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Full Length Research Paper

In vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants

S.E. Atawodi[1*], T. Bulus[1], S. Ibrahim[1], D.A. Ameh[1], A.J. Nok[1], M. Mamman[2], M. Galadima[3]

[1]Biochemistry Department, Ahmadu Bello University, Zaria, Nigeria.
[2]Veterinary Physiology and Pharmacology Department, Ahmadu Bello University, Zaria, Nigeria.
[3]Microbiology Department, Ahmadu Bello University, Zaria, Nigeria.

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Methanol extracts from twenty three plants harvested from the Savannah vegetation belt of Nigeria were analyzed *in vitro* for trypanocidal activity against *Trypanosoma brucei brucei* and *Trypanosoma congolense* at concentrations of 4 mg/ml, 0.4 mg/ml and 0.04 mg/ml. Extracts of *Khaya senegalensis, Piliostigma reticulatum, Securidaca longepedunculata* and *Terminalia avicennoides* were strongly trypanocidal to both organisms while extracts of *Anchomanes difformis, Cassytha spp, Lannea kerstingii, Parkia clappertioniana, Striga spp, Adansonia digitata* and *Prosopis africana* were trypanocidal to either *T. brucei brucei* or *T. congolense*. These findings provide evidence of the effects of some plants in the traditional management of trypanosomiasis.

Key words: Savannah, medicinal plants, trypanocidal effects, trypanosomiasis, trypanosome, *in vitro* model.

INTRODUCTION

Trypanosoma brucei brucei and *Congolense* are unicellular parasites transmitted by the bite of tsetse fly and is the causative agent of sleeping sickness in humans and related diseases in animals (Warren, 1988; Kuzoe, 1993). The search for vaccination against African trypanosomiasis remains elusive and effective treatment is beset with problems of drug resistance and toxicity (Onyeyili and Egwu, 1995; Gutterridge, 1985; Aldhous, 1994). Four drugs (suramin, pentamidine, melarsoprol and eflornithine) are currently available to treat trypanosomiasis (Kuzoe, 1993), with only melarsoprol and eflornithine being effective against the meningoencephalitis that develops in the late stages of the disease. In addition to emerging cases of drug resistance, all four drugs require lengthy, paranteral administration and all but eflornithine have severe toxic side effects (Onyeyili and Egwu, 1995; Gutterridge, 1985) thus, underscoring the urgent need to develop more effective and safer trypanocidal drugs.

Several reports on the evaluation of different chemicals/drugs for trypanocidal activity have appeared (Bodley et al., 1995; Bodley and Shapiro, 1995) just as are interesting reports on the antitrypanosomal effects of plant extracts and plant derivatives (Freiburghaus et al., 1996, 1997, 1998; Sepulveda-Boza et al., 1995; Nok et al., 1993; Asuzu and Chineme, 1990). Some of these reports have indeed shown that, at least under *in vitro* conditions, some of these plants possess trypanocidal activity (Freiburghaus et al., 1996, 1997, 1998). Such systematic evaluation has not been reported for Nigerian plants.

Recently, Atawodi et al. (2002) reported on plants claimed to be useful in the treatment of African

*Correspondence author; E-mail: atawodi_se@yahoo.com, Tel: +234-69-550 837.

trypanosomiasis in North Central Nigeria. As a follow up to that work, we present in this publication, report on systematic *in vitro* assessment of methanol extracts of some Nigerian savannah plants for their trypanocidal activity using *T. brucei brucei* and *T. congolense* as test organisms.

MATERIALS AND METHODS

Plants

Plants were collected from different Northern Nigerian states corresponding with the savannah vegetational belt. The states include Kaduna, Bauchi, Kogi, Plateau, Adamawa and Kano. The Department of Biological Sciences, Ahmadu Bello University, Zaria or the Department of Botany, University of Jos, Nigeria confirmed the identities of the plants.

