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THERAPEUTIC ANTITRYPANOSOMAL ACTIVITY OF ALCHORNEA LAXIFLORA LEAVES EXTRACT IN RATS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA BRUCEI BRUCEI.

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ABSTRACT

African trypanosomoses has been estimated to render 50 million people and 48 million cattle at risk leading to low productivity and death. The disease is responsible for major health and economic problems in rural sub-Saharan Africa. Caused mainly by extracellular protozoa of the genus, Trypanosoma with specie such as *Trypanosoma brucei brucei*. This study was carried out to evaluate the antitrypanosomal activities of *Alchornea laxiflora* leaf extracts in rats experimentally infected with *Trypanosoma brucei brucei. Invitro* antitrypanosomal analysis was carried out at varied concentrations of 2.5mg/ml, 5mg/ml and 10mg/ml with five different solvent extracts (ethanolic, ethyl acetate, n-hexane, chloroform and aqueous) using diminazine aceturate and normal saline as positive and negative controls respectively. The invivo assay was carried out by intraperitoneal administration of ethyl acetate and chloroform extracts of the plant at graded doses of 200, 400 and 600 mg/kg for three consecutive days. The chloroform, n-hexane and ethyl acetate extracts yielded high percentage DPPH free radical scavenging activity of 86.03, 84. 16 and 76.10 respectively. All extracts tested invitro showed decrease in motility of the parasites at different times. The responses were concentration dependent positively. Complete cessation of motility was recorded at 35 and 45 minutes at 10mg/ml concentration of chloroform and ethyl acetate extracts respectively. Two extracts (ethyl acetate and chloroform extracts) showed the best *invitro* responses and were subjected to invivo analysis. Both extracts caused decrease in trypanosome parasitemia and prolongation of mean survival days of the rats to 15days as compared with 7days in the negative control group. The extracts displayed dose-dependent significant ($p \leq 0.05$) antitrypanosomal activity as compared to the control. The antitrypanosomal values obtained in this study show that the tested leaf extracts of *Alchornea laxiflora* might be a good alternative drug for the treatment of trypanosomoses in Nigeria. Further fractionation, purification and isolation should be done to confirm the active components in *Alchornea laxiflora* leaves responsible for the antitrypanosomal activities recorded.

KEYWORDS: *Alchornea laxiflora*, antitrypanosomal activities, *Trypanosoma brucei brucei*,

I. INTRODUCTION

Trypanosomosis is an important protozoan disease of domestic animals and man. Human African trypanosomosis (HAT) is caused by the tsetse fly-transmitted hemo-flagellates *Trypanosoma brucei rhodesiense* (in East and Southern Africa) and *T. b. gambiense* (in West and Central Africa), while animal trypanosomosis is caused by *T. b. brucei, T. vivax* and *T. congolense.* Sleeping sickness has been on the rise in recent years and is viewed as a major health problem in many African countries, with sixty million people being at risk of infection in sub-Saharan Africa.[1,2]

Chemotherapy and chemoprophylaxis, coupled with vector control programmes, have been the main stay of trypanosomosis control in Africa in the absence of effective vaccine against the disease. Drug control of animal trypanosomosis in Africa is mainly dependent on the use of diminazene and isometamidium chloride. Their continued use and effectiveness have been threatened by serious limitations, which include high cost, serious side effects, long-course of parenteral administration, variable efficacy and emergence of drug resistant trypanosome strains.[3] The presence of drug resistant trypanosomes has recently risen to alarming proportions[4], hence the search for new chemical entities that are effective against trypanosomes, safe and affordable for disease-endemic countries is rational.[5]

Plants are potential sources of new drugs due to the presence of countless number of secondary molecules that have pharmacological effects.[5,6] Exploring traditionally claimed medicinal plant for the biological activity gave humankind a number of antiprotozoal medications. All over the world, pastoralists have old tradition in the use of herbs for the treatment of several animal diseases, including trypanosomosis. *Alchornea laxiflora* (Benth). 'Ewe lya, Pepe in Yoruba' leaves

belong to Euphorbiaceae family, it grows naturally in South-West Nigeria and are mostly used for packaging and preservation of kola nuts in Nigeria. The decoction of the leaves is used to treat inflammatory and infectious diseases. The leaves are also a common ingredient in herbal antiprotozoan preparations.[7]

Validation of medicinal plant for their antitrypanomal activity will guide the society for the best approach to employ their indigenous knowledge and at the same time provide hit compounds to feed future pipeline for antitrypanomal drug development.[8] This study therefore provides a valid scientific proof for the invitro and invivo therapeutic efficacy of crude extracts of *Alchornea laxiflora* against *Trypanosoma brucei brucei* infected wistar albino rats.

