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Original Research Article

Screening of Ethanol extract of *Combretum racemosum* and *Euphorbia hirta* leaves for possible activity on *Trypanosoma brucei brucei* infected mice

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Abstract: Enrichment of medicinal plants with biologically active compounds which induce various chemo-therapeutic effects has made a good turn and philosophy in the science of pharmacology. In the light of the popular notion of the use of *Combretum racemosum* and *Euphorbia hirta* as potent ethnopharmaceutical botanicals, this study was done to determine the trypanocidal activity of the ethanol leaf extracts of the plants against *Trypanosoma brucei brucei* which was induced in Swiss albino mice. The animals were inoculated intraperitoneally (IP) with trypanosome load of 10^6, and were then kept under standard conditions for 10 days to enable circulation and reproduction of the parasite within them. Parasitaemia level was detected and analysed via microscopy. Both plants proved positive by overall reduction in the mean parasitaemia level as the days progressed at concentrations of 50,100 and 200mg/kg body weight respectively. Acute toxic dose for analysis of the high dose extract toleration was also checked by a 1000mg/kg administration of the extracts, while diminazene aceturate, a standard trypanocidal drug was used as control. *Combretum racemosum* exhibited its best trypanocidal activity at the 200mg/kg concentration, and *Euphorbia hirta* was at its best at 50mg/kg. Following the administration of diminazene aceturate (control) the parasites were cleared within four days of administration. The results derived were confirmed with statistical analysis using SPSS 16 software at $p<0.05$, and posits the possible utilization of these extracts of *Combretum racemosum* and *Euphorbia hirta* as trypanocidal agents.
Keywords: Trypanocidal, *Euphorbia hirta, Combretum racemosum,* Parasitaemiae, Mice, Phytochemicals, Extract.

INTRODUCTION

The pharmacological importance of indigenous plant herbs and fibers over the years has been over looked by the scientific world. However, in many parts of the World, ethno therapies are no longer seen as myth, superstition, witchcraft or ungodly practices [1] and their use in ethno pharmaceutical practices has augmented modern medicine. Medicinal plants represent a constituent part of the natural biodiversity of many countries in Africa [2].

The plant *Combretum racemosum* commonly called Ebi-odo among the Urhobos in Nigeria [3], is a straggling shrub widespread across Africa and bears a mass of crimson flowers which is very spectacular gaining it the local English name of Christmas rose in Southern Nigeria [4].The leaves of *Combretum racemosum*are used mostly in Nigeria traditionally as a remedy for the treatment of some parasitic, bacterial and fungal infections, and has a folkloric reputation as an antiulcer [5], trypanocidal [6,7], antihelminthic and antimicrobial [8] agents and also claimed to be effective against stomach pains, dysentery, abdominal disorder, fever [9,10]. Previous phytochemical analysis of *C. racemosum* extracts revealed the presence of alkaloids, steroids, cardiac glycosides, saponins and tannins [8] and also, anthocyanins and triterpenoids [11].

Euphorbia hirta on the other hand, is a pantropical weed, very hairy and grows in open grasslands, roadsides and pathways. It has been shown to kill various types of pathogenic bacteria, i.e. *Plasmodium* [12;13] and also, widely used as a decoction or infusion to treat various ailments including intestinal parasites, diarrhoea, peptic ulcers, heartburn, vomiting, amoebic dysentery, asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs, colds, kidney stones, menstrual problems, pimples, gonorrhoea, digestive problems and tumors, sterility and venereal diseases [14]. It is reported to contain alkalods, triterpenes, phytosterols, tannins, polyphenols and flavonoids.

African trypanosomiasis is an age long protozoan disease of both man and animals. It is caused by flagellated haemoprotozoan parasite (trypanosomes)

and transmitted by tsetsefly(Glossina genus). The most pathogenic trypanosomes belong to the vivax group (Duttonella), congolense group (Nannomonas) and the brucei group (Trypanozoon) subgenera. *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* cause Human African Trypanosomiasis (HAT), whereas African Animal trypanosomiasis (AAT) is caused by *Trypanosoma brucei brucei*, *Trypanosoma viva* and *Trypanosoma congolense*(in cattle, sheep, goats, and dogs), *Trypanosoma equiperdum*(in equidae), *Trypanosoma simiae*(in pigs) and *Trypanosoma evansi*(in camels). The disease causes huge economic losses and has been a major setback to livestock production in sub-Saharan Africa. Some studies show that plants are used in traditional medicine in Africa to treat trypanosomiasis in humans and animals [15; 16 17 &18]. Chemotherapy is faced with problems of parasite resistance [19], toxicity [20], misuse or inefficient application [21]and high costs of the few available veterinary and human trypanocides [22]. The aim of this study was therefore to evaluate the *in vivo* trypanocidal activity of crude ethanol extract of the leaves of *Combretum racemosum* and *Euphorbia hirta*.

