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Full Length Research Paper

In vitro antitrypanosomal activity of aqueous and methanolic crude extracts of stem bark of *Ximenia americana* on *Trypanosoma congolense*

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The *in vitro* antitrypanosomal activity of methanolic and aqueous extracts of stem bark of *Ximenia americana* was evaluated on *Trypanosoma congolense*. Blood obtained from a highly infected mice with *T. congolense* (10^7) was incubated with methanolic and aqueous extract at 20, 10 and 5mg/ml and Diminal^R (diminazene aceturate) at 200, 100 and 50 ug/ml in a 96 well micro titer plate. The results revealed that methanolic and aqueous extracts had activity at 20 and 10 mg/ml however, the methanolic extracts were more active than aqueous extract at 10 and 5 mg/ml. Phytochemical screening of the methanolic and aqueous extract of the bark showed that they both had flavonoids, anthraquinone, saponnin, terpenes and tannin. The aqueous and methanolic extract appears to show some potential activity against *T. congolense*.

Keyword: *Ximenia americana*, trypanocidal, *Trypanosoma congolense*

INTRODUCTION

African trypanosomosis remains a disease with unsatisfactory medical control. To date, the control of trypanosomosis continues to rely principally on chemotherapy and chemoprophylaxis using the salts of the three compounds, diminazene; an aromatic diamidine; homidium, a phenanthridine; and isometadium, phenanthridine – aromatic amidine (Leach and Roberts, 1981; Ilrad, 1990; Anene et al., 2001). However, the therapeutic and prophylatic use of trypanocides is beset by numerous limitations, including toxicity and the development of resistance by the parasites (Gutteridge, 1985). The emergence of drug resistance trypanosome strains is considered a very serious problem in trypanosomosis control, particularly for the resource – poor at risk populations and farmers in Africa. Recent surveys in Eastern and Southern Africa (Ndung'u et al., 1999) and in West Africa (McDermott et al., 2000; Maikai et al., 2007) have shown that the prevalence of trypanocidal drug resistance might even be higher than hitherto expected. The limited availability and affordability of pharmaceutical medicines emphasizes the need for research into a more comprehensive, formidable and cheaper sources of trypanocide.

It is estimated that some 20,000 species of higher plants are used medicinally throughout the world (Tagboto and Townson, 2001). Plants have provided the basis for traditional treatment for different types of diseases and still offer an enormous potential source of new chemotherapeutic agents. Plants present a spectrum of biological compounds with activities against virus, cancer and parasites.

These plants contain compounds mainly secondary metabolites such as alkaloids, Glycosides, flavonoids, terpenes and coumarins (Rates, 2001). They have been reported to provide better and cheaper alternatives (Nwude and Ibrahim, 1980; Secoy and Smith, 1983; Phillipson and Wright, 1991; Freiburghans et al., 1996; ITDG and IIRR, 1996; Nok et al., 1996; Adewummi et al., 2001; Nok, 2005). *X. americana* has been reported (Zachariya et al., 2000; Maikai et al., 2007) to be used

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by local herdsmen in the treatment of Trypanosomiasis, this work was therefore, carried out to verify this claim.

MATERIALS AND METHODS

Collection of plant material

The stem bark of *X. americana* was collected from Afaka village 35 km to Kaduna (11^{o} 10' N, 7^{o} 38' E) and taken to Department of Biological Sciences, Ahmadu Bello University Zaria for identification the voucher No. 1612 was deposited. The stem bark was dried at room temperature before crushing into powder then stored in air tight container and kept at 4^{o}C until needed.

Parasites

Stabilates of *T. congolense* (Nasarawa strain), were obtained from Nigeria Institute for Trypanosomiasis Research Vom, Plateau State and passage in mice.

Plant extraction

The powdered plant (100 g) was then extracted using 500 ml of methanol or water for 24 h. The filterate obtained was concentrated in vacuum. The extracts were stored at 4^{o}C in tightly sealed containers till needed.

Phytochemical screening of extracts

The aqueous bark extract was screened as described by Sofowora (1993), Trease and Evans (1989), and Harborne (1973).

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A blood – incubation infectivity test (BIIT) was performed. Infected blood were incubated in 96 well microtiter plates as described (Nok et al., 1992) in presence of aqueous and methanolic stem bark extract. 0.5 g of the extracts was dissolved in 25 ml of 0.5% DMSO to give a stock solution of 20 mg/ml. Other concentrations were made by dilution (10 and 5 mg/ml) and Diminal^{R} (standard drug) was similarly prepared at 200, 100 and 50 µg/ml. The control was 0.5% Dimethyl sulfoxide (DMSO). The blood was checked at 5 min. interval for inactivity of the parasites in the blood using a microscope at x 400 magnification.