Sample preparation and extraction

Appropriate parts of plants were harvested, dried under the shade or in open air in the laboratory (to avoid heat destruction of the active components). Dried materials were pounded in laboratory mortar into small particles. Fifty grams (50 g) of the pounded dried plants materials were weighed and extracted with 300 ml methanol by reflux following prior extraction with petroleum ether and chloroform. The extracts were dried *in vacuo* and stored in the refrigerator at 4^{o}C until required.

Exactly 10 mg of the different plant extracts were weighed into Eppendorf tubes and dissolved in 10% dimethylsulfoxide (DMSO) in PBS to produce extract solutions of 20.0 mg/ml (stock). Two other extract concentrations (10.0 mg/ml and 2.0 mg/ml) were prepared from the 'stock' extract solution by appropriate dilution with PBS. Extract solutions were prepared just before use.

Test organisms

T. congolense and *T. b. brucei* were obtained from stabilates maintained at the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. The parasites were maintained in the laboratory by continuous passage in rats until required. Passage was considered necessary when parasitamea was in the range of 16 – 32 parasites per field (usually 3 - 5 days post infection). In passaging, 1 X 10^3 parasites were introduced intraperitonially or intramuscularly into rats in 0.1 - 0.2 ml blood/PBS solution. For several passages, approximately 80% blood solution (v/v) was obtained by cardiac puncture into 1ml syringe containing 0.2 ml EDTA (1% w/v). About 0.1 - 0.2 ml of the blood collected as described above or blood (diluted with PBS to contain approximately 1 X 10^3 parasite/ml) was injected into clean rats acclimatized under laboratory condition for at least one week.

Determination of parasitamia

Parasitamia was monitored in blood obtained from the tail, pre-sterilized with methylated spirit. The number of parasites was determined microscopically at X 400 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 buffered phosphate saline (PBS, pH 7.2). Logarithm 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.

In vitro test for trypanocidal activity

Assessment of *in vitro* trypanocidal activity was performed in duplicates or triplicates in 96 well micro titer plates (Flow laboratories Inc., McLean, Virginia 22101,USA). 20µl of blood containing about 20-25 parasites per field obtained as described under "determination of parasitaemia" was mixed with 5 µl of extract solution of 20.0 mg/ml, 10.0 mg/ml and 2.0 mg/ml to produce effective test concentrations of 4 mg/ml, 2 mg/ml and 0.4 mg/ml, respectively. To ensure that the effect monitored was that of the extract alone, a set of control was included which contained the parasite suspended in 10% DMSO only. For reference, tests were also performed with the same concentrations of *Diminal*^{R} *(445mg diminazene diaceurate*+ 555 mg phenazone/g, Eagle Chemical Company LTD, Ikeja, Nigeria) - a commercial trypanocidal drug.

After 5 min incubation in closed Eppendorf tubes maintained at 37^{o}C, about 2 µl of test mixtures were placed on separate microscope slides and covered with cover slips and the parasites observed every 5 min for a total duration of sixty minutes. It should be noted that under this *in vitro* system adopted, parasites survived for about 4 h when no extract was present. Cessation or drop in motility of the parasites in extract-treated blood compared to that of parasite-loaded control blood without extract was taken as a measure of trypanocidal activity.

RESULTS

Methanol extracts from twenty three plants harvested from the savannah vegetational belt of Nigeria were analyzed for their *in vitro* trypanocidal activity against *T. b. brucei* and *T. congolense* at effective concentrations of 4 mg/ml, 2 mg/ml, 0.4 mg/ml. Complete elimination of motility or reduction in motility of parasites when compared to control were taken as indices of trypanocidal effects.

