TLC. Three major absorption bands of interest in the sub fraction D were 3268cm^{-1}, 2926 cm^{-1} - 2855 cm^{-1} and 1748 cm^{-1} revealing the presence of O-H_{str}, C-$H_{str.}$and C=$O_{str.}$ stretching vibrations (Fig. 1).

3.2 Bioassay

In vitro assessment of the effect of *A. paniculata* (sub fraction D) on *T. b. brucei* indicated 100% inhibitory effect on the trypanosomes after 18h incubation (Table 1). It was observed that the isolate exhibited more trypanocidal effect compared to standard control (diminazene diaceturate) at lower concentration. This result is scientifically significant (*P<0.05*), which implies that the isolate is a potential trypanocidal. Moreover, from *in vivo* assay of sub-fraction D on *T. b. brucei* showed that trypanocidal activity of the isolate is dose-dependent. Parasitic replication was faster among the experimental rats treated with lower concentrations in comparison with higher concentration (Fig. 2). However, all the treated animals showed lower parasite count compared to positive control (untreated group). This is scientifically significant in term of survival rate of animals in each group (Table 2) at the end of the study. However, it was observed that diminazene diaceturate (standard drug) completely cleared trypanosomes in the blood after treatment but active trypanosomes were cited microscopically from the blood of animals treated with sub fraction D.

Fluctuation in body temperature can be an indication of infection both in animals and humans. Decrease in body temperature was observed among the animals infected with trypanosomes after 9 days post infection (9dpi) with statistical significance (*p<0.05*).

However, all the experimental animal body temperature between 11dpi and 29dpi indicated stable temperature (Table 3). Feed in-take of any trypanosome infected animals is another indicator used in assessing the effect of trypanosomes. From this study, it was observed that there was drop in feed in-take among the infected animals before treatment. A significant increase ($p<0.05$) in body weight was record on 19dpi among animals treated with 20 mg of the isolate (Table 4). Moderate average packed cell volume (PCV) was recorded among the treated animals compared with the positive control. Also, there was no significant difference ($p>0.05$) in PCV of the animals treated with sub fraction D compared with the group treated with diminazene diaceturate (Fig. 3).

4. DISCUSSION

Over the years, plants have been one of the sources of medicinal agents (drugs) for management of various diseases both in animals and human most especially within the very poor settlement in developing countries. The activities of these plants could be as a result of single component (molecule) or synergetic effects of complex molecules. Flavonoid, a common component of many plants is a potent antioxidant agent with ability to scavenge free radicals such as hydroxyl radicals, superoxide anions and lipid peroxide generated in animals and human as a result of pathogenic effect on red cells (Anand *et al*., 2011). Presence of several metabolites in *A. paniculata* leaves probably responsible for the multifunctional effect of the plant on both animals and humans (Verma *et al*., 2012; Agbonlahor *et al*., 2014). A spot-on TLC with a moderate R_f value (0.69) observed is an indication of relatively pure isolate. However, other chromatographic and spectroscopic techniques are required to ascertain the class of compound the isolate belongs. The presence of -OH, -CH- and C=O functional groups may be responsible for the biological activities of the plant. Functional groups in organic molecules are important because they are the portion of a molecule that is capable of undergoing chemical reactions. They, therefore, determine the properties and chemistry of many organic compounds.