II. MATERIALS AND METHODS

A. Collection of plant leaves

The fresh leaves of plant were collected from their natural habitats in Akure, Ondo State, Nigeria during the day time. Authentication was done by expert from Department of Forestry and Wood Technology, Federal University of Technology, Akure.

B. Extraction of plants

The leaves were air dried and crushed with the aid of a mechanical grinder to powdered form. Extraction with five different solvents (chloroform, ethanol, hexane, ethyl acetate and distilled water) were done. A dry weight of 200 g of powdered plant leaf sample was soaked in the 1.0 liter of solvents to extract the bioactive components of plants. The mixture was daily stirred for 72 hours. The resultant mixtures were filtered with muslin cloth followed by No. 1. Whatman filter paper of pore size 2.5µm. The extracts were lyophilized using rotary evaporator (R110) at 40°C to obtain a dry powder extract. Percentage yield of each plant extracts were calculated. The respective extracts obtained were kept in a refrigerator until use.

C. Phytochemical Analysis for the Plant Extracts

Extracts were subjected to qualitative and quantitative phytochemical screening according to the method described by Trease and Evans[9] and Ejikeme *et al*.,[10] to detect the presence or absence of plant secondary metabolites such as; saponins, tannins, alkaloids, flavonoids and phenol.

D. DPPH free radical scavenging activities

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-dipheny 1-2-picrylhydrazyl (DPPH) free radical will be determined by the method described by Villano *et al*.[11]

E. Trypanosomes and experimental animals

Trypanosoma brucei brucei was gotten from the National Institute of Trypanosomosis Research, Vom, Jos, Nigeria. The parasites were passaged serially in a donor rat. Parasitaemia of 'donor rat' was estimated by "rapid matching" method, as described by Herbert and Lumsden.[12]

F. Acclimatization of Experimental rats

Healthy adult wistar albino rats of both sexes weighing 130-180g were procured from the Animal facility of Obafemi Awolowo University Ile Ife, Osun State, Nigeria. They were housed in clean dry cages and fed standard pellets and watered ad libitum. They were acclimatized for seven (7) days and cared for according to the international guidelines for the use and maintenance of experimental animals.[13]

G. Evaluation of invitro antitrypanosomal activity

Invitro test was performed in triplicates to detect any motile trypanosomes in a 96 well microplate. Twenty microliters of blood containing about 16-32 organisms per field were mixed with 5µL of the test substance at concentrations of 2.5, 5, 10mg/mL to produce test concentrations of 0.5, 1, 2.0mg/mL, respectively.

Phosphate buffer saline (pH 7.2) and standard trypanocidal drug, diminazene aceturate (DA) were used to serve as untreated control and treated controls, respectively. The mixtures were incubated at 37°C for up to 2hours. During the period, motility of the parasites was checked in 5 minutes' interval under microscope (X40 objective lens). Briefly, about 2µL of test mixtures was placed on microscope slide and covered with cover slips. The parasites were observed for reduced motility or complete cessation of motility. Chloroform and ethyl acetate extracts had the best invitro activities and were subjected to invivo analysis..

