

Full Length Research Paper

# ***In vitro* and *in vivo* animal model antitrypanosomal evaluation of ten medicinal plant extracts from south west Nigeria**

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Accepted 23 January, 2009

The relative antitrypanosomal and haemolytic activities of the methanol extracts of 10 Nigerian medicinal plants extracts comprising of the leaf, stem bark and root bark of *Azelia africana*, *Khaya senegalensis* and *Terminalia superba* as well as the leaf extract of *Lannea welwistchii* were investigated. All the extracts showed varying degrees of trypanocidal activity *in vitro*. The roots of *T. superba* and *K. senegalensis*, the stem of *T. superba* and the leaf of *A. africana* were the most effective with a minimum lethal concentration (MLC) of 3 mg/ml; compared to 5.4 mg/ml for diminazene aceturate (Berenil<sup>®</sup>), the reference drug. *A. africana* stem bark also had an MLC of 5.4 mg/ml in the study. The extracts were also analyzed for hemolytic activity, using washed bovine red blood cells in order to ascertain if any relationship between their hemolytic and antitrypanosomal activities. In the *in vivo* analysis, only the *T. superba* root bark extract totally inhibited the growth of parasites in both rats and mice; all the other root bark extracts resulted in parasite clearance in rats only. The duration of clearance in all cases was 48 h, with relapse parasitaemia occurring on the 3<sup>rd</sup> day post-administration of the extracts.

**Key words:** *In vitro* antitrypanosomal activities, *in vivo* in mice and rats, *Azelia africana*, *Khaya senegalensis*, *Terminalia superba*, *Lannea welwistchii*.

## **INTRODUCTION**

*Trypanosoma brucei brucei* are unicellular parasite transmitted by the tsetse fly and it is the causative agents of African Animal Trypanosomiasis (AAT). Trypanosomiasis is one of the major constraints on animal production in the Africa region and it is the greatest potential for significant increases in domestic livestock population and productivity. Currently, trypanocidal drugs constitute the principal method of control, as development of vaccines against AAT is still in progress. Effective chemotherapy of AAT is beset with problems of poor clinical efficiency, drug resistance and toxicity (Onyeyili and Egwu, 1995; Geerts and Holmes, 1998;

Legros et al., 2000). The current drugs used as veterinary trypanocides include diminazine aceturate (Berenil<sup>®</sup>) and isomethamidium (Samorin<sup>®</sup>). These are also used as prophylactics for control of the disease in cattle (WHO, 1995). Resistance to both chemotherapeutic agents has however been documented in field studies (Kuzoe, 1993). This presents an urgent need to develop efficacious chemotherapeutic agents from locally available ethno-medicinal plants for use trypanocides.

Several reports of different chemicals and plant derivatives with trypanocidal effects have been reported (Bodley and Shapiro, 1995; Freiburghaus et al., 1996, 1997, 1998; Atawodi et al., 2003). Plants used in traditional medicine are rich sources of bioactive secondary metabolites that can be effective as haemolysins and cytolysins (Eric, 1937; Yuldasheva et al., 2005). There is a possibility that the cytolytic activities of these

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plants share similar mechanisms of action. Several secondary plant metabolites including alkaloids, tannins and anthraquinones have been indicated with trypanocidal activity (Sepulveda-Boza, 1996; Hopp et al., 2004).

As part of our efforts to screen Nigeria medicinal plants for antiparasitic activities, we present herein the *in vivo* and *in vitro* (animal) trypanosocidal activities of ten plant extracts (Ajaiyeoba and Okogun, 1996; Ajaiyeoba et al., 2004; 2005).

## MATERIALS AND METHODS

### Plant collection and authentication

The leaves, stem bark and root bark of *Azelia africana*, *Khaya senegalensis*, *Terminalia superba* and *Lannea welwistchii* were obtained in October, 2005 from the Botanical Gardens of the University of Ibadan and authenticated at the Herbarium of both Botany Department of the University of Ibadan and Forestry Research Institute of Nigeria (FRIN), Ibadan. Voucher specimens were deposited in FRIN under *Terminalia superba* 107601, *Khaya senegalensis* 107602 and *Lannea welwistchii* 107603.

