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Preliminary Evaluation of Anti-trypanosome Impact of Methanol, Alkaloid and Flavonoid Extracts of *Sarcocephalus latifolius* in *T. Brucei* Infected Mice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The menace of ravaging infection of trypanosomiases in Africa is of concern in view of the parasites resistance to drug coupled with the cost and extended treatment practice with adverse effect. The prospect of exploiting medicinal plants as alternative in treating illnesses is heightened. This study evaluated the anti-trypanosome activities of the methanol, alkaloid and flavonoid extracts of *sarocephalus latifolius* leaves. Acute toxicity of the extracts was obtained using the Lorke's method. *In vitro* and *in vivo* analysis of the test plant leaves was determined using different concentrations. The *in vitro* anti-trypanosome effect of the crude methanol, alkaloid and flavonoid extracts of *Sarocephalus latifolius* leaf showed that the activity was determined based on dosage with more influence at 4 mg/mL in relation to 0.4 and 0.04 mg/ml. There is significant different ($p<0.05$) between mice treated with extract and the reference drug. The acute toxicity test was safe as no mortality was recorded at 5000mg/kg of bodyweight of mice. However, *in vivo* Anti-trypanosome activity of the crude extract at 250 and 500mg/kg bodyweight exposed a trend of increase in the parasite level which was observed when compared with standard control. But, elongation of life in mice was observed. Methanol treatment of mice lasted for about nine (9) days before mortality

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occurred while, that of alkaloid and flavonoid lasted for about five (5) days respectively subsequent to mortality. Consequently, the *in vivo* anti-trypanosome activity of methanol, alkaloid and flavonoid extracts did not eradicate the parasite in the blood of the poisoned mice but, appear to have prolonged the life of the treated animal when compared with the infected untreated group. Therefore, methanol extract of *Sarocephalus latifolius* leaf having the most influence on mice has illustrated more potential in anti-trypanosome activity which can be utilize in controlling African trypanosomiasis.

Keywords: Alkaloid; flavonoid; methanol extract; trypanosomiasis; Trypanosoma brucei; Sarocephalus latifolius.

1. INTRODUCTION

The sickness known as trypanosomiasis is initiated by a parasite (trypanosome species) which infect the blood of its host. It has been an issue to humans and animal mostly in African region. The parasite "*tryapnosoma brucei"* is a flagellated unicellular pathogen which have impacted adversely to health and economic benefits [1]. However, humans and animals are infected by two different classes of trypanosomiases which includes hematic groups (*T. congolense* and *T. vivax*) and tissue invading group (*T. brucei, T. evansi, T. rhodesiense, T. gambiense and T. equiperdum*). This parasites mechanism of action is distinct owing to their unique nature. The hematic groups occur within the plasma of the blood cell while, tissue groups are located intravascularly or extravascular [1]. The infection occurs in two phases which are haemolymphatic spread and late central nervous system infection. When the infection is within the latter phase, it usually difficult to cure [2]. Furthermore, the parasite mode of transmission is through a vector mostly species of *Glossinia* and *Stomoxy* which are commonly referred to as Tse-tse fly. The insect is known to cause sleeping sickness when it bites the host. The degree of virulence differs probably because of the various strains and its host [3]. Infection of host with trypanosomiases poses a constraint in the production of both animal and plant products and also, limit the efficiency of animals in farm work [4]. The two major approaches involved in constraint of the infection is through elimination or reduction of tse-tse fly (insecticides) and prophylaxis treatment by trypanostatic or trypanocide drugs [5-7]. Medical indicators of trypanosomiasis include but not limited to psychological, endocrinological, dermatological, cardiovascular impact [8]. Consequently, the efficiency of programs aimed at combating the menace caused by trypanosomiases remain to be reviewed owing to minimal success recorded [9]. Indications of resistance suggest the proficiency of parasite to initiate immunosuppression which prevent the host from eliminating the trypanosome after drug administration [10]. Therefore, efforts intended at treatment and cure of trypanosomiasis through therapeutic efficacy of ethnomedicinal plants is sustained. Remarkable trypanocide effect demonstrated through plants elucidate the efficacy of plants extract in trypanosomiasis treatment [11,12]. The root and bark extract of *Terminalia superba* inhibited trypanosomiasis growth entirely in rats and mice [13] while, complete elimination of the parasite through the root and bark extract of *Afzelia africana* and *Khaya senegalensis* [14]. *Sarcocephalus latifolius (Nuclea latifolia*) is a common plant in Nigeria. This plant belongs to the family of *Rubiaceae* and locally it is known with different names when considering the tree major tribes of Nigeria. The plant is referred to as egbesi (Yoruba), *tafashiya* or *tuwon biri* (Hausa), *ubuluinu* in (Igbo), and African Peach in English. This plant species has been observed to have various therapeutic effect and as such been utilized in treatments of different ailment such as tooth decay, indigestion, wounds, diabetes fever, malaria, constipation, diabetes, wounds, cough, gonorrhea, septic mouth, diarrhea and dysentery [15,13]. Hence, the motive to exploit the medicinal benefit of *Sarcocephalus latifolius* in treating trypanosomiasis by evaluating the anti-trypanosome activity of the crude methanol, alkaloid and flavonoid extracts in *T. brucei* infected mice.

