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EVALUATION FOR ANTI-TRYPANOSOMAL EFFECTS OF *Acacia nilotica* STEM BARK METHANOL EXTRACT FRACTIONS IN WISTA RATS INFECTED WITH *Trypanosoma brucei brucei*

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ABSTRACT

The present study was designed to assess the *invivo* effect of stem bark methanol extract of *Acacia nilotica* in 40 wistar rats infected with *Trypanosoma brucei brucei*. The rats weighing abouthe aim of this study was to determine the *invivo* effect of stem bark methanol extract fractions of *Acacia nilotica* in 40 wistar rats infected with *Trypanosoma brucei brucei.* The rats weighing about 110grams were randomly allotted to different groups of 4 rats each. The groups were treated with 300, 400 and 500mg/kg doses of each of the fractions. The methanol extract was further fractionated into different fractions using 100% ethyl acetate, 100% n- butanol, 100% n- hexane and water. The yield shows ethyl acetate with highest percentage of fraction followed by butanol. N-hexane with the lowest percentage. Three (3) days pre patent period was observed after inoculating of the parasite. Parasiteamia was monitored daily while Parked Cell Volume (PCV) was determined at one-day interval during the course of infection. The 100% ethyl acetate and 100% n- butanol fractions showed antitrypanosomal activities against *T. b. b.* in infected experimental animals. The Butanol fraction (500mg/kg dose) cured the rats after 5 days' treatment period while Ethyl acetate fraction at 400mg/kg and 500mg/kg doses cleared the parasite after 6 days' treatment. Water and n-hexane fractions shows little or no effects against *Trypanosoma brucei brucei* in experimental animals. The study concluded that butanol and ethyl acetate faction contains active ingredients with therapeutic potentials against trypanosomiasis.

Keywords: *Trypanosoma brucei brucei, Acacia nilotica,* Stem bark, anti-trypanosomal.

INTRODUCTION

Trypanosoma brucei brucei is a unicellular parasitic protozoan of genus *Trypanosoma* that causes trypanosomiasis in cattle and other domestic animal by infecting their blood plasma. This parasite is transmitted by a vector tsetse fly of the family Glossinidae. The disease is prevalent in north central state of Nigeria. Susceptible animals when infected become weak, emaciate, and reproductively breeding animals may abort and become infertile (ILRAD, 1994). The protozoa belong to the genus *Trypanosoma. Trypanosomes* like *Trypanosoma Vivax, Trypanosoma congolense* and *Trypanosoma brucei brucei* are the main species responsible for African animal trypanosomiasis (AAT) called Nagana in West Africa. Human African Trypanosomiasis (HAT) or

sleeping sickness is a disease caused by two subspecies of *Trypanosome brucei, i.e. T. brucei gambiense and T. brucei rhodesiense*, Surra and Dourine are caused by the other *Trypanosoma species T. evansi and T. equiperdum* respectively. The diseases are transmitted by the vector-tsetse fly (*Glossina species*) (D'Archivio *et al*., 2011). Sleeping sickness occurs in 36 sub-Saharan Africa countries where there are tsetse flies that transmit the disease (WHO 2016).

In Nigeria, the wide distribution of the disease is due to the abundance of its biological and mechanical transmitting vectors which are tsetse flies and biting flies, respectively. It affects human and livestock production in Nigeria causing significant losses which ranges from a decrease in milk production to death of animal (Diall *et al.,* 2017). All warm-blooded animals including wildlife species have been implicated in the transmission cycle of the disease. Tsetse flies cover an approximately 80% of the landmass in Nigeria (Anene *et al.,* 1991), hence AAT continues to thrive, and losses incurred have not reduced, it seems to be re-emerging as a significant livestock disease and extending to areas that were previously designated as tsetse- free zones (Ayodele *et al.,* 2013).