The isolate obtained from *A. paniculata* leaves exhibited trypanocidal activity by completely clear trypanosomes (*T. b. brucei*) when examined on microscope after 18 h incubation of trypanosome/isolate. It was observed that the isolate (sub fraction D) exhibited trypanocidal effect compared to standard control (diminazene diaceturate). The activity observed supported previous report on prophylatic activity of *A. paniculata* against *Trypanosoma b. brucei* (Olanrewaju *et al*., 2017). The biological activity indicates that the isolate is a potential agent that could be used for the management of trypanosomiasis. Also, the effect could be attributed to the major active component of *A. paniculata* (Andrographolide) which has been reported to be effective as anti-microbial, anti-oxidant, anti- cancer, anti-diarrheal, anti-hapatitis, anti-HIV, anti-inflammatory, anti-malarial as well as managing cardiovascular diseases (Agbonlahor *et al*., 2014; Hossain *et al*., 2014; Joselin and Jeeva, 2014). Also, Dua *et al*. (2009) and Mishra *et al*. (2011) had earlier report inhibitory effect of diterpene lactone and xanthones derived from *A. paniculata* on *Plasmodium falciparum* and *T. b. brucei* respectively. Moreover, quercetin and its derivates are polyphenolic flavonoids present in *A. paniculata* had been documented to induce apoptosis of *Trypanosoma brucei gambiense*; a sub species of *Trypanosoma brucei* that causes sleeping sickness in human within West and Central Africa (Maria *et al*., 2004). Our findings from *in vivo* assay of sub-fraction D on *T. b. brucei* showed that trypanocidal activity of the isolate is dose-dependent. The result obtained could be linked to the ability of the plant; *A. paniculata* to boost immune system and also serve as scavenger of free radicals as earlier reported by Koh *et al*. (2011). Mechanism of action of most of the naturally source anti-trypanosomal agents is not yet properly understood. Some scientists related their activity to ability of the plants (isolates) to accelerate activities of anti-oxidant enzymes (superoxide dismutase, catalase, gluthathione peroxide) thereby ameliorate free radicals effects on the cells (Shen *et al*., 2000; Trivedi *et al*., 2001; Verma and Vinayak, 2008). Moreover, activities of some plants have been attributed to saponins and tannins which assumed to interact with parasite membrane sterols, protein and phospholipids and cause rupture of the parasitic cells (Godwin and Theodore, 2001; Bulus *et al*., 2008). It was observed in this study that sub-fraction D relatively stabilized body temperature of the infected animals. Hyperpyrexia remains one of the indications of parasitic infection in domestic animals. Pyrexia (normal temperature) observed after 14 dpi which is 1 day post treatment could be an indication that the isolate used is a potential anti-pyretic agent (Akintola *et al*., 2012). Loss of appetite is another clinical symptom of trypanosome infection in domestic animals which usually causes weight loss because of rapid decrease in food in-take. Stable body weight gain immediately after administration of the isolate (drug) for 5 days (Table 4) suggests that *A. paniculata* possesses anti-protozal agent(s) which can be further optimized to generate less toxic cheaper chemotherapy for the treatment and management of African Trypanosomiasis (Mishra *et al*., 2011).

Anaemia remains a major indication of trypanosome infection both in animals and human. This can be determined using packed cell volume (PCV) percentage. The low PCV observed in untreated group of trypanosome infection (positive control) could be an indication of depletion of antioxidant enzymes. Relatively stable PCV in the animals treated with the isolate reflects that the isolate reduced the severity of anaemia and increased survival rate (Ekanem *et al*., 2008; Saleh *et al*., 2009)

Table 1: *In-vitro* assay of sub-fraction D on *Trypanosoma brucei* after 18 h incubation

Concentration (µg/mL)	Number of surviving trypanosomes	
	Sub-fraction D	Diminazine diaceturate
500	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
250	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
125	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
62.5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
31.25	0.00 ± 0.00^{a}	7.00 ± 1.53^{b}
15.625	0.00 ± 0.00^{a}	9.33 ± 0.88^{c}
7.8125	0.00 ± 0.00^{a}	15.0 ± 1.16^{d}
control	19.6 ± 0.33^{b}	19.6 ± 0.33^{e}
p value	< 0.001	< 0.001

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

Table 2: Survival rate of animals treated with sub-fraction D

	8dpi		13dpi		18dpi		23dpi		29dpi	
	No. of rat	Survival rate	No. of rat	Survival rate	No. of rat	Survival rate	No. of rat	Survival rate	No. of rat	Survival rate
Negative Control	4	100	4	100	4	100	4	100	4	100
Positive Control	4	100	4	100	1	25	1	25	1	25
Diminazene diaceturate	4	100	4	100	4	100	4	100	4	4
5mg	4	100	4	100	4	100	4	100	3	75
10mg	4	100	4	100	4	100	4	100	4	100
20mg	4	100	4	100	4	100	4	100	3	75

Table 3: Mean ± SEM Temperature (^{0}C) of wistar rats infected with *Trypanosoma brucei brucei*