RESULTS

Table 1 shows the result of the phytochemical screening showed that aqueous extract had saponnins, tannins and terpenes (highly present), alkaloids, glycosides and flavonoids (moderately present) while anthraxquinones (faintly present) methanolic extracts, revealed flavonoids (highly present), while alkaloids and glycolsides were absent. At concentration of 20 mg/ml the parasites were completely immobilized within 60 min of incubation with aqueous and methanolic extracts, while the standard drug Diminal^{R} immobilize / eliminated the parasite within 45 min of incubation at concentration of 200 µg/ml (Table 2). The control consisting of the parasites incubated with 0.5% Dimethyl sulfoxide (DMSO) showed the presence of very active parasites at the end of 2 h.

DISCUSSION

Parasite motility constitute a relatively reliable indicator of viability of most zoo flagellate parasites (Peter et al., 1976; Kaminsky et al., 1996) cessation or drop in motility of trypanosomes may serve as measure of antitrypanosomal potential of the crude extract when compared to the control phosphate buffer saline. Atawodi et al. (2003) reported that complete elimination of motility or reduction in motility of parasites when compared to the control could be taken as indices of trypanocidal effects. The result obtained is similar with that reported (Freigburghaus et al., 1996; Adewumi et al., 2001; Atawodi et al., 2003; Sara et al., 2004; Nok, 2005) that some plants had promising activity against trypanosomes. The concentration value of the extracts were high (20 mg/ml) when compared to the values used for the trypanocide (Diminal^{R}) which was at lower concentration of micrograms (µg). The extracts are still crude and could have complex composition; hence purification might lead to pure compounds with highly increased activity which could also be used at microgram concentration. The morphology of the blood cells was maintained while that of the parasites was affected when compared to the control that still had very active parasites. The mechanism by which the extracts eliminate / immobilize the parasites is not immediately known at this stage of the work. Sepulveda – Boza and Cassels (1996) suggested that many natural products exhibited 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.

Natural products are thought to possess structures capable of generating radicals that may cause peroxidative damage to tryanothione reductase that is very sensitive to alterations in redox balance. To obtain information on type of compounds which could be responseble for the activity, phytochemical screening revealed the extracts to contain alkaloids, flavonoid, tannins, cardiac glycosides, and others. Phytochemicals in contrast to synthetic pharmaceuticals based upon single chemicals, may exert their effects through the additive or synergistic action of several chemical compounds acting at a single or multiple target sites associated with a physiological process (Kaufmann et al., 1999; Tyler, 1999). Some literatures have reported that some flavonoids had antitrypanosomal activity (Tarus et al., 2002) while Nok, (2002) reported anzaanthraquinone to have activity against *T. congolense*. The aqueous and methanolic extracts have shown the presence of some of these phytochemicals, at this stage of the work we can not say which could be responsible, a definite statement can not be made until they are tested *in vivo* and a column chromatography carried out. Currently we

Table 1. Phytochemical screening of *Ximenia americana*

Extract	Alkaloids	Anthraquinones	Cardiac glycosides	Flavonoids	Pylobatannins	Saponnins	Tannins	Terpenoids
Crude methanolic extract (CME)	++	+	++	++	++	+++	+++	+++
Aqueous methanolic extract (AME)	-	+	-	+++	-	++	++	++

+++ - highly present, ++ - moderately present, + - faintly present, - absent

Table 2. *In vitro* effect of crude aqueous and methanolic stem bark extracts of *Ximenia americana* on *T. congolense*

Plant name	Family name	Plant parts screened	Extract used	Time taken to immobilize parasites	inhibitory concentration (IC) (mg/ml)	Activity on *T. congolense*
Ximenia americana	Olacaceae	Stem bark	Methanol	55 min.	20	+
			Aqueous	52 min.	20	+
		Diminal	-	45 min.	200 µg/ml	+
		Control (DMSO)	-	-		Parasites very active even after 2 h

+ active against *T. congolense* - not active against *T. congolense* IC- concentration at which no trypanosome with a normal morphology/or motility was found when compared to the controls

are carrying out the *in vivo* experiment to confirm its activity.

Conclusion

Aqueous and methanolic extracts of *X. americana* stem bark possess antitrypanosomal activity. This could be the reason why Fulani herdsmen use it locally to treat their animals, since the plant is easily found and the plant soaked and given to the animals to drink.

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REFERENCES

Adewummi CO, Agbedahunsi JM, Adebajo AC, Aladesanmi AJ, Murphy N, Wando (2001). Screening of Nigerian medicinal plants for trypanocidal properties. J. Ethnopharm. 77: 19-24 .

Anene BM, Onah DN, Nawa Y (2001). Drug resistance in pathogenic African *trypanosomes*: What hopes for the future? Veterinary Parasitology 96:83 -100.

Atawodi SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman M, Galadima M(2003). Invitro trypanocidal effect of methanolic extract of some Nigerian Savannah plants. Afr. J. Biotechnol. 2(9). 317-321.

Freiburghaus F, Basel N (1996). African medicinal plant used in the treatment of sleeping sickness – an evaluation. Weittever service Ag, Bern. pp 1 –23.

Gutteridge WE (1985). Existing chemotherapy and its limitation. British Med. Bull. 4 (2): 162 – 168.

Harborne JB (1973). Phytochemical methods. London Chapman and Hall Ltd. pp.49-188.

