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In vitro anti trypanosomal activity of crude extracts of some Nigerian medicinal plants

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ABSTRACT
Objective: To evaluate the in vitro antitrypanosomal activity of crude extracts of eight medicinal plants from Nigeria,viz: *Anthocleista vogelii ,Blighia unijugata, Cussonia arborea, Gardenia erubescens, Hymenocardia acida, Lophira lanceolata ,Stereospermum kunthianum* and *Uapaca togoensis*.
Methodology and results: Aqueous and ethanolic extracts of the plants were used in this study. Blood obtained from an infected donor rat by cardiac puncture was incubated with varying concentrations of the extracts at 37° C for 10 minutes. The mixtures were observed under the microscope for parasites' motility. The results showed that *Hymenocardia acida* extracts were active against *Trypanosoma brucei brucei* at minimum inhibitory concentration of 2.5 mg/ml. This is the first report of antitrypanosomal activity of *Gardenia erubescens* and *Lophira lanceolata* which were effective at minimum inhibitory concentration of 20 mg/ml. Phytochemical screening of H. acida extracts showed the presence of saponins, tannins, alkaloids and flavonoids.
Conclusion and application of findings: The present study is a step towards validation of folkloric use of these medicinal plants as antitrypanosomal agents. However, further study is needed to determine their in vivo efficacy and suitability in the treatment of African trypanosomosis.

Key words: Antitrypanosomal, in vitro activity, medicinal plants, *Trypanosoma brucei brucei*

INTRODUCTION
African trypanosomosis is a protozoan disease of man and livestock. It is caused by trypanosomes and transmitted by tse tse fly. While *Trypanosoma rhodiense* and *Trypanosoma gambiense* cause African Human Trypanosomosis (AHT, sleeping sickness), *Trypanosoma brucei brucei, Trypanosoma vivax, Trypanosoma congolense, Trypanosoma evansi* and *Trypanosoma equiperdum* are the agents of African Animal Trypanosomosis (AAT). The huge reproductive losses in livestock due to AAT are attributed to low foetal weights, premature births and neonatal losses, poor reproductive performance and poor lactation (Faye *et al*., 2004). Low quality ejaculate, reduced sperm motility and concentration are other problems (Akpavie *et al*., 1987). Adamu *et al*.

(2007) also reported trypanosome induced testicular degeneration, poor sperm characteristics and lack of libido making infected bull unfit for breeding. Despite efforts during most of the last century, the disease has proved difficult to eradicate.

Chemotherapy, the main means of controlling the disease is under threat due to parasite resistance (Maser *et al*., 2003) and toxicity of the trypanocidal drugs (Amaechi, 2001). The poor prospect for a vaccine due to antigenic variation of the parasite (Nantylya & Moloo, 1989) is further compounded by unwillingness of the pharmaceutical industry to develop new compounds because of uncertain and unprofitable market or perhaps the localized nature of the disease. The few commercial trypanocides(diminazene aceturate, isometamidium and homidium) have been in use for well over 40 years. Thus, the search for medicinal plants with trypanocidal activities continue to generate a lot of research interest (Hoet *et al*., 2004; Samson, 2005).

Although recent reports indicate antitrypanosomal activity exists in some medicinal plants (Wurochekke *et al*., 2004; Ibrahim *et al*., 2008; Shuaibu *et al*., 2008), the potentials of many other plants used in folkloric medicine in Nigeria are yet to be investigated. This study was designed to determine the in vitro antitrypanosomal activity of some selected medicinal plants in Nigeria.

MATERIALS AND METHODS

Plant materials: *Anthocleista vogelii, Blighia unijugata, Cussonia arborea, Gardenia erubescens, Hymenocardia acida, Lophira lanceolata, Stereospermum kunthianum* and *Uapaca togoensis* were selected on ethnopharmcological basis. The appropriate parts (leaf, stem root) of the plants were collected in March, 2008 within the environs of Makurdi, capital of Benue state, Nigeria and identified by Mr. Patrick Ekwuno of College of Forestry, University of Agriculture, Makurdi , Nigeria. Voucher specimens were deposited at the College herbarium.