The current chemotherapy of Trypanosomosis relies on drugs which were been used for donkey years and are expensive with toxic side effects and many studies shows the emergence of strains that develop resistance to these drugs (Perez-Morga 2007). In view of these the development of new inexpensive, effective drugs in the treatment of trypanosomiasis is urgently required in order to control the disease. However it has been observed that natural products obtained from plants and recent discovery of novel drugs such as artemisinin, atropine, digitoxin, digoxin, emetine, pilocarpine, quabain, quinidine, quinine, reserpine, vinblastine, vincristine, etc., from medicinal plants implies that vast potential still exist for the production of numerous more novel drugs (Abdulhamid *et al*., 2018). Consequently, the area of ethno pharmacology of medicinal plants has attracted increasing attention in new drugs research and development (Monier M., 2016). This is the main reason why this research were design to study the Antitrypanosomal properties of the components of Stem bark extract of *Acacia nilotica* plant.
Acacia nilotica, is popularly known as Gum Arabic tree. It is called Bagaruwa in Hausa, Gaude in Fulfulde and Kangar in Kanuri. It is mostly found in Guinea and Sudan Savannah vegetation in Nigeria. The plant is widely used in traditional medicine in Africa, it has been found to possess significant antimicrobial activity, antioxidant, antidiarrhoeal, anticancer, antimutagenic properties, anthelmintic activity, antiplatelet aggregatory activity and vasoconstrictor among others (Singh *et al.,* 2015). The aim of this study was to determine the invivo anti-trypanosomal effect of *Acacia nilotica* stem bark methanol extract in wistar rats infected with *Trypanosoma brucei brucei.*

MATERIALS AND METHODS

This research paper report is a further continuation on the authors' previous work carried out as titled; (*In vivo* antitrypanosomal activities of *Acacia nilotica* stem bark methanol extract, as cited in Goronyo et al.,2022. (https://doi.org/10.53858/arocnpr02012127). Therefore, materials and methods were re-described in sections of this paper.

Animal ethical clearance

An approval letter on the use and care of laboratory animals was obtained from Animal Right Ethical Committee of Ahmad Bello University, Zaria.

Sources of Plant Materials

Acacia nilotica plant was collected from Goronyo town in Goronyo local government Sokoto State, North West Nigeria, between the months of December 2020 and January 2021.

Identification of Plant

The plant sample was identified at the Herbarium unit of the Department of Botany, Faculty of Life Sciences, Usmanu Danfodio University Sokoto, where a Voucher specimen number of UDUSH/ANS/0751 was given and deposited.

preparation of plant Sample

The plant materials were washed with tap water and dried under shade. The dried materials were grounded using pestle and mortar. Eight hundred grams (800g) of the ground stem bark was weighed and stored in a clean tied up polythene bag at room temperature until required.

Extraction and Fractionation of plant

500grams of the ground stem bark was used for extraction with methanol using a Soxhlet apparatus for 24 hours. The liquid extract was concentrated to dryness under rotary evaporator to remove the solvent for 12 hours. The recovered extract was weighed and subjected to fractionation using n- hexane, ethyl acetate, n- butanol and water. Sequential portioning was carried out because the polarity nature of the plant was not known.

Test Organisms

An isolate of *Trypanosoma brucei brucei* was obtained from the Department of Veterinary Parasitology and Entomology Ahmadu Bello University Zaria and maintained in the laboratory by serial passage in rats. The culture was confirmed microscopically.

Experimental animals

40 Wista rats of both sexes weighing 110g were purchased from the Department of Pharmacology Faculty of pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were maintained on commercially prepared feed and housed in disinfected cages for one week to acclimatize prior to the commencement of the experiment. The experiment was conducted at protozoology lab, Department of Veterinary parasitology and entomology. Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Experimental groups

Wistar rats were randomly assigned to different groups based on the number of fractions obtained and doses of treatment (group A to H).

Test for anti trypanosomal activities in Rats Infected with *Trypanosoma brucei brucei* using Stem bark Methanol extract Fractions

Ethyl acetate fraction, Butanol fractions, N-hexane fraction and Aqueous fractions of the methanol stem bark extract of *Acacia nilotica* were used to treat the infected rats at 300, 400 and 500mgkg^{-}bw doses for 7days. Each fraction contains 3 groups. For example Ethyl acetate Fraction, there are 3 groups- A, B, C. Group A was administered 300mg, while group B and C were administered 400 and 500mg respectively. The same applied to other fractions. However, two Groups consisting of 3 mice each were used as positive and negative controls. The positive group was infected and treated with 3.5mgkg-1bw diminazene aceturate (standard drug) while negative group was infected but not treated.

Statistical Analysis

All values were shown as mean values along with their standard deviations (±SD). SPSS software was used to analyze data. Statistical comparisons were carried out by Analysis of Variance (ANOVA), Tukey's multiple comparison test, and p values <0.05 were considered significant**.**

RESULTS

Yield of Extracts

Table 1 Shows the result of Percentage Yield (%) obtained from Fractionation and concentration of Acacia Nilotica Stem bark Methanol extract using ethyl acetate, n- hexane, n- butanol and distilled water as solvents.