H. Evaluation of invivo antitrypanosomal activity

Forty-eight wistar albino rats of both sexes weighing between 130g-180g were used for the invivo phase of this study. The animals were randomly divided into eight groups (I-VIII) consisting 6 rats each. All groups were infected intraperitoneally with 0.2ml of T. brucei (at 7.2 antilog) suspension. Groups I-III and IV-VI were treated with the chloroform and ethyl acetate extracts respectively at daily doses of 200, 400 and 600mg/kg body weight for three days intraperitoneally. Groups VII and VIII were administered Diminacene aceturate DA (3.35mg/kg) and 0.3 mL normal saline intraperitoneally respectively to serve as treated and untreated controls. Treatment began on fourth day post inoculation. Body weight, parasitemia, rectal temperature and environmental temperatures were monitored daily for 21days.[14]

Parasitemia was monitored by examining blood from the tail of rats under microscope at × 400 magnications using the "Rapid Matching" method of Herbert and Lumsden 1979. Monitoring of parasitemia was performed daily until the 21st day post-treatment initiation.[15, 16]

Body weight of experimental animals were recorded on the day of parasite challenge and everyday thereafter for 21days.[17]

Rectal temperature was measured using digital rectal thermometer (Mettler Toledo, Switzerland) on the day of parasite inoculation and everyday thereafter for 21days.[18]

III. RESULTS

A. Yield

Ethanol gave the highest yield followed by n-hexane, chloroform, aqueous and ethyl acetate in that order.

Table I: Percentage Yield of The Different Solvents of Extraction.

S/N	EXTRACTS	% YIELD
1	EEAL	14.6
2	EAAL	8.8
3	NHAL	10.49
4	AEAL	9.24
5	CEAL	9.65

EEAL: Ethanolic extract of *A. laxiflora*
EAAL: Ethyl acetate extract of *A. laxiflora*
NHAL: N- hexane extract of *A. laxiflora*
AEAL: Aqueous extract of *A. laxiflora*
CEAL: Chloroform extract of *A. laxiflora*

B. Phytochemical screening

Phytochemical screening revealed the presence of saponins, tannins, phenol, flavonoids and alkaloids in varying quantities with the various solvents used for the extraction (Figure I).

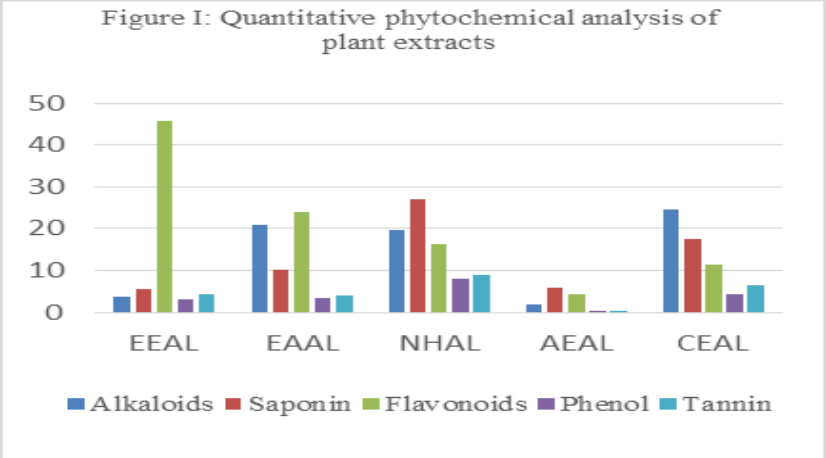

Figure 1: Quantitative phytochemical analysis of plant extracts.

C. Invitro antitrypanosomal activity

Chloroform and ethyl acetate extracts of *A. laxiflora* leaves ceased motility of the trypanosomes within 50 minutes at 10 and 5 mg/ml concentrations (Table II).

Table Ii: Effect Of Different Extracts Of Alchornea Laxiflora On Motility Ceasation Time Of Trypanosoma Brucei Brucei Invitro.

	Time (min) After Which Motility Ceased						
Concentration	**EEAL**	**EAAL**	**NHAL**	**AEAL**	**CEAL**	**DA**	**NC**
10mg/ml	65	45	60	75	35	15	NE
5mg/ml	75	50	70	85	45	20	NE
2.5mg/ml	95	70	80	95	55	35	NE

D. Effect of treatment on parasitemia

Parasitemia level assessed at day 5 post-treatment in all experimental animals for the different groups showed that all doses of the extract including the standard drug suppressed parasitemia ($p<0.05$). The best result was obtained with chloroform extract of *A. laxiflora* at 400mg/kg on day 11 (Figure II).