MATERIALS AND METHODS

Collection of Plant Material

Fresh leaves of *Combretum racemosum* and *Euphorbia hirta* were collected from the surroundings of Federal University of Technology Owerri, Imo State (South-East), Nigeria. The plants were identified and authenticated by Mr. Iwueze Francis of the Department of Forestry and Wildlife Technology, FUTO.

Ethical Approval

The research was approved by the Research Grant and Experimentation Ethics Committee of the Federal University of Technology, Owerri, Imo State, Nigeria.

Preparation of Plant Material

After collection, the leaves of *Combretum racemosum* and *Euphorbia hirta* were rinsed with water to remove dust and dirt, followed by air drying under the shade for about two weeks. The dried leaves were then pulverized (ground into fine powder) using a properly cleaned grinder. Ethanol extraction was carried out for 72 hours at room temperature with intermittent shaking, while resultant mixture was filtered using Whatman ® filter paper and the extracts then stored in a refrigerator using sterile universal tubes until its use.

Phytochemistry

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out at Nobel scientific laboratory, Owerri, Imo State, using the standard procedures as described by [23]; [24] & [25].

Experimental Animals

Albino mice weighing between 19g - 35g of either sex were used for the study. The animals were procured from the department of Veterinary Medicine, University Of Nigeria, Nsukka (UNN) and kept in clean wire meshed cages under standard animal house conditions in accordance with recommendations in Guide for the Care and Use of Laboratory Animals (DHHS, NIH Publication No. 85-23, 1985). The mice were given standard pellet diet and water *adlibitum* during the entire period of experimentation.

Parasite

The *Trypanosoma brucei brucei* was procured from the department of Veterinary Medicine, University Of Nigeria, Nsukka (UNN). Each mouse was inoculated intraperitoneally i.e. IP, with a parasite load of 10^6 trypanosomes in whole blood of infected mice. The quantitative estimation of trypanosomes was done by rapid matching method for blood trypanosome estimation developed by [26].

In Vivo Evaluation of the Extract

Forty mice randomly divided into ten (10)groups of four (4) mice each were used for this study and administration of extract and standard drug (Diminazeneaceturate) was done using 2ml syringes. The animals were inoculated intraperitoneally (IP) with trypanosome load of 10^6, and were then kept under standard conditions for 10 days to enable circulation and reproduction of the parasite within them. Parasitaemia level was detected and analysed via microscopy, animals of groups 1 – 4 received test extract of *Combretum racemosum* at 50, 100, 200 and 1000mg/kg body weight respectively by IP route, daily for six consecutive days from day eleven post infection (PI) and also same procedure for group 5 – 8 which received the test extract from *Euphorbia hirta*. Animals of group 9 were treated with diminaze (Diminazeneaceturate 445mg/g and antipyrine 555mg/g, Pantex Holland BV, obtained from Forthright Pharmaceuticals LTD, #174 Tetlow Road: Onyeche Street, Owerri) at 3.5mg/kg body weight IP from day 11 PI (serving as positive control), while those in group 10 did not receive treatment (served as negative control). The efficacy of the test extract was assessed on the basis of differences in parasitaemia (microscopy) and animal body weight measurements. The administration of the 1000mg/kg dosage served as acute toxicity test (to check the toleration of the extract in high concentrations in the body). Trypanosome microscopy and animal weight measurements were done and used to analyse effect of extract administration and parasite load during the experiment.

Statistical Analysis

The data collected were summarized as means ± standard error of means (S.E.M). Statistical comparisons between the treatment groups were made by one-way analysis of variance (ANOVA) and the

means were separated with Duncan's multiple range, using SPSS 16 software. Means were considered significant at $P<0.05$.