Test for anti trypanosomal activities in Rats Infected with *Trypanosoma brucei brucei* using Stem bark Methanol extract Fractions

Antitrypanosomal activities of *Acacia nilotica* Stem bark Methanol extract fractions were tested using two pathological parameters (Changes in Parasiteamia level and Packed Cell Volume (PCV)) as presented in figure 1and 2 respectively.

Table 1: Weight (g) recovered and percentage (%) yield of the Stem bark crude methanol extracts and fractions of *Acacia nilotica*.

Methanol /Fraction	Weight (g)	Percentage yield (%)
Methanol	95	19.75
N – Hexane Fraction	0.28	0.37
Butanol Fraction	32.04	42.72
Ethyl Acetate Fraction	33.58	44.77
Aqueous Fraction	7.02	9.36

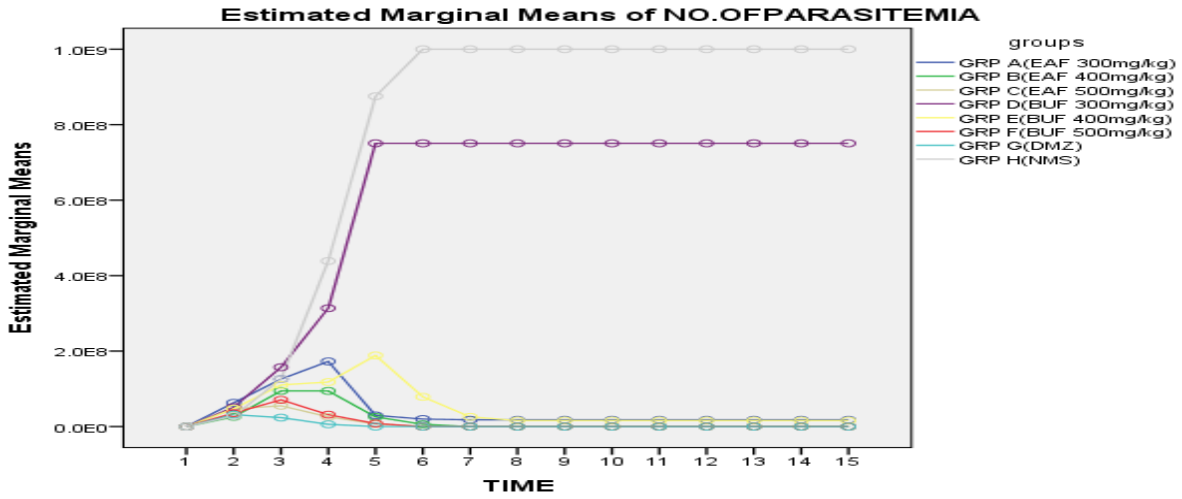

Figure 1: Changes in Parasitaemia levels of Rats infected with *Trypanosoma brucei brucei* and treated with *Stem* bark Methanol extract Fractions of *Acacia nilotica*.

