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In vitro and *in vivo* Anti-trypanosomal Potentials of *Afrormosia laxiflora* and *Khaya senegalensis* against *Trypanosoma brucei brucei*

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SUMMARY

Animal African trypanosomiasis (AAT) also known as Nagana is a resurgent disease in Africa. Medicinal plants are being used in less developed countries for the treatment of various diseases including trypanosomiasis, due to the high cost of currently available drugs. Most of these plants have been useful sources of treatment of various diseases based on information obtained from folk medicine but have not been scientifically certified. Here, we investigated the in vitro and in vivo anti-trypanosomal potentials of the methanol extract of *Aformorsia laxiflora* and *Khaya senegalensis* against *T. b. brucei*. Phytochemical screening as well as LD_{50} of the plant extracts was carried out following standard procedures. Parasitemia was monitored daily while Packed Cell Volume was determined at three time points (days 1, 4 and 7) during the course of the infection. The phytochemical analysis showed the presence of saponins, alkaloids, flavonoids, antraquinones, resins and tanins. However, steriods/terpenoids were absent in *K. senegalensis* but present in *A. laxiflora*. The toxicity of methanol extract of both *A. laxiflora* and *K. senegalensis* was above 5000mg/kg body weight. Methanol extracts of *A. laxiflora* (leaves) and *K. senegalensis* (stem bark) showed promising trypanocidal potential in vitro against T. b. brucei at concentrations of 10, 15, 25mg/ml and 40 and 20mg/ml respectively. At these concentrations, both extracts immobilized the parasites within 55mins post-incubation. In general, *A. laxiflora* leaf extract demonstrated prophylactic activity against T. b. brucei in vivo at a dose of 500mg/Kg body weight particularly in group C animals where a delayed pre-patent period (6 days post-infection), extended survival (14 days post-infection) and significant (P<0.05) reduction in the parasite burden confirmed by an absence of anemia (PCV 47.00±0.8 %) was observed when compared to the infected untreated control group. *K. senegalensis* extract on the other hand did not show anti-trypanosomal activity in the treated groups (1, 2, and 3). Based on these observations, it was therefore deduced that the methanol extract of leaves of *A. laxiflora* possessed the ability to ameliorate the burden of the disease and could be a plausible candidate for drug development against the disease.

Key words: *Trypanosoma brucei brucei*, *Afromosia laxiflora, Khaya senegalensis*, anti-trypanosomal, in vitro, in vivo

INTRODUCTION

African animal trypanosomiasis (AAT) is still a major and the most important constraint to livestock and agricultural production in tropical Africa (Isaac *et al.,* 2017; Courtin *et al.,* 2008). It has been considered as a threat to poverty alleviation programs in the continent. AAT is mainly caused by *Trypanosoma congolense,* and to a lesser extent by *Trypanosoma vivax* and *Trypanosoma brucei brucei* species (Vanden, 2001). Tsetse flies, which are the vectors of the African trypanosomes, are responsible for the cyclical transmission of this parasitic protozoan between numerous vertebrate hosts. All domestic animals can be affected by Nagana and the symptoms are fever, listlessness, emaciation, hair loss, discharge from the eyes, oedema, anemia and paralysis. As the illness progresses the animals weakens more and more and this will eventually lead to the death of the animal if untreated. Interestingly, these parasites cause relatively mild infections in wild animals while in domestic animals they cause a severe, often-fatal disease (Chechet, 2015).

There is currently an absence of effective alternative drug for the treatment and control of trypanosomiasis, therefore, the search for active substances of natural origin is of paramount important (Adeiza 2010). Coupling to the side effects of the available trypanocidal drugs, the resistance mechanisms employed by trypanosomes are major factors that contribute to the difficulties encountered in the managements and treatment of the disease. This challenge therefore calls for an urgent search for a novel chemotherapy for the treatment and management of AAT.

It is estimated that two thirds of the world population still rely on traditional medical remedies, mainly plants, because of limited availability and affordability of pharmaceutical medicines (Tagboto and Townson, 2001). This explains why a lot of current research focuses on natural molecules and plant-derived products as they can be sourced easily, locally available and can be selected on the basis of their ethno medicinal use (Verpoorte *et al*., 2005). The use of an alternative drug has been recommended as a measure to avoid the development of resistant pathogenic organism including trypanosomes (Abdul, 1990). The use of herbal preparations for the treatment of this disease still holds a strong potential in that some ethnomedicinal plants have been demonstrated to contain potent trypanocides (Igweh and Onabanjo, 1989; Owolabi *et al.,* 1990; Nok *et al.,* 1993; Atawodi, 2005).

Natural products are still major potential sources of innovative therapeutic agents for various conditions, including infectious diseases as they represent an unmet source of chemical diversity (Clardy and Walsh, 2004). Indeed, several antiparasitic drugs have been derived directly from natural sources, such as quinine, artemisinin and atovaquone as antimalarials and amphotericin B as antileishmanial drug. Plants possess valuable organic substances, which have potential value for the treatment of diseases therefore, there is the need to harness these potentials in the treatment and control of diseases therefore making it necessary to investigate such plants. To curb the menace of this disease Human African Trypanosomiasis, the use of alternate drugs has been recommended as a measure to avoid the development of resistant pathogenic organism (Abdul, 1990).