	5dpi	7dpi	9dpi	11dpi	13dpi	15dpi	17dpi	19dpi	21dpi	23dpi	25dpi	27dpi	29dpi
Negative Control	34.20 ± 0.372^a	35.73 ± 0.648^a	36.75 ± 0.519^a	35.13 ± 0.851^a	34.78 ± 0.487^a	34.00 ± 0.545^a	35.18 ± 0.620^a	35.43 ± 0.656^a	35.33 ± 0.539^a	35.53 ± 0.401^a	35.78 ± 0.315^a	35.43 ± 0.512^a	35.43 ± 0.512^a
Positive Control	35.28 ± 0.485^a	34.75 ± 0.253^a	36.20 ± 0.255^a	36.25 ± 0.512^a	33.80 ± 0.412^a	35.38 ± 1.066^a	35.40 ± 0.390^a	36.33 ± 0.577^a	34.15 ± 0.350^a	35.10 ± 0.400^a	36.20 ± 0.300^a	36.50 ± 0.500^a	36.50 ± 0.500^a
Diminazene diaceturate	34.20 ± 0.752^a	33.23 ± 0.371^{ab}	34.65 ± 0.525^{ab}	33.60 ± 0.502^a	34.38 ± 0.544^a	35.30 ± 0.420^a	33.85 ± 0.366^a	34.33 ± 0.489^a	33.80 ± 0.392^a	33.88 ± 0.459^a	35.08 ± 0.352^a	35.00 ± 0.294^a	35.00 ± 0.294^a
5mg	34.83 ± 1.129^a	34.30 ± 0.647^a	34.68 ± 0.411^{ab}	34.00 ± 0.731^a	34.83 ± 0.512^a	33.53 ± 0.560^a	33.63 ± 0.506^a	33.83 ± 0.527^a	33.85 ± 0.533^a	35.60 ± 0.555^a	35.40 ± 0.208^a	34.70 ± 0.551^a	34.70 ± 0.551^a
10mg	34.65 ± 0.842^a	33.88 ± 0.673^a	34.20 ± 0.212^{ab}	35.33 ± 0.407^a	35.30 ± 0.698^a	34.75 ± 0.492^a	35.28 ± 0.473^a	35.85 ± 0.562^a	35.27 ± 0.426^a	35.33 ± 0.320^a	35.88 ± 0.111^a	35.63 ± 0.676^a	35.63 ± 0.676^a
20mg	34.10 ± 0.600^a	33.90 ± 0.721^a	35.87 ± 0.033^a	36.03 ± 0.567^a	35.27 ± 0.384^a	35.30 ± 0.524^a	35.73 ± 0.058^a	35.20 ± 0.153^a	36.30 ± 0.252^a	34.97 ± 0.203^a	35.07 ± 0.186^a	35.25 ± 0.250^a	35.25 ± 0.250^a
p value	0.93	0.03	0.0002	0.054	0.053	0.18	0.13	0.08	0.058	0.07	0.27	0.27	0.27

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

Table 4: Mean ± SEM Weight (g) of wistar rats infected with *Trypanosoma brucei brucei*

	5dpi	7dpi	9dpi	11dpi	13dpi	15dpi	17dpi	19dpi	21dpi	23dpi	25dpi	27dpi	29dpi
Negative Control	118.00± 8.456^a	130.00± 9.704^a	123.80 9.681^a	121.30± 8.577^a	121.80± 7.920^a	117.50± 8.617^a	125.30± 8.459^a	116.80± 8.025^a	132.30± 9.123^a	131.50 ± 8.568^a	124.30± 8.816^a	135.30± 8.635^a	125.80 ± 8.673^a
Positive Control	120.30± 7.465^a	120.50± 6.589^a	122.80± 3.400^a	116.00± 5.672^a	109.50± 6.185^a	123.30± 7.028^a	131.50± 7.240^a	118.50± 6.946^a	120.00± 3.000^a	129.00 ± 1.000^a	136.00± 1.000^a	139.50± 0.500^a	141.00 ± 1.000^a
Diminazene diaceturate	129.80± 10.31^a	130.80± 12.080^a	138.80± 13.310^a	136.30± 11.870^a	141.80± 11.730^a	131.80± 11.350^a	137.50± 11.180^a	130.00± 11.100a	142.30± 10.700^a	146.00 ± 12.460^a	132.80± 11.100^a	146.00± 12.800^a	141.30 ± 11.090^a
5mg	155.3 ± 15.84^a	157.00± 16.190^a	154.00± 13.990^a	156.50± 14.040^a	154.00± 13.030^a	142.30± 12.330^a	159.00± 13.350^a	166.00± 13.450^a	152.80± 12.070^a	161.00 ± 12.300^a	131.00± 8.972^a	173.30± 18.980^a	170.00 ± 18.580^a
10mg	145.00± 8.963^a	124.30± 9.223^a	144.80± 9.141^a	136.50± 10.350^a	137.00± 11.170^a	131.50± 10.050^a	144.80± 11.210^a	137.80± 9.995^a	152.80± 10.550^a	148.50 ± 10.370^a	137.00± 10.170^a	153.30± 11.020`^{a}	146.80 ± 12.150^a
20mg	140.70± 9.244^a	136.30± 8.950^a	140.70± 9.244^a	139.00± 10.020^a	144.00± 8.718^a	141.30± 9.955^a	151.00± 10.690^a	156.70± 9.684^{ab}	159.00± 7.572^a	158.00 ± 8.327^a	158.00± 9.018^a	164.30± 7.219^a	163.50 ± 13.500^a
p value	0.21	0.12	0.17	0.06	0.14	0.36	0.19	0.008	0.21	0.31	0.47	0.28	0.24