RESULTS/DISCUSSION

The extract derived for *Combretum racemosum* leaves was dark greenish-brown in colour and pasty in consistency and the percentage yield was 8.566% (w/w). While for the leaves of *Euphorbia hirta*, the extract possessed dark brown colour, also exhibiting pasty (sticky) consistency with percentage yield of 23.631% (w/w). Phytochemical screening of *Euphorbia hirta* reveals the presence of saponins, tannins, flavonoids, phenols, steroids, terpenoids and alkaloids at various degrees of concentrations from low to high concentration (as shown in Table 1), while the screening of *Combretum racemosum* showed presence of saponins, tannins, phenols, steroids, terpenoids, anthraquinones and alkaloids at varying concentrations (Table 2). From the results derived, it is noticed that *E. hirta* lacked anthraquinones which was present in *Combretum racemosum*. On the other hand, *C. racemosum* lacked flavonoids whose concentration was moderately high in *Euphorbia hirta*. These differences in their content give reason for their differences in curative properties and preference in use among locals for various ailments. For example, flavonoids are known to possess a variety of biological activities at nontoxic concentrations [27], and together with other secondary metabolites identified has been severally reported in other plants to show curative activity against diverse pathogens, used traditionally for ethnopharmacological functions [28]; [29]. It is also noteworthy that the result derived correlates with screening results published [30]; [14] & [3] for the plants.

Table 1: Phytochemical screening result for *Euphorbia hirta*

PHYTOCHEMICAL	RESULT	DEGREE OF PRESENCE
SAPONINS	++	MODERATE
TANNINS	+++	HIGH
FLAVONOIDS	++	MODERATE
PHENOLS	+++	HIGH
STEROIDS	+++	HIGH
TERPENOIDS	+	LOW
ANTHRAQUINONES	-	ABSENT
ALKALOIDS	+	LOW
+ = LOW CONC., ++ = MODERATE CONC., +++ = HIGH CONC.		

Table 2: Phytochemical screening result for *Combretumracemosum*

PHYTOCHEMICAL	RESULT	DEGREE OF PRESENCE
SAPONINS	++	MODERATE
TANNINS	+++	HIGH
FLAVONOIDS	+++	HIGH
PHENOLS	-	ABSENT
STEROIDS	++	MODERATE
TERPENOIDS	+++	HIGH
ANTHRAQUINONES	+	LOW
ALKALOIDS	++	MODERATE
+ = LOW CONC., ++ = MODERATE CONC., +++ = HIGH CONC.		

First detection of trypanosomes in the Mice was on day 4 PI subsequently followed by progressive increase in mean parasitaemia in all the infected groups of mice (Figure 1 and 2). Treatment with the extract from day 10 PI (Post Infection) caused significant ($p<0.05$) reduction in mean parasitaemia by day 16 PI when compared to the infected untreated group. The administration of Diminazeneaceturate led to clearance of the parasites by day 14 PI. Meanwhile, resurgence in parasitaemia in the extract treated groups started occurring by day 20 PI. There was continual reduction in weight in all mice from day 4 PI, but improvement in mean body weight started to gradually increase by day 12 PI for all treated groups; both extract and Diminazeneaceturate (Figure 3 and 4). The total mice death recorded was four, for which occurrence is suspected for pregnant conditions (two mice; groups 4 and 6) and handling during extract administration (two mice; groups 1 and 7), no deaths were recorded among members of the untreated control group.