Four plants, namely, *Khaya senegalensis*, *Piliostigma reticulatum*, *Securidaca longepedunculata* and *Terminalia avicennioides* caused complete cessation of motility of both *T. b. brucei* and *T. congolense* within 60 min (Table 1). Extracts of five other plants (*Anchomanes difformis, Cassytha spp, Lannea kerstingii, Parkia clappertoniana, Striga* spp) eliminated motility in only *T. brucei* within 60 minutes, while extracts of *Adansonia digitata* roots, and *Prosopis africana* exclusively eliminated motility in *T. congolense* within 60 min. Whereas extracts of *Afzelia africana*, *Annona senegalensis*, *Lawsonia inermis, Magnifera indica, Momordica balsamina, Sterculina setigera* and *Vernonia* spp had drastic or slight effect on parasite motility, that of *Tamarindus indica* (stem bark) showed effect on neither *T. brucei nor T. congolense*.

In two instances where different parts of the same plants were tested, differences in trypanocidal effect were observed. For example while roots of *Adansonia digitata*

Table 1. Effect of different concentrations of methanol extracts of some Nigerian savannah plants on motility of *T. b. brucei and T. congolense*.

S / No	Plants	Part Of Plant	Time (Min) after which motility ceased, reduced drastically (*) or reduced slightly (**) with different effective concentrations of extracts (mg/ml)					
			T. b. brucei			*T. congolense*		
			4	2	0.4	4	2	0.4
1	*Adansonia digitata*	Root	55**	-	-	50	-	-
		Leaves	-	-	-	SRM	SRM	-
2	*Afzelia africana*	Whole plant	40**	-	-	45**	-	-
3	*Anchomanes difformis*	Rhizome	45	-	-	15**	45**	60**
4	*Annona senegalensis*	Root	-	-	-	30**	50**	-
5	*Anogeissus leiocarpus*	Root	50*	-	-	SRM	SRM	-
6	*Cassytha sp.*	Leaves	25	55	-	55**	-	-
7	*Guiera senegalensis*	Leaves	NT	NT	NT	SRM	SRM	-
8	*Khaya senegalensis*	Stem bark	35	-	-	5	35	-
9	*Lannea kerstingii*	Root	30	-	-	50*	-	-
10	*Lawsonia inermis*	Leaves	-	-	-	5*	-	-
		Root	SRM	SRM	SRM	NT	NT	NT
11	*Magnifera indica*	Root	30**	-	-	5**	-	-
12	*Mormordica balsamina*	Whole plant	55*	-	-	-	-	-
13	*Parkia clappertoniana*	Root	55	-	-	-	-	-
14	*Piliostigma reticulatum*	Leaves	5	-	-	30	-	-
		Stem	-	-	-	-	-	-
15	*Prosopis africana*	Stem bark	SRM	SRM	-	40	-	-
16	*Pseudocedrella kotschi*	Stem bark	45*	-	-	55*	-	-
17	*Saba florida*	whole plant	-	-	-	NT	NT	NT
18	*Securidaca longepedunculata*	Root	5	20	50	5	5	30
19	*Sterculia setigera*	Root	SRM	-	-	SRM	SRM	-
20	*Striga spp.*	Leaves	40	-	-	10**	45**	-
21	*Tamarindus indica*	Stem bark	-	-	-	-	-	-
22	*Terminalia avicennioides*	Stem bark	10	25	45	10	30	55
23	*Vernonia Spp.*	Leaves	35**	-	-	SRM	SRM	-

- = No noticeable effect on motility after 60 minutes; SRM = slightly reduced motility; NT= not tested.

eliminated motility in *T. congolense* and drastically reduced motility in *T. brucei* extract of leaves of the same plant had little or no effect on the two organisms (Table 1).

Diminal^{R} eliminated trypanosomal motility within 60 min even at the lowest concentration tested (0.4 mg/ ml). The effect was such that after 20 - 30 min of incubation, no motility was visible with drug concentrations of 4.0 and 2.0 mg/ml.