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

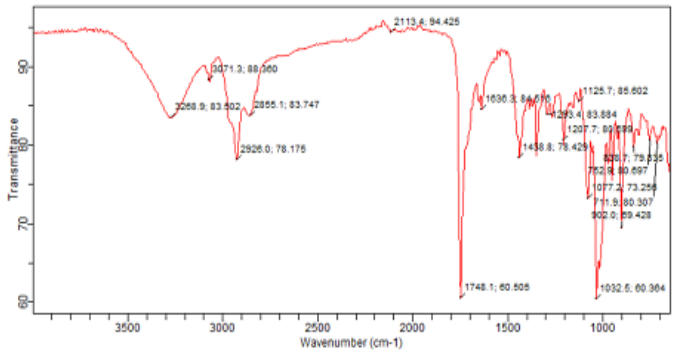

Fig. 1: FTIR spectrum of sub fraction D

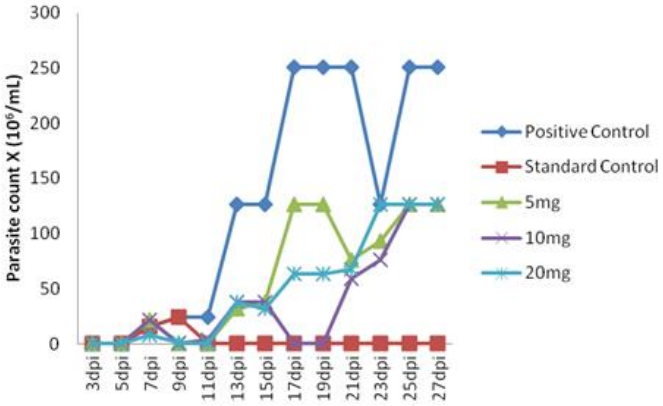

Fig. 2: Parasitemia of experimental rats

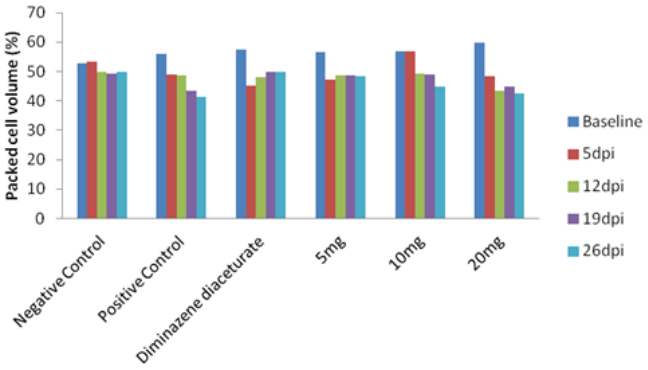

Fig. 3: Packed cell volume of experimental rats

5. Conclusion

Natural products from plants remain one of the sources of alternative medicine for management of diseases associated the poor. *Andrographis paniculata* evaluated in this study demonstrated inhibitory effects on *T. b. brucei* and boost immune response of the experimental animals. This indicated that the plant is a potential trypanocidal agent that can be explore for development of cheaper less toxic drugs for treatment and management of Trypanosomiasis. We recommend further investigation on the plant for characterization of the active molecule(s) responsible for the activities observed in this study.

Declaration of competing Interest

The authors declare that there is no any conflicting interest that could have influence the report of the findings in this study.

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