Preparation of crude extracts: The different parts (leaf, stem and root) were washed, air dried at room temperature (28^{o}C - 30 ^{o}C) for one week, pulverized and stored in air-tight containers until required. The powdered material (100g) was soaked in 500ml of distilled water or ethanol and stirred intermittently for 48 hours at room temperature (28^{o} C - 30^{o} C). The choice of water was to mimic the traditional method of preparing crude plant extracts and that of ethanol was to enhance the extraction of more constituents.

The material was filtered using sterile cotton wool and Whatman (No.1) filter paper; the residue was resuspended in the same amount of solvent and then filtered three more times. The pooled aqueous filtrates obtained were concentrated to dryness over water bath at 50^{0}C whereas the ethanolic filtrates were dried under the electric fan. The extracts were stored in air-tight containers at 4^{0}C until needed.

Parasite: Stabilates of *T. brucei brucei* (federe strain) were obtained from the National Institute for Typanosomosis Research, Vom, Jos, Nigeria. The parasites were maintained in the laboratory by passage in mice. Blood harvested from a donor animal at peak parasitaemia (10^{7} parasites/ml of blood) was used for in vitro antitrypanosomal assay.

In vitro antitrypanosomal activity of crude extracts: Different concentrations (2.5 to 40.0 mg/ml) of crude extracts were prepared in phosphate buffered saline (PBS) or 10% DMSO (dimethyl sulphoxide). Ten micolitres of extract was mixed with 60µl of infected blood and the mixture was incubated at 37^{o} C for 10 minutes in wells of microtitre plates. For negative control, 10µl of extract was replaced with PBS or 10% DMSO, In a pilot study, 10% DMSO was confirmed to have no adverse effect on trypanosomes' motility. A standard trypanocide (Diminazeme aceturate – Dimivet SKM Pharma PVT LTD. India) was used as positive control. After incubation, parasites were observed under the microscope (x 400) for a drop or cessation of motility every 5 minutes for a period of 60 minutes.

RESULTS

Of 30 extracts from the eight plants tested, 13 of them showed some activity on *T. brucei brucei* (table 1). At 2.5 mg/ml, both water and ethanolic extracts of *Hymenocardia acida stem bark* exhibited strong trypanocidal activity within 30 – 35 minutes. At this concentration, morphology of red blood cells were unaffected. Extracts of *Lophira lanceolata (*leaf and stem*)* and *Gardenia erubescens (*stem bark*)* showed activity at 40 and 20 mg/ml. However, water extract of *Gardenia erubescens* leaf had no activity, whereas ethanolic extract of the leaf exhibited activity at 40 and 20 mg/ml. While both water and ethanolic extracts of the root bark of *Anthocleista vogelii* showed activity at 40 and 20 mg/ml, the stem bark had no activity at the test concentrations.

The standard trypanocide (Diminazene aceturate) used as positive control completely killed the parasites at concentration of 100µg/ml within 5 – 10 minutes of incubation. The negative control consisting of parasites incubated with PBS or 10% DMSO showed very active parasites even after 2 hours.

DISCUSSION

Since the few trypanocides developed over 40 years ago are expensive and toxic (Amaechi, 2001), it has become necessary to search for new compounds that are safe and efficacious, especially those of plant origin. The plants screened in the present study have folkloric medicinal uses as fever remedies (Tor-anyiin *et al*., 2003). In a survey, Atawodi *et al (*2002) documented several plants which are used by Fulani herdsmen and other livestock farmers to treat trypanosomosis. In folkloric medicine of Idoma people of North Central Nigeria, H. acida is used alone or in combination to treat trypanosomosis and other fever related diseases(Ada & Claffey,2003). Results of the present study showed that the extracts of H.acida stem bark exhibited trypanocidal activity. There have been previous reports of in vitro antitrypanosomal efficacy of leaf (Hoet et al., 2004) and root bark (Atindehou et al., 2004) extracts of H. acida of Beninese and Ivorien origins respectively. However, the strong trypanocidal activity of stem bark extracts of *H.acida* observed in the present study contradicts the report of Kubata *et al* (2005). Screening the extracts of *Kola acuminata* nuts, *Garcinia cola* and *H. acida* using cytoflourometric assay, these researchers found that the bark of *H. acida* did not show any activity against *T. brucei*.