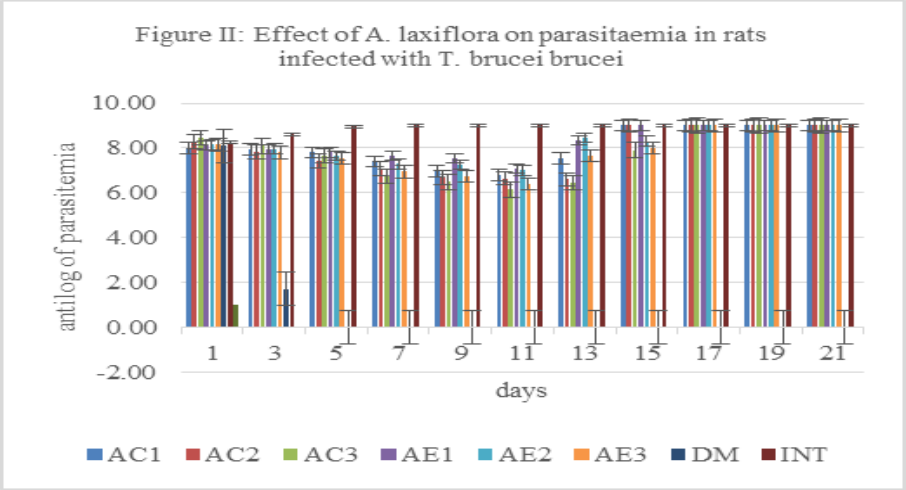

Figure II: Effect of A. laxiflora on parasitaemia in rats infected with *T. brucei brucei.*

Notes
AC1: Chloroform extract of *A. laxiflora* at 200mg/kg
AC2: Chloroform extract of *A. laxiflora* at 400mg/kg
AC3: Chloroform extract of *A. laxiflora* at 600mg/kg
AE1: Ethyl acetate extract of *A. laxiflora* at 200mg/kg
AE2: Ethyl acetate extract of *A. laxiflora* at 400mg/kg
AE3: Ethyl acetate extract of *A. laxiflora* at 600mg/kg
DM : Diminazene aceturate at 3.35mg/kg
INT: Infected not treated

E. Effect of treatment on body weight

There is statistically significant ($p<0.05$) body weight changes in 600mg/kg and DA 3.35mg/kg treated groups compared with 200mg/kg, 400mg/kg, treatment groups with both chloroform and ethyl acetate extracts from day 8 to day 14 post-treatment initiation and with untreated control on day 5 post-treatment (Figure III).

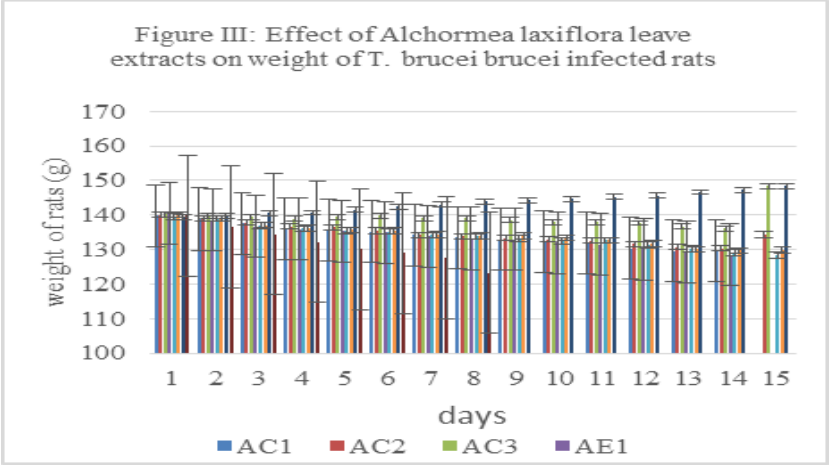

Figure III: Effect of Alchormea laxiflora leave extracts on weight of T. brucei brucei infected rats.

F. Effect of treatment on mean survival days

The average survival days of infected rats were increased to 13.33 and 14 days in 600mg/kg of ethyl acetate and chloroform extracts respectively compared with 6.83 days in negative control group (Figure IV).