Afrormosia laxiflora, is a plant that is widely distributed in tropical Africa, extending from Senegal to Sudan (Von Ffranziska, 1996). Various parts of this plant have been used previously in Nigerian traditional medicine as medication or remedy for a

number of diseases. The bark is widely used as a remedy for fever, malaria and other diseases with symptoms of icterus. An aqueous extract of the leaves given to patients orally for a long time has been used as a prophylactic remedy for malaria (Von Ffranziska, 1996).

Khaya senegalensis (Juss), is highly reputed for its numerous medicinal uses and has been reported to be used indigenously in the treatment of trypanosomiasis (Atawodi *et al.,* 2001). The plant has also been reported to possess an *in vitro* antitrypanosomal activity (Wurochekke and Nok, 2004; Atawodi, 2005). The *in vitro* activities of the aqueous and ethanol extracts of the leaves, root bark and stem bark of *K. senegalensis* against *T. evansi* has been reported (Umar *et al.,* 2010). However, the *in vitro* and *in vivo* anti-trypanocidal potentials of methanol extracts of *A. laxiflora* (leaves) and *K. senegalensis* (stem bark) are yet to be evaluated. This study therefore is aimed at investigating the *in vitro* and *in vivo* anti-trypanocidal potentials of *A. laxiflora* leaves and *K. senegalensis* stem bark against *T. b. brucei*.

MATERIALS AND METHODS
Parasites
Trypanosoma brucei brucei was obtained from the Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria Kaduna State, Nigeria. Two (2) donor rats were infected with *T. brucei brucei blood* from a donor rat at peak parasitemia. The parasites were subsequently maintained by serial parasite passages in albino rats. The experimental animals were inoculated with 0.2ml containing 10^6 parasites/ml estimated using Rapid Matching Method (Herbert and Lumsden, 1976).

Parasite authentication by Polymerase Chain Reaction (PCR)
Polymerase Chain Reaction (PCR) was performed using Phusion Blood direct kit (Finzymes) and ITS-1 generic primers to further authenticate the identity of the parasites. Briefly, 1µl of blood from an infected animal was included in the first round of PCR. The PCR was performed in a 20µl reaction volume containing 10µl of the Phusion buffer (Finzymes), 1.0µl of each outer primer (10 µM), 0.4µL of Phusion enzyme, 6.6µL of double distilled water and 1µL of blood that served as template in the first reaction. The cycling conditions were as follows: 1 cycle at 98^0C for 300 seconds followed by 35 cycles at 98^0C for 1 second, 57^0C for 5 seconds, 72^0C for 20 seconds and a final extension at 72^0C for 60 seconds. At the end of the reaction, the tubes were centrifuged at 1800 rpm for 3 minutes and 1µL of the supernatant was used as template in the second reaction. The nested PCR was performed using a 25µL reaction volume containing 1µL (1ng/µL) template DNA, 25µM of each primer, 10mM dNTPs, 25mM 10x Dream Taq polymerase buffer, 5U/µL Dream Taq polymerase to a reaction volume of 25µL. Cycling conditions were as follows: 1 cycle at 95^0C for 300 seconds followed by 30 cycles at 95^0C for 60 seconds, 56^0C for 60 seconds, 72^0C for 30 seconds, and final extension 72^0C for 220 seconds. The products were resolved by electrophoresis (45 minutes to 1 hour at 100V) on 1.5 or 2% Agarose gels stained with G-Stain (Serva®) and bands were visualized using GelDoc-It^2 Imaging System (Analytik Jena AG Jena, Germany).

Experimental Animals
Thirty albino rats, which weighed between 130-178g, were obtained from the Department of Pharmacology, Faculty of Pharmaceutical sciences, Ahmadu Bello University Zaria Kaduna State, Nigeria. Each group of experimental animals was housed in a 15x30cm cage with metal tops that was thoroughly cleaned. Bedding materials consisting of wood shavings (saw dust) were used and changed ones a week. The rats were fed daily with commercial rat chow (Vital Feeds, Jos, Nigeria) with drinking water *ad libitum*. All experimental

animals were acclimatized for two (2) weeks before the commencement of the experiment.

Sample Collection and identification

Afrormosia laxiflora leaves and *Khaya senegalensis* stem bark was collected from the botanical garden of Biological Sciences, Department, Ahmadu Bello University Zaria, Nigeria. Both plants were identified, authenticated and voucher numbers was assign to them.

Sample Preparation

The leaves of *A. laxiflora* and the stem bark of *K. senegalensis* was collected and dried under shade for 3 weeks in Mary Hallaway Teaching Laboratory, Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria. The dried samples of both plants were ground to fine powder with mortar and pestle and then stored in dry containers until needed.