DISCUSSION

That some of the plants tested showed promising trypanocidal effect (table 1) is not surprising since earlier reports (Freiburghaus et al., 1996, 1997, 1998; Asuzu and Chineme, 1990; Nok et al., 1993; Owolabi et al., 1990) have clearly indicated that plants of different families could possess potent trypanocidal activity. In fact, natural products with trypanocidal activity and belonging to a variety of phytochemical classes have been identified (Hopp et al., 1976; Oliver-Bever, 1986; Sepulveda-Boza and Cassels, 1996). Although our investigation did not involve structure elucidation, literature search revealed that extracts showing potent trypanocidal activity in this investigation (Table 1) have also been reported to contain either alkaloids, flavonoids, phenolics and/or terpenes. For example, the presence of quercetin and kaempferol has been reported in the leaves of *P. reticulatum* (Le Grand, 1986) while

alkaloids have been found in *A. differmis* (Chukwurah and Ajali, 2000).

The reported therapeutic non-activity of stem bark extracts of *P. reticulatum* against mouse and rats *in vivo* (Youan *et al*., 1997; Asuzu and Ugwuja 1989) is confirmed by this *in vitro* assay. But the significant trypanocidal activity of the leaf extracts of this plants in our study underscores the need to study all parts of a plant before any generalization is made on the plant's pharmacological and therapeutic potentials.

It is not known why extract of some plants such as *Pseudocedrela kotschi* will strongly reduce trypanosome motility within the first few minutes but could not completely eliminate motility. However, it appears reasonable to speculate that these extracts may belong to the group that acts by static action affecting growth and multiplication of trypanosomes rather than eliminating them altogether.

Our observation that methanol extract of the stem bark of *A. senegalensis* is not effective *in vitro* is consistent with earlier findings (Freiburghaus et al., 1996), although a much earlier report suggested an encouraging *in vivo* trypanocidal effect of the aqueous extract of the root bark of this plant (Igweh and Onabanjo, 1989). This finding also collaborates our earlier observation (Atawodi et al., 2002) that the popularity of an herbal recipe in traditional medical practice may not necessarily be an indication of its effectiveness. Similarly, the high trypanocidal activity observed for *S. longepedunculata* in this experiment is also consistent with the findings of Freiburghaus et al. (1996).

Put together, our findings on *A. senegalensis* and *S. longepedunculata* vis-à-vis other reports (Freiburghaus et al., 1996), clearly validate the relatively simple *in vitro* system employed in this investigation as a fast and reliable system for *in vitro* screening of plants and other materials for trypanocidal activity.

The observation that *Cassytha* spp*, Striga* spp and *Lannea kerstingii* were strongly trypanocidal to *T. brucei brucei* but only weakly so to *T. congolense* appears to suggest that species-dependent factors may play a role in susceptibility. For instance, unlike some other species mammalian *T. brucei* has no functional kinetoplast DNA (Fairlamb, 1982) while *T. congolense* possess no functional flagellum (Hoare, 1972)

It is difficult to speculate the mechanism by which these extracts exhibit their trypanocidal action. However, accumulated evidence (Sepulveda-Boza and Cassels, 1996) 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 defenses against oxidative stress. 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. It is also known that some agents act by binding with the kinetoplast DNA of the parasite.

The result of Freiburghaus (1996) has clearly indicated that different solvent extracts of the same plant may exhibit different trypanocidal activity just as extracts of different parts of the same plants. Therefore, the statement that a plant is trypanocidal or not should be taken within the context of the solvent used and the parts investigated. Moreover, a plant with high *in vitro* trypanocidal activity may have no *in vivo* activity and vice versa, because of peculiarities in the metabolic disposition of the plant's chemical constituents. Therefore, plants found to be active in this report must be tested *in vivo* before a definite statement can be made on their trypanocidal potentials.

Currently, we are investigating the toxicology and the *in vivo* trypanocidal activity of extracts that have shown potent trypanocidal activity *in vitro*. Preliminary results suggest that some plants with promising *in vitro* trypanocidal action may also possess similar activity *in vivo*.

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