2. MATERIALS AND METHODS

2.1 Sample Collections

The plant sample utilized in this investigation were acquired from katcha area of Bida Local Government. *Sarcocephalus latifolius* leaves were identified at department of Forestry and Wood Technology, Federal University of Technology, Minna, Niger state and

authenticated at Herbarium Department of National Institute of Pharmaceutical Research and Drug Development (NIPRD) Idu-Abuja, Nigeria [16]. While, male and female mice(20-25g) were purchased from Biological Science department Niger State Polytechnic, Zungeru. They were sustained to adjust to the environmental conditions within a period of two weeks prior to the experiment.

2.2 Collection *Trypanosome brucei*

National Institute for Trypanosomiasis Research (NITR) Kaduna State, Nigeria supply *Trypanosoma brucei* parasites. The parasites were inoculated and maintained in three uninfected albino mice earlier to the experiment.

2.3 Plant Preparation and Extraction

About 200g of plant sample was weighted and poured into 1000ml volumetric flask. Methanol(800ml) was added and mixed thoroughly before heating at 45^{O}C for 2hrs. Whatman paper filter of 20µm pore size was utilized for filtering and the extract evaporated at 40^{O}C and dried in water bath at 35^{O}C before transferring to a sterile flask and stored at 4^{O}C [3]. The percentage yield was obtained using the equation below:

$$\%\, Yield = \frac{Weight\ of\ extract}{Weight\ of\ dry\ sample}\ X\ 100$$

2.4 Determination of Phytochemical Contents of Test Plant

Flavonoid was determined through method [17]. An aliquot (0.5 ml) of the methanol extract was blended with methanol (1.5ml), aluminum chloride (0.1m of 10%)0.1ml), 1M sodium acetate and distilled water(2.8ml) which was preserved for 30 min at standard condition. It was calibrated using standard concentration of 12.5 to 100g/ml of methanol and reading of absorbance was obtained at 415nm. However, Alkaloid was obtained according to method [8].

2.5 *In vitro* Analysis of Leaf Extracts

Estimation of crude methanol, flavonoid and alkaloid extracts of *S. latifolius* for *in vitro* trypanocide activity was ascertained using micro titer plate with 96 wells [18]. About 20mg/ml of the extracts concentration was suspended in 1ml of phosphate buffer saline (PBS). Poisoned blood of about 20µl was mixed with 5µl of extract solution(20mg/ml) to formulate different concentrations of 4, 0.4, 0.04mg/ml. Suspended parasite in normal saline was prepared as negative control while, the same concentration treated with 3.5 mg/kg bodyweight of berenil was retained as positive control. The Eppendorf tubes containing the test mixture were incubated at 37 ^{O}C for 2min. Slide containing about 2µl of the test mixture was examined using light microscope within an interval of 5mins for about 60mins to observe parasite motility.