Sample Extraction and storage

Cold extraction of both plant samples was carried out. 100g each of the fine powdered *A. laxiflora* and *K. senegalensis* was soaked in 300ml of 70% methanol and sequentially extracted by shaking for 6 hours on wrist action shaker. The preparations were left at room temperature to stand for 24 hours. Both preparations were filtered through Whatmann's No.1 filter paper and extracts were concentrated to dryness on a water bath at 40^0C. The two extracts were packed separately in polythene bags and stored in the refrigerator at 4^0C until needed (Atawodi, 2005).

Qualitative phytochemical screening of plants

Test for flavonoids

For the confirmation of flavonoids in the leaves powder, 0.5 g of plant extract was placed in a test tube and 10 ml of distill water as well as 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of the plant extract followed by addition of 1 ml concentrated H_2SO_4. Indication of a yellow color shows the presence of flavonoids in each extract (Wadood *et al.*, 2013).

Test for alkaloids

The procedure used for determination of alkalolids in the plant samples is similar to that described by (Wadood *et al.*, 2013). Briefly, 0.2 g of leaves powder was added to a glass test tube that has 3 ml of hexane. The powder and hexane were thoroughly mixed, shaken well, and filtered. To the hexane and leaves extract mixture, 5 ml of 2% HCl were added, and the mixture heated until boiling. The mixture was then filtered, and 1-3 drops of picric acid were added to the hexane, HCl and leaves extract filtrate. The presence of alkaloids in the samples was confirmed by the yellow- colored precipitate that was formed.

Test for terpenoids

An amount of 0.8 gram of the leaves powder was placed in a test tube, then 10 ml. of methanol was poured in it, shaken well and filtered. Five ml of the plant extract of plant samples was taken. Then 2 ml of chloroform were mixed with the extracts and 3 ml of sulphuric acid were added. Formation of reddish brown color indicates the presence of terpenoids in the selected plants (Wadood *et al.*, 2013).

Test for tannins

Crude extract of the plants powder was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of tannins (Yadav and Agarwala, 2011).

Test for steroids

Leaves powder from both plants was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each

of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids (Yadav and Agarwala, 2011).

Test for saponins
The crude extracts were mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins (Yadav and Agarwala, 2011).

Test for Anthraquinones
The ether and chloroform maceration from both plants was filtered and 1mL of this solution was treated with 1mL of 10% sodium hydroxide solution. A red coloration indicates the presence of quinines.

In vitro **assessment**
Plant extracts were prepared from a 40 mg/ml stock solution from which dilutions were made. For *A. laxiflora*, four different concentrations of 2, 4, 10 and 15mg/ml were prepared while three different concentrations of 10, 20 and 40mg/ml were prepared for *K. senegalensis*. 25mg/ml of the standard drug (Diminazene aceturate) was also constituted. The *in vitro* assays were performed in triplicates in microtitre plates. Aliquots of 50µl of $1x10^9$ parasites/ml of parasitized blood were incubated with 50µl of the different aforementioned concentrations of both plant extracts and the standard. A positive control containing 50µl of parasitized blood incubated in 50µl Phosphate buffered Saline Glucose (pH 7.4), was also prepared. 5 µl of incubated cocktail was placed on microscope slides and observed under a light microscope at 5 minutes intervals over a period of 1 hour. A cessation or drop in motility of the parasites in treated blood compared to that of parasite-loaded control blood without extract was taken as a measure of anti-trypanosomal activity, since motility constitutes a relatively reliable indicator of the viability of most zooflagellate parasites (Atawodi,

2005). The shorter the time of cessation of motility of the parasite, the more active the extract was considered to be (Atawodi *et al.*, 2001)

Median Lethal Dosed (LD$_{50}$)
The acute toxicity of the methanolic extracts of *A. laxiflora* and *K. senegalensis* was determined by Lorke's method 1983. The experimental animals were administered with the preparation of the methanolic extracts of both plants intraperitoneally, and it was conducted in two phases. In the first stage, nine experimental rats were divided into three different groups on the basis of their weights with each group containing three rats. Following Lorke's method, in the first phase of experiment, 10, 100 and 1000mg/kg body weight of the crude extracts solution was administered to the first, second and the third groups intaperitoneally respectively. The animals were placed under observation for 24 hours to monitor their behaviors and some signs and symptoms of toxicity as well as if mortality will occur. In the second phase of the experiment, three rats were divided into three different groups on the basis of their weight consisting of 1 animal per group. To the three different groups, 1600, 2900 and 5000mg/kg body weight of the crude methanolic extracts was administered to the first, second and the third group intraperitoneally respectively. The animals were placed under observation for 24 hours to monitor their behaviors and some signs and symptoms of toxicity as well as if mortality will occur.
The LD$_{50}$ was calculated by the following formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

In vivo **Prophylactic Studies of** *A. laxiflora* **and** *K. senegalensis*
A total of 30 rats were used for the in vivo experiment. The rats were divided into five

different groups with each group containing three rats. Rats to be administered with *A. laxiflora* were labelled groups A-E while those to be administered with *K. senegalensis* were labelled groups 1-5.

