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In vitro Antitrypanosomal Activity and Phytochemical Screening of Aqueous and Methanol Extracts of *Terminalia avicennioides*

*T. Bulus[1], S.E. Atawodi[2] and M. Mamman,[3]
[1]Department of Biochemistry, Kaduna State University, Kaduna.
[2]Department of Biochemistry, Ahmadu Bello University, Zaria.
[3]Department of Veterinary Physiology and Pharmacology,
Ahmadu BelloUniversity, Zaria.
Corresponding Author. Email: tbulus@yahoo.com

Aqueous and methanolic extracts of *Terminalia avicennioides* (TA) were tested *in vitro* for their antitrypanosomal potentials. The test involved incubating the parasites, *Trypanosoma brucei brucei*, with 20,10,5 or 0 mg/ml of the extracts in 96-well microtitre plate. Cessation or drop in the parasite motility, determined microscopically, was taken as a measure of efficacy of the extract against control wells. The test organisms were immobilized by 20 mg/ml of the stem and root extracts within 25-30 minutes of incubation. Anthraquinones, saponins, tannins and flovanoids were the major phytochemicals found in the crude extracts. Alkaloids were not detected. Further studies with column chromatographic fractions of the stem aqueous extract showed that the active principles were pooled into ethyl acetate/methanol (19:1) fraction which was found to be rich in saponins. These findings are discussed in relation to chemotherapy of African trypanosomiasis.
Key words: *Terminalia avicennioides,* antitrypanosomal potentials, *Trypanosoma brucei brucei*, phyto chemicals.

INTRODUCTION

African trypanosomiasis, popularly referred to as African sleeping sickness, is a parasitic disease of man and animals. Trypanosome, the causative organism of this disease, is a single cell protozoan parasite belonging to the genus *Trypanosoma* and is transmitted from host to host via the bite of blood-sucking insect, the tsetse fly, and vector. Where endemic proportions of the disease occurred in domestic animals, it was impossible to rear such animals. The impact of this is low production of high quality protein (Vickernman, 1978) and farming tasks that could be performed by animals are still done by hand in large parts of Africa (William, 1985).The disease poses a serious threat to the African tourist industry, particularly in region like Zambia, Kenya and Tanzania with high prevalence of the infection (David *et al*, 2002). The disease is known to occur in 36 African countries, and that over 50 million people are at risk of acquiring the infection (Kuzoe, 1993).

Trypanosomes establish their presence in host animals by releasing numerous toxic substances (Mellors and Samad, 1989) and by mediating alternate expression of an extensive repertoire of variant surface glycoproteins (Vickerman, 1975), a process known as antigenic variation. These survival mechanisms employed by the parasite are major factors that have made the chemotherapy and development of vaccine against trypanosomosis difficult.

In recent times, the search for effective, less toxic, cheaper plant-derived drugs for the treatment of animal and human sleeping sickness is growing and is being encouraged, particularly in developing countries of the world, where orthodox medicines are usually too expensive for the majority of the citizenry (Nwude and Ibrahim, 1980; Farnsworth *et al*., 1985). It seems rather justified that the search for plant-derived drugs for the treatment of African trypanosomosis should continue.

Terminalia avicennioides Guill and Perr, is a tropical plant abundant in the Savannah region of West Africa . In Nigeria , the plant is commonly found in the North Central region where most of the country's cattle are reared. It is locally known as *baushe* (Hausa), (Keay *et al.,* 1960). The antimicrobial properties of this plant have been reported of recent (Akinshide and Olukoya, 1995; Abdullahi *et al*., 2001), but little known scientific study on its antitrypanosomal effect has been reported. This work, therefore, aimed at exploring the antitrypanosomal efficacy of aqueous and methanolic extracts of *Terminalia avicennioides.*

MATERIALS AND METHODS

Plant Sample

Different parts of *Terminalia avicennioides* were obtained from Tashan Fulani village in Zaria Local Government Area in Kaduna State. The plant's identify was confirmed at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Its

voucher number is 900239.

Animals

White albino rats (Wistar stock) were bought from the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria. The animals were fed on diet specially prepared from chick Grower's mash (Pfizer) and were given water *ad libitum* throughout study period. The weight-range of the rats and mice just before the experiment were 10g – 200g and 15g – 30g, respectively.

Test Parasite

The parasite (Trypanosoma brucei brucei) was obtained from Dr. R.C. Ezeokonkwo of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. The parasite was isolated from a hunting dog in March 2002 and has since been maintained in laboratory rats by continuous passage until required for the experiment.

Preparation of the Crude Aqueous and Methanol Extracts of *T. avicennioides*

Fresh plant samples (leaf, stem and root) were cut into small pieces and then dried under shade for a week. Exactly 200g of each dried sample was ground and boiled in 1 litre of distilled water contained in a conical flask for one hour. The extract was thereafter filtered hot first with muslin cloth and then with filter paper. The filterate was concentrated in a water bath set at 50^0C for 2 days. The concentrated extract was finally exposed to air to complete drying. The dried extract was stored in a refrigerator at 4^0C until required. The methanol extracts were prepared by maceration in methanol and allowing standing for 24 hours before filtration. Each filtered extract was dried at 50^0C in water bath.