2.6 Evaluation of Acute Toxicity

Toxicity test was performed [4]. This was done to obtain the lethal dose (LD_{50}) of the crude extracts. Three different mice were injected with 10,100 and 1000ml/kg of crude extracts and observed for changes including death for 24hrs.The same treatment was carried out with different dosages 1600,2900,5000mg/kg. The lethal dose was obtained using the equation below:

$LD_{50} = \sqrt{(D_0 \times D_{100})}$
D_0 is the maximum dose with no death
D_{100} is the minimum dose with total death

2.7 Infection and Treatment of Experimental Mice

Blood sample infected with *trypanoma brucei* was obtained and thin out with normal saline to 1x10^{6}. Prior to mice infection, tail blood microscopy was carried out to determine the status of the experimental mice in *trypanosoma brucie* infection. About 0.2ml was injected intraperitoneally into healthy mice. Different doses of plant leaf extracts were introduced into the mice to examine the level of parasite inhibition [1] as listed in the table.

The animals were treated daily after the establishment of infection and parasitemia was monitored at 2 days interval. The packed cell volume (PCV) was obtained in earlier and later treatment. The physical characteristics such as weight of the albino mice was also monitored. All data was obtained in triplicate.

2.8 Statistical Analysis

The entire data were expressed as mean ± Standard Error Mean and One Way Analysis of Variance (ANOVA) test and Dunnet multiple comparison were obtained to test for significance at ($p< 0.05$).

Table 1. Treatment of infected mice

Extract/Reference Drug	Dosage(250mg/kg)	500mg/kg
Alkaloid	3	3
Flavonoid	3	3
Methanol	3	3
Berenil(control+)	3	-
Not treated(control-)	3	-

3. RESULTS

3.1 *In vitro* Anti-Trypanosome impact of Methanol, Alkaloid and Flavonoid Extracts

Table 2 (a and b) represent the *in vitro* anti-trypanosome action of methanol, alkaloid and flavonoid extract of *S. latifolius* leaf at different concentrations. In table 2a, there was significant difference ($p<0.05$) between the positive control and the various extracts utilized. However, increase in parasitemia was observed across the extracts treated mice with reduction in concentrations. This indicates that the potency of the extracts is dose dependent. Though, methanol demonstrates more potential as fewer parasitemia of about 16.14 count was observed. Mice treated with flavonoid and alkaloid had a higher count of parasitemia within different concentrations exploited. Mice infected but not treated retain the highest parasitic load.

Table 2b shows that methanol reduced the motility of the parasite (*Trypanosoma brucei*) at concentration 0.4mg/ml and 4mg/ml while, at 0.04mg/ml slightly reduced motility was observed. Furthermore, alkaloid and flavonoid had no noticeable effect on motility at 0.04mg/ml and the parasite motility were slightly reduced at concentration 0.4mg/ml and 4mg/ml respectively. This suggests that methanol is more potent that alkaloid and flavonoid in treatment of trypanosomiases. There is no noticeable effect on motility of mice (not treated).

3.2 *In vivo* anti-trypanosome Impact of Methanol, Flavonoid and Alkaloid Extracts

In vivo anti-rypanosome activity of extract of *S. latifolius* leaf is shown in Fig. 1(a,b and c). Extreme inhibition of the parasite was observed on mice preserved with berenil (+control). Parasite level was increasing in the untreated mice with infection as days progressed.Generally, increment in treatment days were observed in mice treated with extracts. However, Fig. 1a depicted the most extended day indicating that there was elongation of life in methanol treated mice.The treatment days were up to nine (9) days. But, the parasite appears to be multiplying increasingly. However, flavonoid and alkaloid in Fig. 1b and c shows life extension as the treatment lasted for about five(5) days before motility occurred.However, the parasite count displays growth.

Fig. 2(a and b) represents the *in vivo* anti-trypanosome effect of the extracts on the weight and pack cell volum(PCV) of the mice after treatment. Variations in weight was observed in mice after the treatment was carried out with respect to the different doses utilized. This reveals that the extracts treatment is dose dependent as shown in Fig. 2a. However, in Fig. 2b the pack cell volume (PCV) was lower after the treatment.