Animals in groups A, B, C and 1, 2, 3 were administered 500mg/kg body weight of *A. laxiflora* and *K. senegalensis* respectively, at different time intervals prior to the experimental infection. Groups C and 3 were administered three doses (once daily for 3 days) of 500mg/kg body weight of the crude extracts at 24 hours interval. Groups B and 2 were administered two doses (once daily for two days) of 500mg/kg body weight of the crude extracts at 24 hours intervals. Groups A and 1 were administered a single dose of 500mg/kg body weight of the crude extract. Groups B/2 and A/1 received the first doses of extracts at 24 and 48 hours intervals respectively after groups C/3 received the first dose of extracts. Groups D and 4 were positive controls that were infected but untreated. While groups E and 5 were the negative controls (uninfected and untreated). Animals in the infected groups received 1×10^6 parasites intraperitoneally 24 hours after groups A and 1 received prophylactic treatment.

Determination of Parasit emia

Parasite count was monitored daily in the infected groups and determined microscopically at 40 magnification using the "Rapid Matching" method of Herbert and Lumsden 1976. Briefly, the method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with phosphate buffered saline (PBS, pH 7.4). Logarithmic values of these counts obtained by matching with the table of Herbert and Lumsden (1976) is converted to antilog to provide absolute number of trypanosomes per ml of blood (Atawodi, 2003).

Pre-patent period was determined when the first *T.b.brucei* parasite was seen in the blood of the infected animals.

Packed Cell Volume (PCV)

The PCVs of all the animals from each group were determined on days 1, 4 and 7 post infection by Microhaematocrit method described by Coles (1974). The blood was directly taken from the tails of the rats (also known as tail bleeding) into heparinized capillary tubes by capillary action to about 75% of its length. The ends of the tubes were sealed with a flame using Bunsen burner. The sealed tubes were placed in a microhaematocrit centrifuge, with the sealed ends near the outside rim of the centrifuge (touching the rim). The cover of the centrifuge was tightened to prevent blood spillage and centrifuged for 5 minutes. Thereafter, the capillary tubes were placed in a graphic reader and the packed cell volume (PCV) was directly read in percentage.

Statistical Analysis

All data was analyzed using One Way Analysis of variance (ANOVA) expressed as means ± standard deviation of duplicate measurements using Gr aph Pad Prism 7 software Version 7.0a. The significant level was set at ($P < 0.05$).

RESULT

Polymerase Chain Reaction analysis

The band in plate 1 runs at about 450bp indicating that the parasite is *T. b. brucei.*

Qualitative phytochemical analysis

The percentage yield of crude *Afromosia laxiflora* and *Khaya senegalensis* leaf extracted using methanol was 11.27 and 15.3% respectively.

The phytochemical screening of *Afromosia laxiflora* and *Khaya senegalensis* leaves showed that the methanol extracts contain Saponins, Alkaloids, Flavonoids, Antraquinones, Resins and Tanins and Steriods/Terpenoids. However, Steriods/Terpenoids was absent in *Khaya sensgalensis* (Table 1).

Figures 1 and 2 showed a dose-dependent trypanocidal effect of methanolic extract of *A. laxiflora* and *K. senegalensis* against *T. b. brucei* trypomastigotes. After 35 minutes post-incubation a reduction of 83.91%, 99.94%, 93.93%, and 53.82% (6.31 ± 0.44, 0.25 ± 0.21, 2.38±0.17, and 20.88 ± 1.01 x10^7 Trypanosomes/ml of blood) of live parasites was observed at concentrations of 25mg/ml (Diminazene aceturate), 40, 20 and 10mg/ml of *K. senegalensis*. After 50 minutes, a reduction of 98.45%, 96.84%, 96.84% and 87.40% (0.79 ± 0.47, 1.59 ± 0.43, 3.16 ± 0.84, 3.16 ± 0.37 and 12.59 ± 0.19 x10^7 Trypanosomes/ml of blood) of live trypomastigotes was observed at concentrations of 25mg/ml (Diminazene
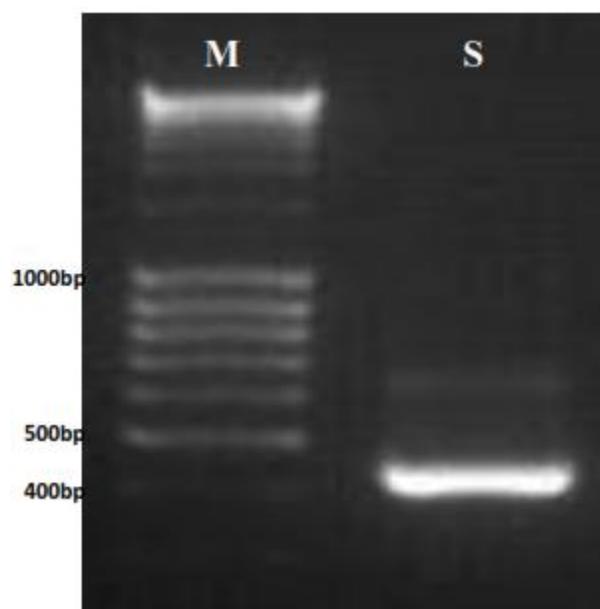
aceturate), 15, 10, 4 and 2mg/ml of *A. laxiflora* respectively. After 60 minutes in maintenance medium, there were no living parasites observed at 40 and 20mg/ml (*K. senegalensis*) as well as 15 and 10mg/ml (*A. laxiflora*). However, a significant decrease (P<0.05) in number of live trypomastigotes was observed in medium containing 4, 2mg/ml (*A. laxiflora*) and 10mg/ml (*K. senegalensis*) after 60 minutes. After 40 and 55 minutes of incubation, the highest concentrations of *K. senegalensis* (40mg/ml) and *A. laxiflora* (15mg/ml) respectively cleared parasites in the medium earlier or at the same time when compared with the group incubated in the standard drug. The positive controls remained motile throughout the duration of the experiment.