### Plant preparation and extracts

The plant parts were air-dried in the laboratory at RT (29°C). Plant samples were extracted by the Soxhlet. Crude extract (50 mg) was each solubilized in 100 µl dimethylsulfoxide (DMSO) and made up to 1 ml in phosphate buffered saline (PBS, pH 7.4) to produce a stock solution of 50 mg/ml respectively. All subsequent dilutions were made in PBS.

### Laboratory animals

Albino rats and mice were used for the *in vivo* analysis. They were obtained from the laboratory animal colonies of the Institute of Malaria Research and Training (IMRAT), University College Hospital, Ibadan. They comprised female white mice (8 - 10 weeks old) weighing between 14 to 22 g and albino rats of mixed sexes (5 - 8 weeks old), weighing 50 to 164 g. Pelletized growers mash (from Ladokun feeds Ltd.) containing 18 % crude protein and supplying 2800 Kcal of metabolisable energy was used to feed the animals throughout the course of the experiment. The animals were fed *ad libitum*.

### Test organism and determination of parasitaemia

*T. b. brucei* (Federe strain) was obtained from stabulates maintained at the Nigerian Institute for Trypanosoma Research (NITR), Vom, Plateau State, Nigeria. The parasites were maintained in the laboratory by continuous passage in rats and mice to give parasitaemia of approximately log<sub>10</sub> 5.4 - 6.2 parasites/ml at 4 - 5 days post inoculation (pi). Parasitaemia of log<sub>10</sub> 8.1 - 8.7 parasites/ml is expected by day 12 -14 pi. Mortality is normally produced in laboratory rats and mice in 14 -15 days.

Blood from the tail was used as estimation of parasitaemia. The trypanosome count was determined by examination of the wet mount microscopically at X 40 magnification using the "rapid matching" method of Herbert and Lumsden (1976).

### *In vitro* antitrypanosomal activity of plant extracts

Assessment of the *in vitro* trypanocidal activity was performed in duplicate in round bottom 96 well microtiter plates. Infected rat blood (10 µl) in heparin blood was mixed with 50 µl of 2-fold serial dilution of stock extract solutions to bring the starting parasite concentration to log<sub>10</sub><sup>7.8</sup> parasites/ml. The controls were serial 2-fold dilutions of 25 mg/ml Diminazene (Veterinary Pharmaceuticals Products, Holland) containing 10.75 mg/ml diminazene acetate and blood mixed with 50 µl of PBS containing 10% DMSO. The plates were incubated in a moist chamber at RT (29°C) room temperature for 60 min. Antitrypanosomal activity was determined by microscopic examination of a drop of the mixture at X40 magnification. Complete elimination of motility or a 2 log<sub>10</sub> reduction in parasite count (from 10<sup>7.8</sup> parasites/ml to 10<sup>6.9</sup> or fewer parasites/ml) was taken as significant activity of the extracts.

### *In vivo* antitrypanosomal activity of plant extracts

*T. b. brucei* infection was established in each mouse by intraperitoneal (ip) inoculation with approximately 10<sup>5</sup> organisms/ml. Four days post inoculation; the level of parasitaemia was approximately log 7.8 (10<sup>7.8</sup> parasites/ml). Ten different groups at 2 mice/group were inoculated with the leaf, stem bark and root bark extracts of the selected plants ip. Mice were given 0.4 ml of 25 mg/ml extract solution (10 mg/kg/mouse). A group was also administered ip 0.2 ml diminazene (10 mg/kg), corresponding to 4.3 mg of diminazene acetate/mouse. The control group was administered 0.2 ml PBS containing 10% DMSO ip. The experiment was also done using with 24 albino rats inoculated with 10<sup>5</sup> organism/ml (0.2 ml). The rats were divided into 6 groups. Five of the groups were inoculated with the root bark extract of the selected plants respectively as well diminazene acetate contained in 0.3 ml was administered to each rat on the day 8 pi. The last group was given 0.3 ml of PBS + 10% DMSO. All treatments were *via* the ip route.