Table 2a. Treatment concentrations of S. *latifolius* leaf extract

Treatments	Conc(4.0mg/ml)	0.4mg/ml	0.04mg/ml
Berenil(+control)	7.43±4.35[a]	7.43±4.35[a]	7.43±4.35[a]
Methanol	16.14±6.09	23.57±5.32	27.14±3.18
Alkaloid	26.14±4.06	29.71±4.46	30.57±3.95
Flavonoid	27.29±5.15	32.00±4.83	31.71±3.73
Not treated(-control)	39.86±5.01	39.86±5.01	39.86±5.01

Means not labeled 'a' are significantly different

Table 2b. Concentrations effect of *S. latifolius* leaf extract on Motility of parasite

Extracts	0.04mg/ml	0.4mg/ml	4mg/ml
Beneril(+control)	25*	10*	5*
Methanol	60***	50**	30**
Alkaloid	-	60***	45***
Flavonoid	-	55***	40***
Not treated(-control)	-	-	-

No effect on motility after 60mins (-), inhibited motility (*), Moderated motility drastically (**), Moderated motility slightly (***)

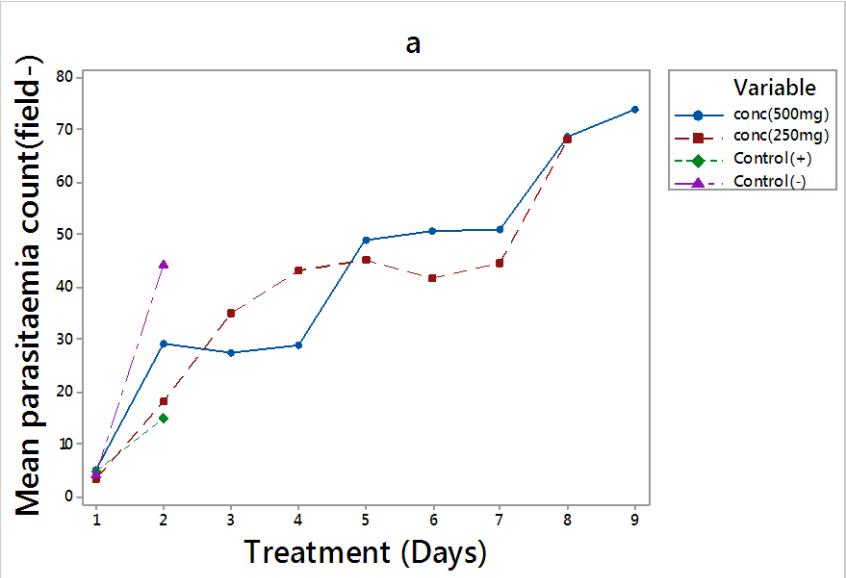

Fig. 1a. *In vivo* anti-trypanosome activity of methanol

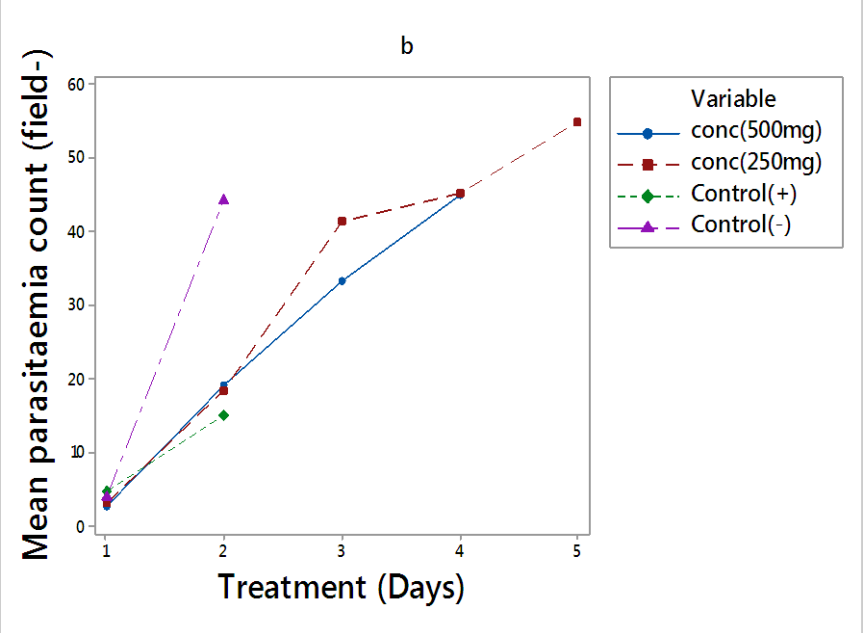

Fig. 1b. *In vivo* anti-trypanosome activity of flavonoid

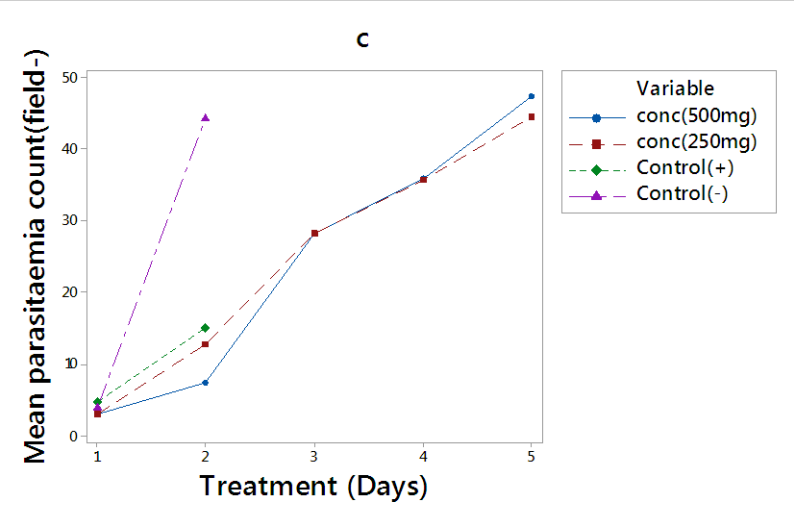

Fig. 1c. *In vivo* anti-trypanosome activity of alkaloid

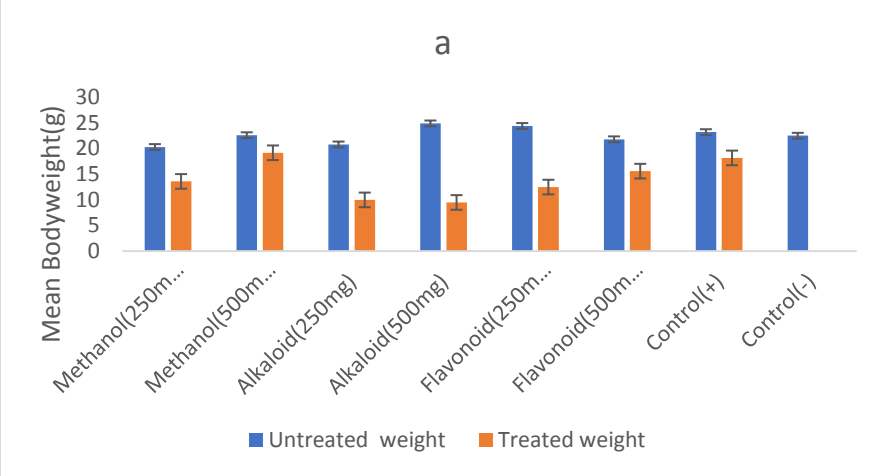

Fig. 2a. Extracts effect on weight of Infected Mice

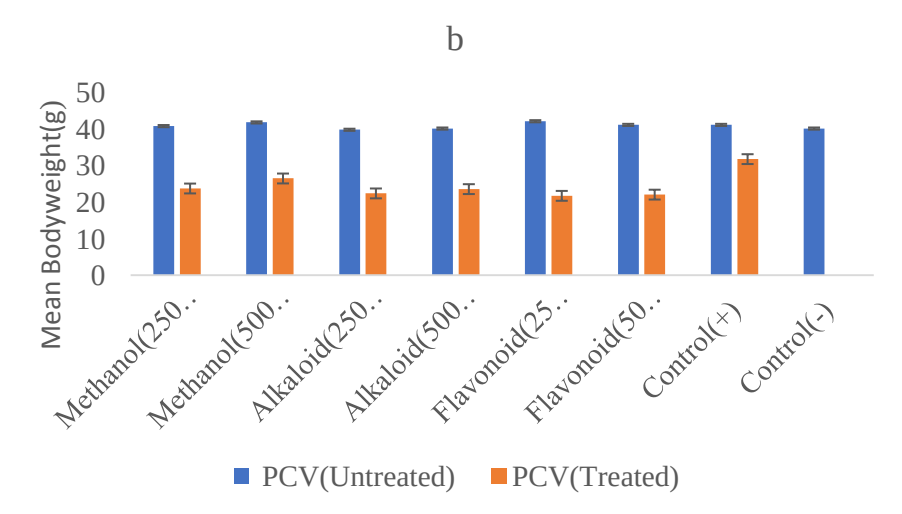

Fig. 2b. Extracts effect on PCV of Infected Mice

4. DISCUSSION

Phytochemicals are necessary as the success of therapeutic effect of medicinal plants has been accredited to it. This probably validates the utilization of plants by traditionalist in treating ailments. Phytochemicals obtained from medicinal plants exerts its effect on different ailments [18]. However, phytochemical is toxic to parasite cells and inhibits their growth [17]. Consequently, the *in vitro* anti-trypanosome activity of the methanol, alkaloid and flavonoid extracts of *S. latifolius* leaf at various concentrations demonstrates that the activity of the extract was dose dependent and appears to be more potent at 4 mg/ml. Steady decrease in the parasitemia count of the mice preserved with methanol extract as time elapses was observed when compared to alkaloid and flavonoid extracts at all concentrations evaluated. Although, there was significant difference ($p<0.05$) with the positive control which was preserved with standard drug. This indicates that the extracts did not exert the desired effect of eliminating the parasite [19-21]. Furthermore, the findings from *in vivo* analysis suggest that the extracts could be responsible for the prolonged survival of exposed mice. This could be attribute to the degradation of active compound which is toxic to the parasite. Trypanocide property of extract is probably because of the phytochemical constituents [22]. Moreover, methanol had the most effect on motility of the parasite. This shows that it elicited trypanocide effect. Parasite motility establishes a moderately dependable guide of viability in most trypanosome. Thus, extensive eradication or decline in motility associated with control group is assumed as a measure of trypanocide activity [23]. Additionally, continuing decrease in weight of the infected mice during treatment supports the observation on parasitemia. This might be as a result of weakening of the body system which may affect the food consumption of these mice. The energy deficit and tissue damage of mice reveal lack of food intake subsequent to parasite infection [22]. The pack cell volume which assists in determination of the anemic state of the mice exhibits lack of blood which is consistent with trypanosome infection on animal. This may be due to radicals released during the parasite's manifestation. Reactive oxygen species could induce oxidation which can lead to hemolysis [24]. Generally, methanol extract has revealed similar prospect with berenil (positive control) in reducing PCV deficiency. This may not be unconnected to rummaging impact of the extract. In the untreated mice, the different concentrations of flavonoid and alkaloid extracts could not decrease the parasite. The parasite counts indicate that there was increased while, the PCV decreased daily till mortality occurred [25].

5. CONCLUSION

The methanol, alkaloid and flavonoid extracts of *S. latifolius* leaf have demonstrated anti trypanosome potential on *T*. *Brucei* infected mice even though, it could not eliminate the parasite. Nonetheless, effect was exerted through extended life expectancy of the infected mice by methanol than alkaloid and flavonoid. This effect of methanol might be synergistically stimulated. The LD_{50} of the methanol extract of *Sarcocephalus latifolius* leaf was more than 5000mg/kg bodyweight. This reveals it is not toxic.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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