In Vitro Evaluation

At the end of the LD$_{50}$ analysis, the methanol crude extract of both *A. laxiflora* and *K. senegalensis* was observed to have no toxicity effect on the experimental animals at concentrations of 10, 100, 1000, 1500, 2900 and 5000mg/kg body weight since no mortality was observed at the concentration of 5000mg/kg b.w for 24 hours. Therefore, it was concluded that the toxicity of methanol extract of both *A .laxiflora* and *K. senegalensis* was above 5000mg/kg body weight, hence the choice of the dose concentration for the *in vivo* experiment.

In Vivo Prophylactic Studies

The *in vivo* prophylactic effect of the methanol extract of *A. laxiflora* and *K. senegalensis*

Plate 1: Electrophorograph of *T. b. brucei*: M= DNA Mass ruler and S=sample (*T. b. brucei*)

TABLE I: Phytochemical analysis of methanol extract of *Afromosia laxiflora* and *Khaya sensgalensis* leaves

Component	*Khaya senegalensis*	*Afromosia laxiflora*
Saponins	+	+
Alkaloids	+	+
Flavonoids	+	+
Antraquinones	+	+
Tannins	+	+
Steroids/Terpenioids	+	-

+ indicates the presence of the test compounds, while − indicates the absence of the test compounds

against *T. b. brucei* is shown in figures 3 and 4. Animals in groups A, B, C and D developed mean parasitemia (pre-patent) of 1.59 ± 0.45, 0.79 ± 0.14, 0.79 ± 0.45, 1.59 ± 0.47 (x10^7 Trypanosomes/ml of blood) on days 3, 4, 6 and 4 respectively. The mean parasitemia peaked at 50.12 ± 0.51, 25.12 ± 0.92, 12.59 ± 0.48 and 100.00 ± 0.45 (x10^7 Trypanosomes/ml of blood) on days 9, 10, 14 and 9 respectively. On the other hand, animals in groups 1, 2 and 3 developed parasitemia of 0.53 ± 0.07, 0.26 ± 0.03 and 0.26 ± 0.03 (x10^7 Trypanosomes/ml of blood) on day 2, group 4 animals showed parasitemia of 1.06 ± 0.35 on day 3. While groups 1, 2 and 3 peaked at mean parasitemia of 5.01 ± 0.42, 4.17 ± 0.49 and 3.35 ± 0.28 (x10^7 Trypanosomes/ml of blood) respectively; group 4 peaked at 5.02 ± 0.45 on day 8. The mean parasitemia displayed between the groups was statistically insignificant (P>0.05).

Packed Cell Volume (PCV)
There was significant (P < 0.05) reduction in the mean PCV from on days 4 and 7 post-infection among animals administered *K. senegalensis* such that the animals developed significant anemia on day 7 which is comparable with the mean PCV observed in the infected treated groups. On the other hand, animals administered *A. laxiflora* did not show significant reduction in the mean PCV on day 4 post-infection. However, on day 7 post-infection, animals in groups A and B showed a significant reduction in mean PCV while the mean PCV was significantly higher (P > 0.05) in group C animals when compared
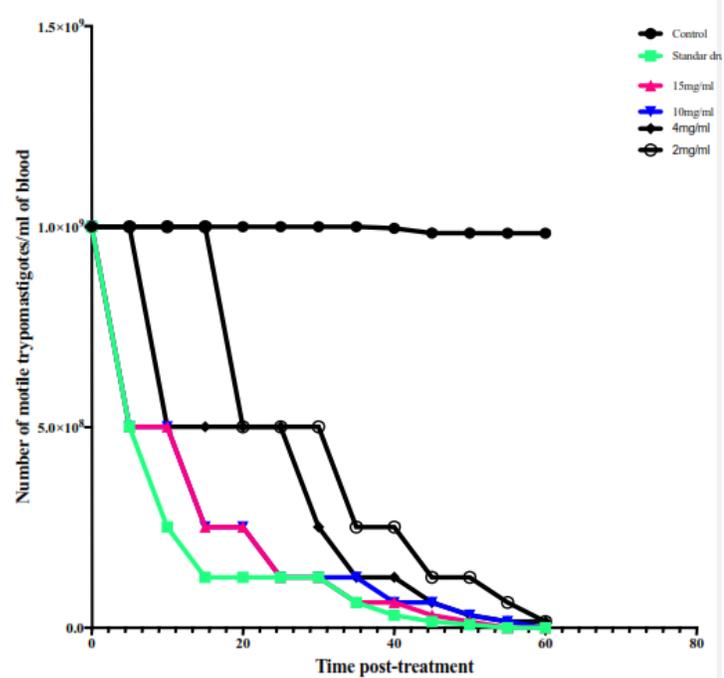