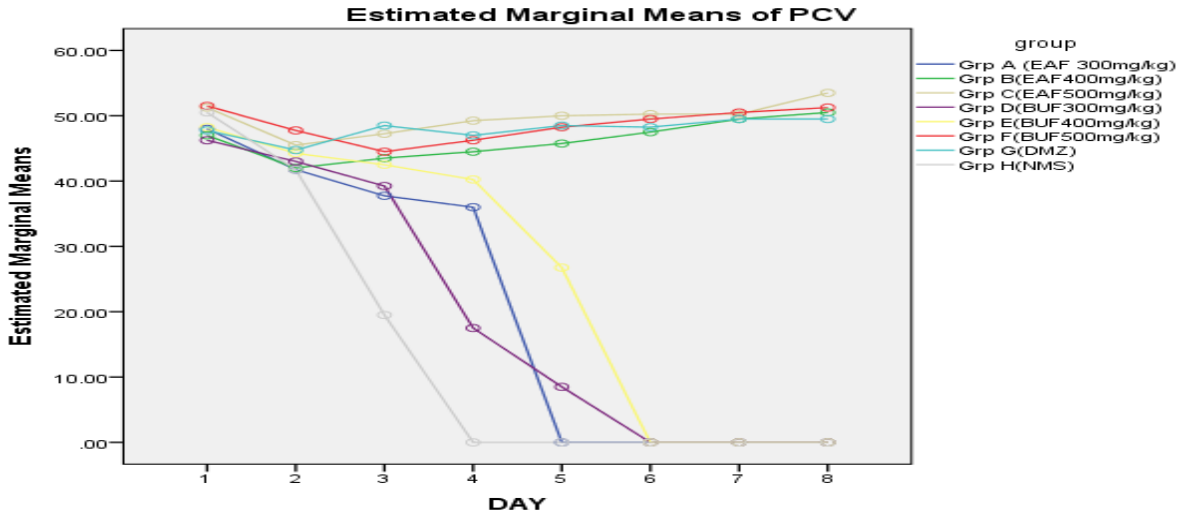

Figure 2: Average parked cell volume (%) of groups of rats (A-H) infected with *Trypanosoma brucei brucei* and treated with fractions of Stem bark methanol extract of *Acacia nilotica*.

DISCUSSION

Sequential Extraction involving solvent of varying polarity (n-hexane, ethyl acetate, n-butanol and water) was used to fractionate varied compounds from the stem bark of *A. nilotica*. As presented in table 1. A sequential fractionation procedure was chosen mainly because the nature and polarity and hence the solubility of the bioactive compound in the stem bark of the *A. nilotica* were unknown (Satyajit *et al.,* 2018). In general n-hexane was used to extract hydrophobic or non-polar compounds such as fatty acids, waxes fatty acids some alkaloids and terpenoids. (Abdulhamid *et al.,* 2019). Ethyl acetate is known to extract both medium polarities and some polar compounds such as phenols, flavonoid, tannin and some terpenoid. On the other hand, butanol and water are known to extract hydrophilic or polar compounds such as carbohydrate, amino acids and their derivatives (Abdulhamid *et al.,* 2019).

The amount (weight in grams) and the percentage (%) yield of the four fractions (and the crude methanolic extract) are presented on table 1. The weight and percentage yield of crude methanolic of *A. nilotica* were 95 grams and 19.72% respectively. Of all the four solvent fractions, ethyl acetate fraction has the highest yield (33.58g), followed by butanol fraction (32.04g), aqueous fraction (7.02g) and lastly n-hexane fraction (0.28g). This result agreed with the findings of Abdulhamid *et al,* (2019) who works on leaves of *A. nilotica* from Kebbi state. These serve as an indicator that the plant is a polar plant and can be extracted using methanol and water solvents.

The antitrypanosomal activities of the partitioned fractions against test organism show different degrees of activities. Out of the four fractions derived from the crude methanolic extract of *Acacia nilotica*, only Ethyl acetate and Butanol fractions show strong activity, This suggests that ethyl acetate and butanol will be good solvents for the isolation and purification of the active compound present in the stem bark of *Acacia nilotica.* This finding is similar to that of Anyam *et al* (2021), who recently isolated two compounds from ethyl acetate fraction of the root extract of *Acacia nilotica* from Benue. The compounds displayed high activity, particularly against *T. brucei, T. evansi, and Leishmania Mexicana* (0.88–11.7 µM) invitro. it also agreed with the work of (El-tahir *et al*., 1999 which revealed that, the ethyl acetate extract holds the highest activity on *Plasmodium* falciparum. Phytochemical analysis indicated that the most active phase contained terpenoids and tannins and was devoid of alkaloids and saponins.

On the other hand, the n-hexane and aqueous fractions showed little or no activity against *T.b.b* and all the animals treated with n-hexane and aqueous fractions died within a week. The inability of this fractions to clear the parasite from the blood could be because of their failure to get to the site of action or rapid metabolization (Dwivedi, 1997; Wurochekke *et al.,* 2005). The fractions were orally administered and lack of activity may be due to biotransformation within the Gastro intestinal tract and liver. Notwithstanding, the fractions may contains active phytochemicals which might be acting individually or synergistically to inhibit the antitrypanosomal activity.

The observed overall anti-trypanosomal effect of the extract of *A. nilotica* 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). The mechanism by which these extracts exhibited their antitrypanosomal activity can only be speculated since the active ingredient(s) were not isolated.

CONCLUSION

Acacia nilotica had significant anti-trypanosomal effect in infected rats, which results from the presence of triterpenes, flavonoid, alkaloids compounds among others. This suggests that ethyl acetate and butanol will be good solvents for the isolation and purification of the active compound present in the stem bark of *Acacia nilotica* against *Trypanosoma brucei brucei*

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Conflicts of Interest

The authors declared no conflict of interest.

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