## RESULTS

### *In vivo* antitrypanosomal activity of the extracts in mice and rats

Intraperitoneal (ip) inoculation of the *T. b. brucei* infected animals was done with the selected plant extracts. Each animal was inoculated with 10 mg in 0.4 ml of extracts. On day 4 post infection when the level of parasitaemia was between 10<sup>7.5</sup> and 10<sup>7.8</sup> parasites/ml, the extracts from *A. africana* (leaf), *T. superba* (leaf and stem) *K. senegalensis* (stem) were toxic to the animals at 10 mg/mouse, causing the death of the animals within 48 h. At this concentration, only the root bark of *T. superba* caused reduction in parasitaemia from 10<sup>7.8</sup> to 10<sup>6.9</sup> parasites/ml. There was total clearance between 3 to 6 days after inoculation. In the diminazene treated group, the parasitaemia was reduced from 10<sup>7.8</sup> to 10<sup>6.9</sup> parasites/ml after 24 h and parasites totally cleared in 2 days, without relapse in the 11 day observation period. The other extracts were not effective in reducing or clearing parasitaemia in a 7 day observation period. The

**Table 1.** *In vitro* antitrypanosomal effects of plant extracts on parasite count.

Plant extract	Concentration (mg/ml)								MIC (mg/ml)	MLC (mg/ml)
	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0.1		
<i>A. africana</i> (leaf)	0*	0	0	7.2	7.5	7.8	7.5	7.5	3.1	3.1
<i>A. africana</i> (stem)	6.9	7.2	7.2	7.8	7.8	7.8	7.5	7.5	12.5	>12.5
<i>A. africana</i> (root)	0	0	6.3	6.9	6.9	7.8	7.8	7.8	0.8	6.3
<i>K. senegalensis</i> (leaf)	6.6	6.9	6.9	6.9	7.2	7.2	7.5	7.5	1.6	>12.5
<i>K. senegalensis</i> (stem)	0	5.7	6.0	6.9	7.2	7.2	7.5	7.5	1.6	12.5
<i>K. senegalensis</i> (root)	0	6.9	7.2	7.5	7.5	7.8	7.8	7.8	6.3	12.5
<i>T. superba</i> (leaf)	0	0	5.4	6.9	7.2	7.2	7.2	7.2	1.6	6.3
<i>T. superba</i> (stem)	0	0	0	6.6	6.6	7.2	7.5	7.5	0.8	3.1
<i>T. superba</i> (root)	0	0	0	6.6	6.6	7.2	7.5	7.5	0.8	3.1
<i>L. welwitschii</i> (leaf)	0	0	7.2	7.5	7.5	7.5	7.5	7.5	6.3	6.3
Diminazine aceturate®	0 <sup>a</sup>	6.9 <sup>b</sup>	6.9 <sup>c</sup>						1.35	5.4

\*Parasite count.

Minimum Inhibitory Concentration (MIC) = First concentration of extract at which parasite count detected was significantly reduced (from log<sub>10</sub> 7.8 to log<sub>10</sub> 6.9).

Minimum Lethal Concentration (MLC) = First concentration of extracts at which no motility was detected.