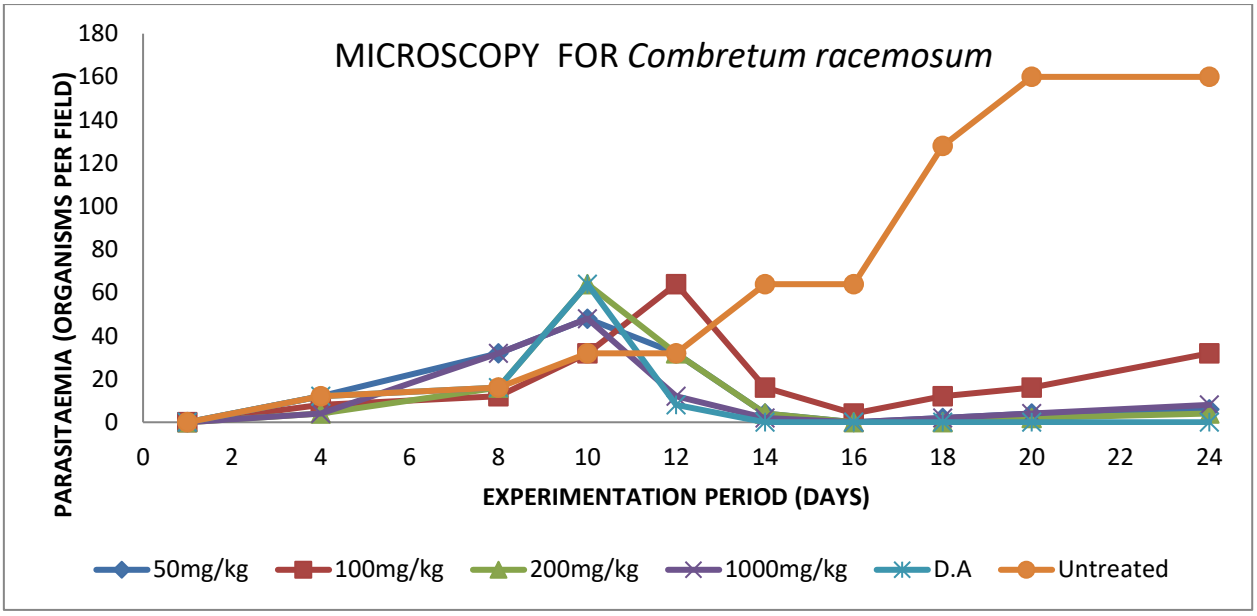

Fig-1: Results for microscopy of the groups treated with diminazeneaceturate and varying doses of *Combretum racemosum* extract.

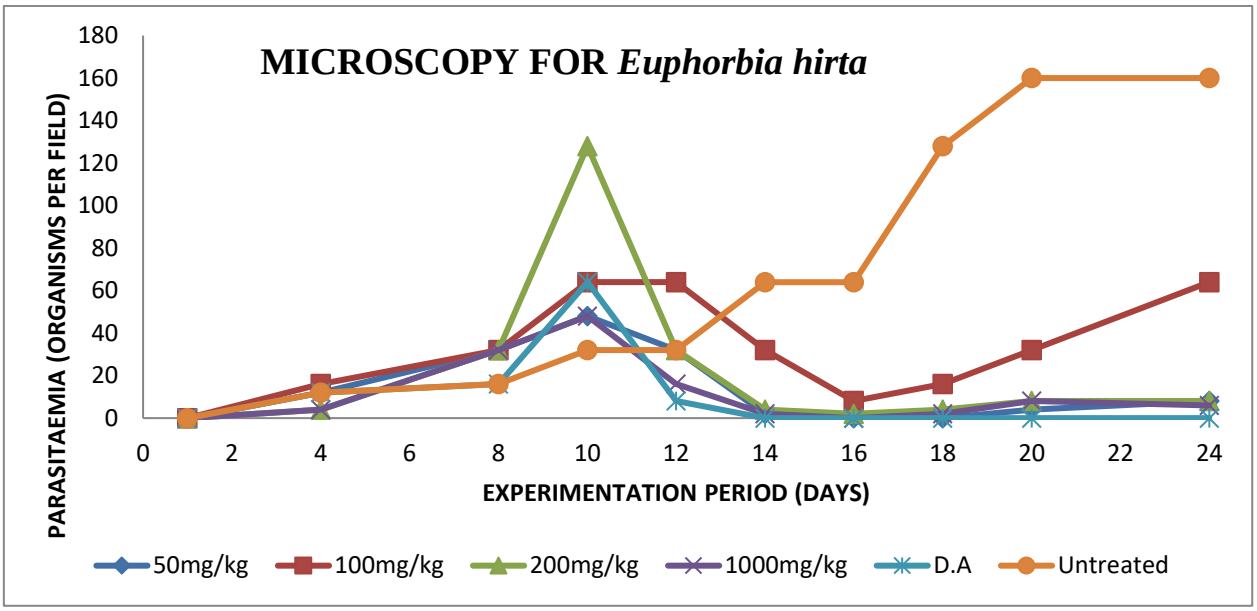

Fig-2: Results for microscopy of the groups treated with diminazeneaceturate and varying doses of *Euphorbia hirta* extract.