Figure 1: In vitro effect of different concentrations of methanolic extract of *A. laxiflora* leaf against *T. b. brucei*

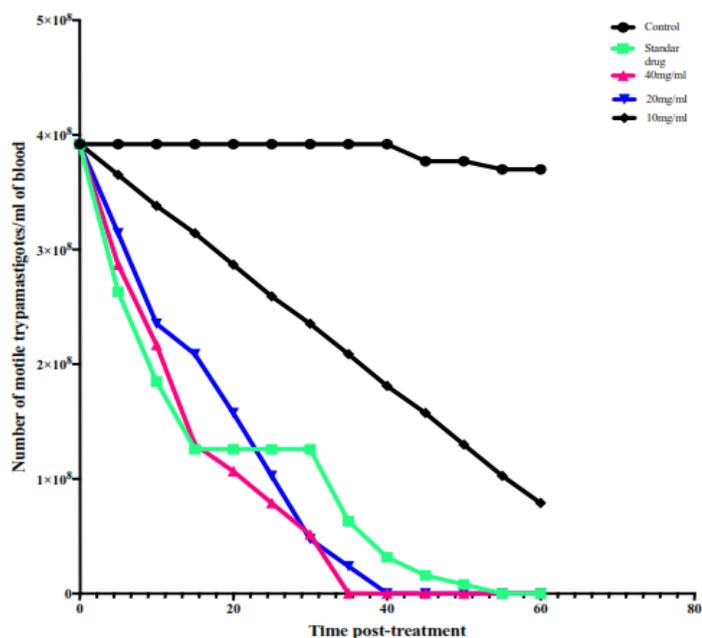|		
I.	●	Control
II	—	Standard Drug
III.	★	15 mg/ml
IV.	◄	10 mg/ml
V.	◆	4 mg/ml
VI	◉	2 mg/ml

Figure 2: In vitro effect of different concentrations of methanolic extract of *K. senegalensis* leaf against *T. b. brucei*

to the animals in other treated groups.

DISCUSSION

Confirming the identity of any parasite using molecular tools in addition to microscopic observations is a basic necessity in any molecular biology related research. Plate 1 confirmed that the specie used in this study is *T. b. brucei* since specie specific primers were used for the amplification. The 450bp band size corresponds to the expected band size of *T. b. brucei* (Adams *et al.,* 2006).

The methanolic extracts of stem bark and leaves of *K. senegalensis* and *A. laxiflora* respectively have been shown to possess *in vitro* antitrypanosomal activity in other studies. Our results support those of Hoet *et al*., 2004 who showed that the leaf extracts of *A. laxiflora* were active *in vitro* against *T.b.brucei*. Whereas Henrietta *et al*., 2015, Wurochekke and Nok, 2004; Atawodi, 2005 showed *in vitro* activity of the stem bark of *K. senegalensis* against *T.b.brucei*. *In vitro*, the antitrypanosomal activities of both extracts compared well with the standard drug suggesting they are efficacious in clearing parasites. The leaf and stem bark of *A. laxiflora* and *K. senegalensis* are used traditionally by local farmers in Northern Nigeria to treat trypanosomiasis infection.

Figure 3: Prophylactic effect of different regimens of methanolic extract of *A. laxiflora* leaf (500mg/kg b.w) on mean parasitemia and pre-patent periods

Figure 4: Prophylactic effect of different regimens of methanolic extract of *K. senegalensis* leaf (500mg/kg b.w) on mean parasitemia and pre-patent periods