Different techniques used in assessment of antitrypanosomal activities present problems associated with comparing results between different laboratories. For example, determining the minimum inhibitory concentration (MIC) requires standardization of the protocols (Likeufack *et al*., 2003) and confirmation using a more accurate assay (Kaminsky & Brun, 1993). The present method which assesses the parasite motility in vitro is a simple and rapid screening test. Atawodi *et al* (2003) and Maikai *et al* (2008) corroborated the report of Kaminsky *et al* (1996) showing that parasite motility is a reliable index for determining antitrypanosomal activity of crude plant extracts. Hoet *et al* (2004) and Atindehou *et al* (2004) also observed that differences in activity of extracts from different parts of the same plant might be due to variations in geographical location, time or period of collection and chemical constituents. Thus, Atawodi *et al* (2003) cautioned that any statement on trypanocidal activity of a plant extract should be made in the context of the method of extraction.

Although the present study did not involve detailed characterization of different compounds that could be responsible for the observed activities, preliminary phytochemical screening showed the presence of saponins, tannins, flavonoids and alkaloids in H. *acida* extracts (data not shown). Several authors have either identified or isolated tannins and phenolic compounds (Shuaibu *et al*., 2008; Kubata *et al*., 2005), flavonoids (Ambrozin *et al*., 2004) and alkaloids (MerschJohann *et al*., 2001) in plants that showed trypanocidal activities. To the best of our

knowledge, this is the first report on antitrypanosomal activity of *Gardenia erubescens* and *Lophira lanceolata*. However, Traore *et al* (2006) showed that oral administration of crude extract of *Gardenia sokotensis* (a similar species to G. *erubescens*) had an in vitro antiplasmodial activity.

Table 1: In vitro antitrypanosomal activity of some Nigerian medicinal plants (expressed in percentages).

Plant	Family	Part(s)	Extracts	Inhibitory concentrations(mg/ml)				
				2.5	5.0	10.0	20.0	40.0
Stereospermum kunthianum	Bignonianceae	LF	Aqueous	0	0	0	0	0
			Ethanolic	0	0	0	0	0
		SB	Aqueous	0	0	0	0	0
			Ethanolic	0	0	0	0	0
Lophira lanceolata	Ochnaceae	LF	Aqueous	0	0	0	100	100
			Ethanolic	0	0	0	100	100
		SB	Aqueous	0	0	0	100	100
			Ethanolic	0	0	0	100	100
Gardenia erubescens	Rubiaceae	LF	Aqueous	0	0	0	100	100
			Ethanolic	0	0	0	100	100
		SB	Aqueous	0	0	0	100	100
			Ethanolic	0	0	0	100	100
Anthocleista vogelii	Loganiaceae	SB	Aqueous	0	0	0	0	
			Ethanolic	0	0	0	0	0
		RB	Aqueous	0	0	0	100	0
			Ethanolic	0	0	0	100	100
								100
Hymenocardia acida	Hymenocardiaceae	SB	Aqueous	100	100	100	100	100
			Ethanolic	100	100	100	100	100
		RB	Aqueous	100	100	100	100	100
			Ethanolic	100	100	100	100	100
Blighia unijugata		LF	Aqueous	0	0	0	0	0
			Ethanolic	0	0	0	0	0
Uapaca togoensis	Euphorbiaceae	SB	Aqueous	0	0	0	0	0
			Ethanolic	0	0	0	0	0
		RB	Aqueous	0	0	0	0	0
			Ethanolic	0	0	0	0	0
Cussonia arborea	Araliaceae	SB	Aqueous	0	0	0	0	0
			Ethanolic	0	0	0	0	0
		RB	Aqueous	0	0	0	0	0
			Ethanolic	0	0	0	0	0

KEY: 100% = Activity, 0% = No activity ; LF - leaf, SB - stem bark, RB- root bark

The present study demonstrated the potential of *H. acida* as an antitrypanosomal agent. H. *acida* is a shrub plant with palatable foliage and widely distributed in the savannah region of Nigeria. The appropriate part of this plant could easily be soaked in water and given to animals to drink. However, considering that a previous study (Wurochekke *et al*., 2004) indicated that a plant

with in vitro activity does not necessarily have in vivo effect, further investigation on in vivo efficacy is necessary, and it is currently in progress in our laboratory.

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