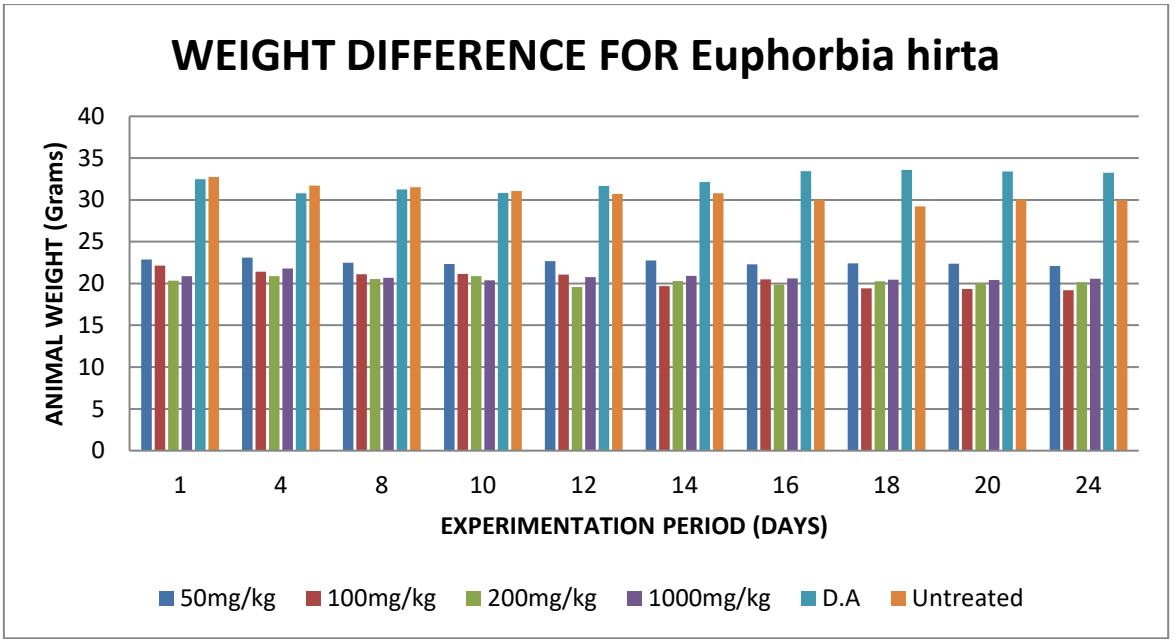

Fig-3: Results for animal weight taken for the groups treated with diminazeneaceturate and varying doses of *Euphorbia hirta* during the work.