<sup>a</sup>Parasite count at 5.4 mg/ml diminazine aceturate; <sup>b</sup>parasite count at 2.7 mg/ml diminazine aceturate; and <sup>c</sup>parasite count at 1.35 mg/ml diminazine aceturate.

assay was done with rats, using only the root bark extracts of *A. africana*, *K. senegalensis* and *T. superba* and the leaf extract of *L. welwitschii*. Extracts (0.3 ml) at 50 mg/ml concentration were inoculated *via* the intraperitoneal route. This corresponded to 15 mg/animal. Diminazone was given at a dose corresponding to 3.01 mg diminazine aceturate/rat. The extracts were administered on the day 8 post-infection with the parasitaemia at 10<sup>8.1</sup>. Parasitaemia was cleared 48 h after inoculation for the entire root extracts. However, this was not sustained, as there was relapse on the day 3 to a parasitaemia of 10<sup>8.1</sup>. Treatment was repeated after 3 days with 0.3 ml of the extracts at 100 mg/ml (30 mg/rat). There was further reduction in parasitaemia in the group inoculated with *T. superba* and *A. africana* to 10<sup>6.0</sup> and 10<sup>7.8</sup> respectively in the day 4 post treatment, after which there was a steady increase in the level of parasitaemia. In the Diminazone treated group, there was reduction of parasitaemia on day 2 and complete clearance on the day 4 which was sustained until the end of the 7 - day observation period.

There was no further reduction in parasitaemia in rats on treatment of the relapse infection with *K. senegalensis* root and *L. welwitschii* leaf extracts.

### ***In vitro* antitrypanosomal activity**

The concentration of extract which resulted in complete elimination of motility (minimum lethal concentration, MLC) or that which caused at least a 1000 times reduction in the number of motile parasites (minimum inhibitory concentration, MIC) compared with PBS control

was taken as an indication of antitrypanosomal activity. *A. africana* (leaf) *T. superba* (stem and root) and *K. senegalensis* (root) extracts were the most effective with MLC value of 3.1 mg/ml). The MLC was comparable to that of diminazine aceturate 5.4 mg/ml. The least effective extracts were the stem bark of *A. africana* and the leaf of *K. senegalensis* (minimum inhibitory concentration, MIC of 12.5 mg/ml; *T. superba* (0.8 mg/ml), diminazone aceturate (1.35 mg/ml) as shown in Table 1.

### **DISCUSSION**

It has been shown in previous studies that different parts of the same plant could show varying levels of antitrypanosomal activity (Atawodi et al., 2003). In the present study the extracts had varying degrees of antitrypanosomal activity. It is interesting to note that the stem and root bark extracts of *T. superba* caused complete cessation of motility of parasites at 3.1 mg/ml extract concentration while the leaf extracts of the same plants only caused cessation at a higher concentration of 6.3 mg/ml. Also the root bark and leaf extracts of *A. africana* caused completed cessation of motility at 6.3 and 3.1 mg/ml respectively while the stem bark extracts did not produce complete cessation of motility at any of the test concentrations. This finding collaborates the earlier observation by Atawodi et al. (2003) for the need to study all plant parts individually in a bid to facilitate drug discovery from medicinal plant.

*T. superba* root, stem and leaf extracts exhibited appreciable antitrypanosomal activity *in vitro*. *T. superba*

root extract also exhibited appreciable activity *in vivo* in rats and mice used in this study. Atawodi et al. (2003) showed in an earlier study that *T. avicenoides* was strongly trypanocidal *in vitro* to both *T. b. brucei* and *T. congolense*.

All the other extracts exhibited mild to moderate antitrypanosomal activity *in vitro* but no significant effect *in vivo*. The absence of outstanding *in vivo* activity of the extracts has also been observed by others (Frieburghaus et al., 1996), and may be attributed to degradation or metabolisation of the active principle through various metabolic processes in the host animal, or to the toxicity of high levels of the crude extract required for therapeutic efficacy. The high level of parasitaemia in the infected mice could also be a factor. On the other hand, it is possible for the animal's metabolic processes to activate a compound with little or no activity *in vitro* (Phillipson et al., 1993)

Sepulveda-Boza and Cassels (1996) reported 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 enzymes that are very sensitive to alterations in redox balance. Some agents also act by binding with the kinetoplast DNA of trypanosomes (Atawodi et al., 2003). Different phytochemical constituents might also be responsible for the antitrypanosomal activities and some saponins tested for antitrypanosomal activity did not show any (Asres et al., 2001).

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