The results of the phytochemical analysis of the leaf methanol extracts of *K. senegalensis* and *A. laxiflora* revealed the presence of Saponins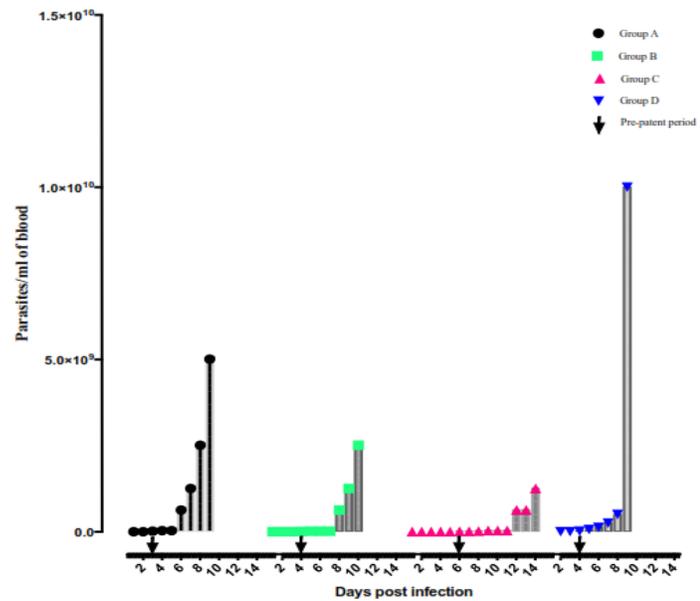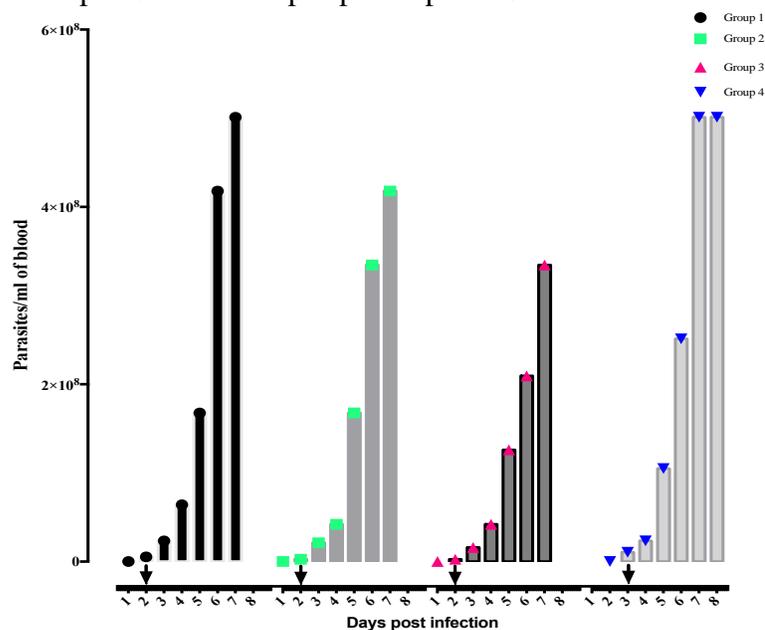, Alkaloids, Flavonoid, Antraquinones, Resins and Tanins and Steriods/Terpenoids (only present in *A. laxiflora*). The antitrypanosomal and antimicrobial activity of some of these compounds, which were detected in high concentrations in the stem, bark methanolic

extracts have been previously demonstrated (Abbiw, 1990, Amvam *et al.*, 1998, Jayanshinge *et al.*, 2003 phytochemical screening and antibiterial activity of the bark extracts of *A laxiflora*). Nok, 2001 and Ibrahim, 2013 attributed the trypanocidal activities of *K. senegalensis* to Azaanthraquinone and phenolics-rich fraction of the stem bark of the plant.

The LD_{50} result showed that, the methanol crude extract of both *A. laxiflora* and *K. Senegalensis* was observed to have no toxicity effect on the experimental animals at concentrations of 10, 100, 1000, 1500, 2900 and 5000mg/kg body weight since no mortality was observed at the concentration of 5000mg/kg b.w for 24 hours. Therefore, it was concluded that the toxicity of methanolic extract of both *A. laxiflora* and *K. senegalensis* was above 5000mg/kg body weight, hence the choice of the dose concentration for the *in vivo* experiment. This result indicated that both plants are save to the test subjects since no any sign of toxicity or mortality was observed after 24 hours.

Figure 5: Mean Packed Cell Volume analysis of rats treated with 500mg/kg b.w of *A. fromosia* and *K. senegalensis* for the all the regimens

■ Group A: To which leaf methanolic extract of *A. laxiflora* at 500mg/Kg/day was administered for one day.

■ Group B: To which leaf methanolic extract of *A. laxiflora* at 500mg/Kg/day was administered for two days.

■ Group C: To which leaf methanolic extract of *A. laxiflora* at 500mg/Kg/day was administered for three days.

■ Group D: Infected with *Trypanosoma brucei brucei* but not treated.

■ Group E: Not infected and not treated.

■ Group 1: To which leaf methanolic extract of *K. senegalensis* at 500mg/Kg/day was administered for one day.

■ Group 2: To which leaf methanolic extract of *K. senegalensis* at 500mg/Kg/day was administered for two days.

■ Group 3: To which leaf methanolic extract of *K. senegalensis* at 500mg/Kg/day was administered for three days.