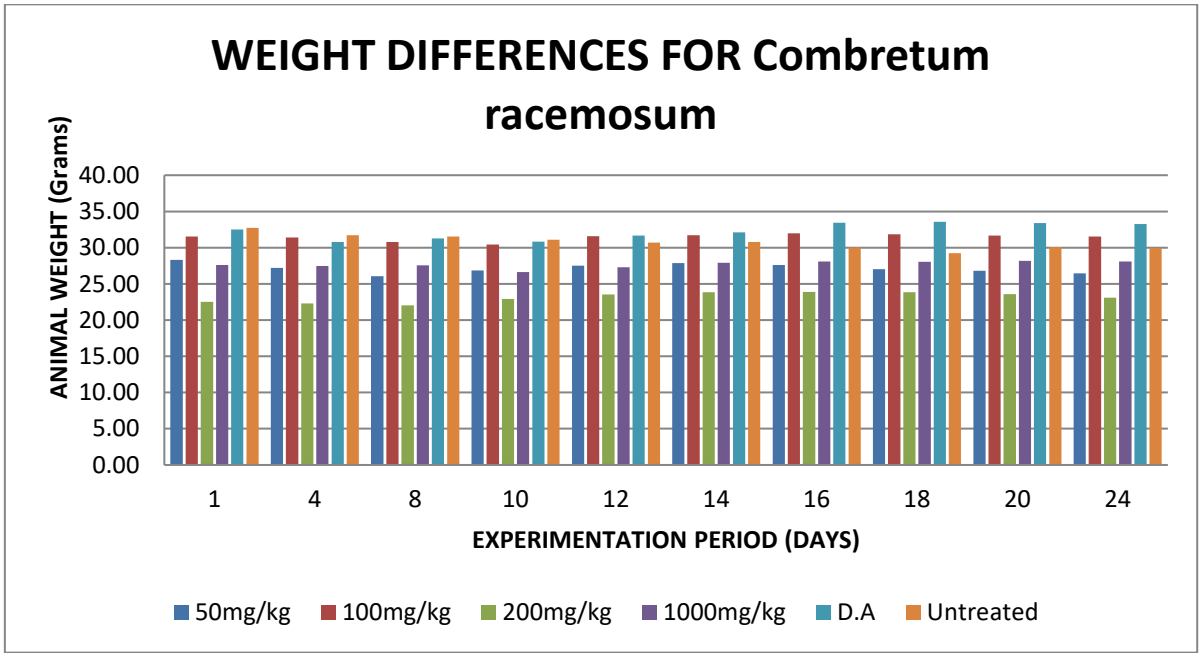

Fig-4: Results for animal weight taken for the groups treated with diminazeneaceturate and varying doses of *Combretum racemosum* during the work.

The result derived in the analysis for trypanosidal activity of the botanicals is very close to that gotten from the work done by [7] for trypanosidal activity of *Combretum racemosum*. The absence of death following administration of the extract at 1000mg/kg showed that the extract was well tolerated. However, it is possible that there existed cumulative toxic effects of administering the extract for 6 consecutive days, because of the notice of general weakness of all treated mice by day 15 PI and also show of aggression in the groups on day 18 PI. Toxicity tests are included to determine the possible dosages at which crude extracts can be administered to experimental animals and it is actually wrong to use drugs/compounds in treatment without carrying out an acute toxicity test, of which this could be fatal occurrences [31]. Academic evidence [32] suggest that many natural products exhibit their trypanocidal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defences against oxidative stress and the antigenic variation of African trypanosomes surface antigens enables them to escape from the host's defense mechanism [33]. This is because natural products

possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. There was a reduction in parasitaemia following administration of *C. racemosum and E. Hirta* extract in groups' 1-4 mice and 5-8 respectively, and also a significant difference in mean parasitaemia between the extract treated groups and the infected untreated group (10)at day 16 PI. Hence, this confirms the anti-trypanosomal activity of the extract. There was complete clearance of the parasites in the blood of the mice of group 9 which was treated with Diminazeneaceturate by day 14 PI, unlike the extract treated groups which had very reduced levels of parasitaemia at day 16 PI. The results of the ANOVA run at 95% confidence interval ($p<0.05$) for *Combretum racemosum* gives a significance of 0.000; for $F(5,59)= 5.309$, which confirms the trypanocidal effect of the extracts used and on arrangement of the means using Duncan multiple range, drug trypanocidal potency moves in the order of Diminazene-200mg/kg-50mg/kg-100mg/kg. While for *Euphorbia hirta*, gives a significance of 0.003; for $F(5,54)= 4.199$. This not only indicate a better trypanocidal effect for the use of *Combretum racemosum*(0.000 at $p<0.05$) but also that it is significant for trypanocidal activity in the order of 50mg/kg-200mg/kg-100mg/kg for the extracts. This shows that diminazeneaceturate was more effective than the extract at the doses (50, 100 and 200mg/kg) tested. There is a report of the curative effects of diminazeneaceturate when used against *T. brucei brucei* infection by [34]. This resurgence in mean parasitaemia in the extract treated groups by day 20 PI can be attributed to the waning effect of the treatment, which the component of the plant conferring the trypanocidal effect only exhibited it transiently and didn't possess strong activity. Since there exists no works for trypanosidal activity of *Euphorbia hirta*, comparison was made with the result gotten from the combretum and standard synthetically derived Trypanosomiasis drug (Diminazeneaceturate)

CONCLUSION

The crude ethanolic extracts from the leaves of *Combretum racemosum* and *Euphorbia hirta* possess trypanocidal activities against *T. brucei brucei*. Though, the extract had issues of non-durability in its activity. Hence, it can be said to be not effective enough to cure mice from the parasitaemia in view. Nonetheless, this study gives credence to the right ethnopharmacological use of *Combretum racemosum* and *Euphorbia hirta*. To achieve a more consistent and durable result from these plants, further phytochemical investigation need to be done using bioassay analysis, and also isolation of pure active compounds, probably through chromatographic means to optimize the efficacy of the extract. This I say because, it is possible certain compounds which are also components present in the crude extract may have activities that enhance or lead to the waning off of the noticed trypanocidal activity with time.

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