Columnar Fraction

The crude aqueous extract of *T. avicennioides* stem bark was fractionated according to the protocol of Nok *et al*. (1993). Slurry was prepared by soaking 30g of silica gel in 100ml of methanol-water solvent mixture (1:1). The slurry was carefully packed in a column (1.5x30). The column was then loaded with the extract solution and subsequently eluted with four solvent-mixtures (ethylacetate/methanol, 19:1; benzene/methanol, 9:1; acetic acid/methanol, 1;1 and water/ methanol, 1:1) in order of increasing polarity.

The eluents were collected in separate beakers and made to dry at 50^o C in water bath.

The dried fractions were kept in containers for *in vitro* experiments.

***In vitro* Study with Crude Extracts**

In vitro study was carried out in accordance with the method of Atawodi *et al*. Briefly, infected rats were sacrificed at high parasitaemic state and the blood collected in eppendorf tubes containing 0.2ml of 1% Ethylinadiaminetetraacetate (EDTA) prepared with Phosphate buffer saline glucose (PBSG).

The aqueous extract and the standard drug were dissolved in PSB while the methanol extracts were dissolved in 10% methanol. For all the extracts and drug, 3 sets of concentrations (20, 10 and 5mg/ml) were prepared by serial dilution.

The in vitro experiment involved incubating 40 µl of infected blood with 10 µl of extract solution in a 96 – well microtitre plate to produce effective test concentrations of 4,2 and 1 mg/ml, respectively. Five microlitre of the mixture were immediately placed on a slide and covered with a cover slip and the parasite's motility monitored microscopically against control wells at 5 minutes intervals for a period of one hour. Here, cessation or drop in motility of parasites was taken as indication of extract's activity against trypanosomes.

Phytochemical Screening

The crude aqueous extracts and column fractions were subjected to a standard phytochemical test for six constituents (Trease and Evans, 1983).

RESULTS AND DISCUSSION

That parasite motility could be a measure of viability among most zooflagelate parasites has long been established (Peter *et al*, 1976). The simple technique employed in this study uses the motility of trypanosome as indicator of parasite viability. We have earlier on reported (Atawodi et al, 2003) that the technique correlated well with other *in vitro* methods.In this study, various concentrations of methanol and aqueous extracts of different parts of *T. avicenioides* were incubated *in vitro* with *T. brucei* infected blood and cessation or drop in parasite motility was taken as a measure of the antitrypanosomal effect of the plant extracts.

Results (Table 1) showed that within 25 to 30 minutes of incubation, the parasites were completely immobilized by stem and root extracts. These observations were comparable to those of diminazine aceturate, an antitrypanosomal drug whose mechanism of action has been reported (Gutteridge and Cooombs, 1977). The leaf extracts had very little effect on the motility of the parasites for the one hour incubation period. The differences observed are partly attributable to

Table 1: Effect of aqueous and methanolic extracts of various parts of *Terminalia mollis* on *in vitro* motility of T. brucei brucei.

		Parasite Motility*										
	Time Post Incubation (Minutes)	**AQ (mg/ml)**				**MT (mg/ml)**				**Berenil (mg/ml)**		
		20	**10**	**5**	**20**	**10**	**5**	**PBS**	**10% MET**	**20**	**10**	**5**
Stem	5	+1	+5	+5	+3	+4	+5	+5	+5	+5	+5	+5
	10	+1	+4	+5	+2	+4	+5	+5	+5	+4	+5	+5
	15	+1	+3	+5	+1	+3	+5	+5	+5	+2	+3	+4
	20	0	+2	+5	+1	+3	+5	+5	+5	0	+2	+3
	25	0	+2	+4	0	+2	+4	+5	+5	0	+1	+3
	30	0	+1	+4	0	+2	+4	+5	+5	0	+1	+2
	35	0	+1	+4	0	+1	+4	+5	+5	0	+1	+2
	40	0	+1	+4	0	+1	+4	+5	+5	0	+1	+2
	45	0	+1	+3	0	+1	+3	+5	+5	0	+1	+2
	50	0	0	+2	0	+1	+3	+5	+5	0	+1	+2
	55	0	0	+2	0	+1	+2	+5	+5	0	+1	+2
	60	0	0	+2	0	+1	+2	+5	+5	0	+1	+2
Root	5	+2	+4	+5	+1	+4	+5	+5	+5			
	10	0	+2	+5	+1	+3	+5	+5	+5			
	15	0	+2	+5	0	+3	+5	+5	+5			
	20	0	+1	+4	0	+3	+5	+5	+5			
	25	0	+1	+3	0	+3	+5	+5	+5			
	30	0	+1	+3	0	+2	+5	+5	+5			
	35	0	+1	+2	0	+2	+5	+5	+5			
	40	0	0	+1	0	+1	+5	+5	+5			
	45	0	0	+1	0	+1	+3	+5	+5			
	50	0	0	+1	0	0	+2	+5	+5			
	55	0	0	+1	0	0	+2	+5	+5			
	60	0	0	+1	0	0	+1	+5	+5			
Leaf	5	+5	+5	+5	+5	+5	+5	+5	+5			
	10	+5	+5	+5	+4	+5	+5	+5	+5			
	15	+5	+5	+5	+4	+5	+5	+5	+5			
	20	+5	+5	+5	+4	+5	+5	+5	+5			
	25	+4	+5	+5	+4	+5	+5	+5	+5			
	30	+4	+5	+5	+3	+4	+4	+5	+5			
	35	+4	+5	+5	+3	+3	+4	+5	+5			
	40	+4	+5	+5	+2	+3	+4	+5	+5			
	45	+3	+4	+5	+2	+3	+4	+5	+5			
	50	+2	+4	+5	+1	+3	+4	+5	+5			
	55	+2	+4	+5	+1	+3	+4	+5	+5			
	60	+2	+3	+5	+1	+3	+4	+5	+5			