An interesting observation was that parasitemia was suppressed in the animals that received three doses of *A. laxiflora* extract suppressed parasitemia at an optimum dose of 500mg/kg body weight, which was clearly seen in the delayed pre-patent period as compared to the other groups which received single, double or no treatment at all. Similarly, animals in this group (which received three doses of *A. laxiflora*) survived even longer than animals that received single, double or no treatment at all. This could be attributed to an increased concentration of active phytochemical components required for parasite clearance. On the other hand, the methanolic extract of *K. senegalensis* did
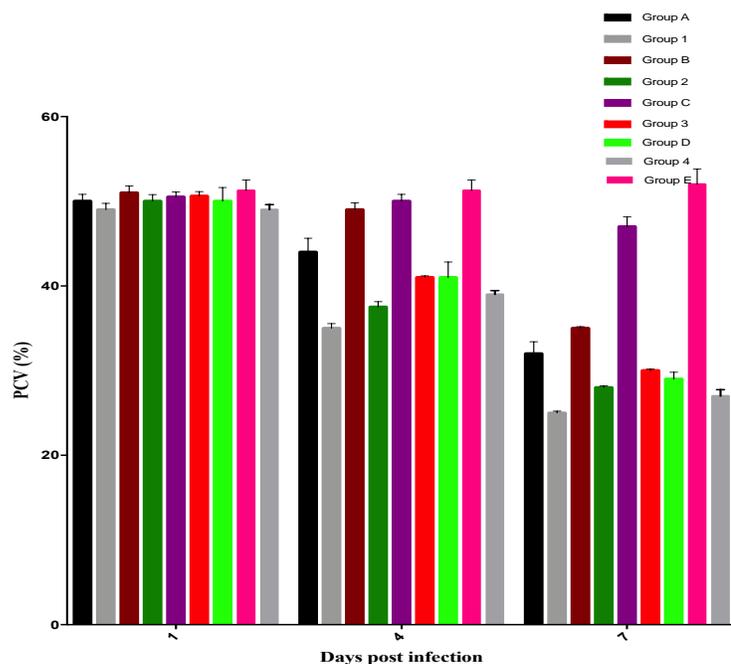
not suppress parasitemia even after three administrations. However, reports have shown that the ethanol extract of stem bark of *K. senegalensis* posses *in vivo* activities against *T. evansi* (Asuzu and Chineme, 1990; Nok *et al.,* 1993; Ibrahim *et al.*, 2008). This could be attributed to the difference in extraction potentials in accordance with the polarity of the different organic solvents.

Interestingly also was that animals in this group exhibited an increase PCV at the same time showing low parasitemia as seen in the experimental data. However, animals in the untreated control group exhibited severe anemia and concomitantly high parasitemia. This result suggests that some components

of the plant extract could likely be responsible ameliorating anemia during the course of the infection.

The observed anti-trypanosomal effect of the extract of *A. laxiflora* in this study was accompanied by corresponding improvement and prevention of further drop in PCV suggesting that they have potentials to ameliorate anaemia. This could possibly be by reducing the proliferating parasite load, neutralizing the toxic metabolites produced by trypanosomes or scavenging the trypanosome associated free radicals (Teka *et al*., 2014).

A common pathological feature observed during trypanosomiasis is anemia. The severity of anemia is often characterized by a drop in the PCV, which is considered as a pointer of the acute nature of the infection (D'Ieteren *et al*., 1998). In the Ndama breed of cattle, which has been known, for sometime to be trypanotolerant, an important aspect of the trypanotolerance is their ability to control the infection-associated anemia. The cytokine-activated macrophages have been suggested to be responsible for the enhanced phagocytosis of parasites and RBCs and also inability to mount a vigorous compensatory erythropoietic response could be responsible for anemia during the course of the disease (Stijlemans *et al*., 2008).

Reactive oxygen species which are generated by trypanosomes can also attack membranes of red blood cells, induce oxidation and eventually hemolysis. Therefore, phytochemicals like flavonoids and some other polyphenolic antioxidants could be used invariably to scavenge the trypanosome associated free radicals thus, ameliorating anemia induced by trypanosome infection (Karori *et al*., 2008). The plant extracts may strengthen the host defense that was already activated because of the presence of trypanosomes in circulation with established infection (Okeke, 2008). The mechanism by which these extracts exhibited their antitrypanosomal activity can only be speculated since the active ingredient(s) were not isolated. Several investigations have suggested that it is often difficult to speculate and elucidate the exact mode of action by which plant extracts exhibit their trypanocidal action. The most likely mechanisms by which plant extracts and phytochemicals carry out this role remains subject to speculations and debate in the scientific community (Gurib-Fakim and Mahomoodally, 2013). Thus, for the methanolic extract of *A. laxiflora* our study, the antitrypanosomal effect produced would most likely be ascribed to either of the saponins, alkaloids, flavonoids, antraquinones, and tannins detected in the extract. However, rare findings have suggested as to how any of these phytochemicals produce their antitrypanosomal activity (Teka *et al*., 2014).

CONCLUSION

We have established the *in vitro* as well as *in vivo* anti-trypanosomal effects of *K. senegalensis* and *A. laxiflora* in this study. Even though the mechanism of action has not been elucidated, a number of reports have associated anti-trypanosomal activities of plant extracts to some phytochemicals present in such plants. *In vivo* assay in the present investigation has shown that methanol extract of leaves of *A. laxiflora* reduced the parasite burden, am eliorated anaemia and extended survival of animals in the infected treated groups. Based on the data obtained, the methanol extracts of *A. laxiflora* exhibited anti-trypanosomal activity when compared to the methanol extract of stem bark of *K. senegalensis* extract. This study has therefore established that the leaves of *A. laxiflora* have anti-trypanosomal activity could be considered a potential source of new drugs for the prevention or management of Animal African Trypanosomiasis.

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