ILRAD (1990) Chemotherapy of trypanosomiasis, International Laboratory for Research on Animal Diseases Reports, Nairobi, pp. 8-17.

ITDG and IIRR (1996). Ethnoveterinary medicine in Kenya. A field manual of traditional animal health care practices. Intermediate Technology Development Group and International Institute of Rural Reconstruction, Nairobi, Kenya. 102.

Kaminsky F, Nkuna MHN, Brun R (1996). Evaluation of African medicinal for their invitro trypanocidal activity. J. Ethnopharm. 55: 1-11

Kaufman PB, Cseke LJ, Warber S, Duke JA, Brielmann HL (1999). Natural Products from plants. CRC Press, Boca Raton, Fl.

Leach TM, Roberts CJ (1981). Present status of Chemotherapy and Chemoprophylaxis of Animal Trypanosomiasis in the eastern hemisphere. Pharm.Therap. 13: 91 –147.

Maikai VA, Salka MN, Adeiza AA, Makeri HK (2007). Assessment of Isometamidium chloride and diminazene aceturate in laboratory mice infected with field isolates of T. congolense from naturally infected cattle.

J. Prod. Agric. Technol. 3(1): 147 - 152

Maikai VA, Umar A,Inuwa T, Al Parisi SA (2007b). Survey of medicinal plants used in treatment of animal trypanosomosis for economic empowerment and development in Kaduna State " 3RD Annual National Conference of the School of Science, Federal College of Education, Zaria. on the April 2 - 5, 2007.

McDermott JJ, Sidibe I, Bauer B, Diarra B, Clausen PH, Woitang T, Ouedraogo D, Kamuanga JMB, Peregrine AS, Eisler MC, Mehlitz D (2000). Field studies on the development and impact of drug resistant animal trypanosomes in market oriented production No2 systems in the southern Guinea Zone of West Africa. Newsletter

of EU concerted Action on.
Ndung"u JM, Murrilla GA, Mdachi RM, Mbwambo H, Sinyangwe L, Machila N, Delespaux V, Geerts S, Brandt J, Peregrine AS, McDermott JJ, Holmes PH, Eisler MC (1999). Area-wide appraisal of drug resistance in trypanosomes infecting cattle in East and Southern Africa. In : Proceedings of the International Scientific council for Trypanosomiasis Research and Control, 25th meeting, Mombasa, Kenya, 27 Sept-1st October 1999. OAU/STRC.Nairobi.
Nok AJ, Esievo KAN, Adaudi A, Ogeshe S, Gimba CE, Kabgu JA (1992). Trypanocidal activity of an organotin compound. J. Clin. Biochem. Nutr. 11 :125-130.
Nok, A.J; Williams, S; Onyenekwe, P.C (1996) *Allium sativum* induced death of African trypanosomes. Parasitol. Res. 82: 634-637.
Nok, A.J.(2005) Effective measures for controlling trypanosomiasis. Expert Opinion. Pharmather. 6 (10): 1-9.
Nwude N, Ibrahim MA (1980). Plants used in traditional veterinary medical practice in Nigeria. J. of Vet Pharm. and Thereap. 3: 261–273.
Peter D, Honigberg BM, Fern AM (1976). An improved method of cryopreservation of *Trypanosoma (Nannomonas) Congolense* brooden in liquid nitrogen. J. Parasitol. 62 (1): 136-137.
Phillipson JD, Wright CW (1991). Medicinal plants in tropical Medicine. Trans Royal Society of Trop. Med Hyg. 85: 18– 22.
Rates, S. M.K. Plants as source of drugs. *Toxicon*. 39: 603–613.2001 Sara H, Federick O, Reto B, Victor A, Joelle Q (2004). *In vitro* antitrypanosomal activity of ethnopharmacologically selected Beninese plants. J. Ethnopharmacol. 91: 37-42.
Secoy DM, Smith AE (1983)Use of plants in control of agricultural and domestic pests. Econ. Bot. 37(1): 28-57.
Sepulaveda-Boza S, Cassels BK (1996). Plant metabolite active against *Trypanosoma cruzi*. Planta med. 62: 98-105.
Sofowora A (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.
Tagboto S, Townson S(2001). Antiparasitic properties of medicinal plants and other naturally occurring products. Adv. Parasitol. 50, 199-295.
Tarus PK, Machocho AK, Langat- Thoruwa CC, Chhabra SC (2002). Flavonoids from *Tephrosia aequilata*. Phytochemistry 60: 375-379
Trease GE, Evans WC(1989). Pharmacognosy. 11th Edition Brailliar Trindel Can. Macmillan Publishers.
Tyler, V.E. (1999) Phytomedicines: back to the future. J. Nat. Prod. 62:1589-1592.
Zachariya TS Kapu SD, Nwinyi FC, Bark T, Gamaniel KS (2000). Medicinal plants used in the treatment of animal trypanosomiasis by Fulani herdsmenin Abuja municipal area concil; Nigeria. Proceeding of International workshop on Ethnoveterinary Practices held 14-18 August 2000, Kaduna Nigeria.