* Level of motility;+0 (Not motile);+1 (very slight motility);+2 (very slight motility)
+ 3 (moderate motility);+4 (very motile);+5 (very active)
Controls; MET (10% Mathanol); PBS (Phosphate buffered saline)

variation in the types and amount of phytocemicals in these plant parts as revealed by the results for the screening (Table 2) The major observed differences between the phytochemical picture of the crude extracts that are active against the trypanasomes and those that are inactive are with respect to the flavanoids and the levels of saponins. *In vitro* tests with column fractions (Table 3) of aqueous extracts of *T. avicennioids* stem back revealed highest activity with the ethyl acetate/ methanol were cessation of motility occurred by the 25th minutes of incubation (Table-4) with extract concentration of 20mg/ml. The acetic acid/methanol fraction was next to Fract-1; here only drop in motility was noticed. Fract-4 on the other hand appeared to have no effect throughout the period of incubation. Results of the phytochemical screening of the column fractions (Table 4) gave an impression that saponins were largely pooled and concentrated into the active ethylacetate/methanol fraction. Tannis and Anthraquinones were seen in all the fractions and so variation in the activity of the extracts against trypanosomes can not be accounted for on the basis of differences in these phytochemicals. From these results, it is hypothesized that the antitrypanosomal potential of the aqueous extract of *T. avicennioides* is perhaps

due to its high content of saponins. *In vitro* studies and further fractionation are required before this can be confirmed. Our team is already working along this direction. Saponins have been reported to possess wide spectrum of antimicrobial activity and their mechanism of action is thought

Table 2: Results of phytochemical screening of aqueous and methanolic extracts of the various parts of *Terminalia mollis*

	Compounds Screened	TM – Parts*		
		Stem	**Root**	**Leaf**
Aqueous	Anthraquinone	+	+	+
	Saponin	+	+	8+
	Tannin	+	+	+
	Flavonoid	+	+	-
	Alkaloid	-	-	-
	Cardiac glycoside	+	+	+
Methanol	Anthraquinone	+	+	+
	Saponin	+	+	-
	Tannin	+	+	+
	Flavonoid	+	+	-
	Alkaloid	-	-	-
	Cardiac glycoside	+	+	+

Table 3: Effect of column fractions of aqueous stem bark extract of *Terminalia avicennioides* on *in vitro* motility of *T. brucei brucei*

	Parasite Motility*						
Time Post Incubation (Minutes)	**Frac-1 (mg/ml)**		**Frac-3 (mg/ml)**		**Frac- 4 (mg/ml)**		**PBS**
	20	**10**	**20**	**10**	**20**	**10**	
5	+1	+5	+3	+4	+5	+5	+5
10	+1	+4	+2	+4	+5	+5	+5
15	+1	+3	+1	+3	+5	+5	+5
20	0	+2	+1	+3	+5	+5	+5
25	0	+2	0	+2	+4	+5	+5
30	0	+1	0	+2	+4	+5	+5
35	0	+1	0	+1	+4	+5	+5
40	0	+1	0	+1	+4	+5	+5
45	0	+1	0	+1	+3	+5	+5
50	0	0	0	+1	+3	+5	+5
55	0	0	0	+1	+2	+5	+5
60	0	0	0	+1	+2	+5	+5

Table 4: Phytochemicals seen in the crude extracts and the active chromatographic fractions of aqueous extracts of *T. avicennioides* stem bark

Phytochemical tested	Crude Extract*	Ethylacetate-Methanol Fraction	Aceticacid -methanol Fraction
Tanins	+	**+**	**+**
Saponins	**+**	**+++**	**d+**
Alkaloids	_	_	_
Anthraquinones	**+**	**+**	**+**
Glycosides	**+**	**+**	_
Flavanoids	**+**	_	_

** The symbols +, -, +++, and **d+** indicate the presence of the test compound, the absence of the test compound, high amount of test compound and the little amount of the phytochemical tested, respectively*

to be via interaction with parasite membrane sterols, proteins and phospholipids (Godwin and Theodore, 2001).

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