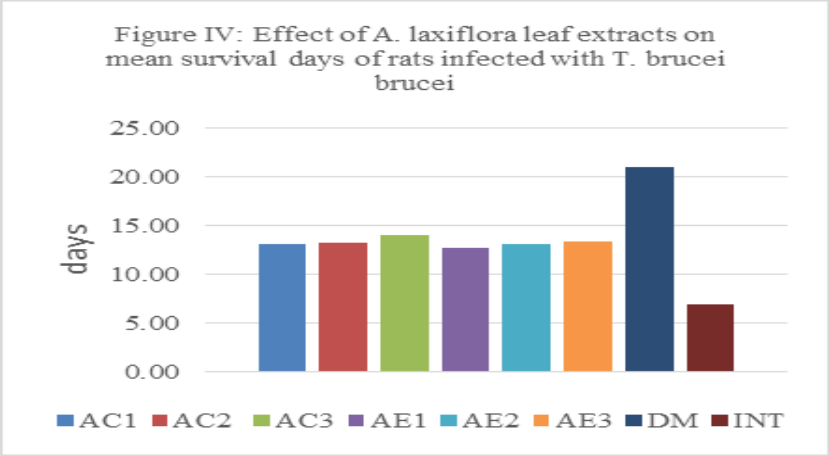

Figure IV: Effect of *A. laxiflora* leaf extracts on mean survival days of rats infected with *T. brucei brucei.*

G. Effects of treatment on rectal temperature

The rectal temperatures of the rats fluctuated throughout the duration of the experiment. There is no observable significant difference nor pattern.

IV. DISCUSSION

The variations in the yield of the different solvent extracts is due to affinity of the chemical composition. Compounds have been found to be more soluble in polar solvent such as methanol when compared with chloroform which is more of non-polar solvent.[19] Omoya and Oloruntuyi[20] also reported variation in yield of *Alchornea laxiflora* using methanol and chloroform.

The qualitative and quantitative phytochemicals obtained from both plants differ with the various solvent used for the extraction. Alkaloids have significant pharmacological functions such as; antimalarial, anticancer, analgesic, and anti-hyperglycemic and antibacterial functions.[21] Terpenoids have antioxidant activity and Steroids are used to remedy inflammatory conditions. Glycosides have antimicrobial and anticancer activities. Saponins have been evidenced to possess anticoagulant, anti-carcinogenic, hypoglycemic, immunomodulator, neuroprotective, anti-inflammatory and antioxidant potentials.[22]

The availability of these constituents in the leaf of Alchornea laxiflora and Annona muricata gives credence for their traditional uses in the remedy of diseases.

Antitrypanosomal and therapeutic properties of medicinal plants have been attributed to the presence of different classes of secondary metabolites or phytochemicals compounds contained in them. The leaf extract of *Alchornea laxiflora* contains alkaloids, saponins, tannins, phlobatannins, and flavonoids. These phytochemicals have been reported earlier to be present in memebrs of the family Euphorbiaceae.[23]

The decrease in body weight in the 400mg/kg and 600mg/kg over a period might be due to anorexia which is one of the clinical features of trypanosomosis. This can further be described by the inverse relationship between level of parasitaemia and weight loss of gain in the treatment groups; a relationship that is positively dose dependent.

Chloroform extracts of *A. laxiflora* showed higher antitrypanosomal activities than its ethyl acetate extract both invitro and invivo. This fact may suggest that the pharmacological active ingredients of this plant responsible for its antitrypanosomal activities may be localized in the chloroform extract to a large extent. Diminazene aceturate treatment group showed highest antitrypanosomal activities in the infected rats because the parasite has been tested sensitive to diminazene being a standard antitrypanosomal drug. The effectiveness of diminazene aceturate at very low dosages is because it has been purified compared with the crude extracts that were used in which relapse was recorded.

V. CONCLUSION

The antitrypanosomal action of the plant may be attributed to the presence of active secondary metabolites in the leaf and this affirms its use in Nigeria local communities in the treatment of parasitic infection. The chloroform and ethyl acetate extracts showed a relatively higher activity over other solvent extracts used in this study.

This study discovered that extract of *A. laxiflora* can be beneficial to human and animal health in the treatment of trypanosomosis.

This study will provide a lead for researchers to uncover the specific bioactive component of *Alchornea laxiflora* that exerts the antitrypanosomal efficacy.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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