

Full Length Research Paper

Comparative *in vitro* trypanocidal activities of water and methanol extracts of three parts of *Khaya senegalensis* on *Trypanosoma evansi*

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A comparative phytochemical and *in vitro* studies of water and methanolic extracts of three different parts of *Khaya senegalensis*, a plant used by herbalists in Nigeria to treat helminthiasis and other ailments revealed the presence of alkaloids, carbohydrates, tannins, saponins, flavonoids, terpenes sugars, cardiac glycosides and phlobatannins. At a dose of 20 mg/ml the water extract (leaf, stem bark and root) immobilised *Trypanosoma evansi* the most at 25 min while the methanol extract showed the most activity in the stem bark at 30 min. The results obtained with these crude extracts showed that *K. senegalensis* is a potential source of trypanocidal drug/chemical leads.

Key words: *Khaya senegalensis*, *Trypanosoma evansi*, trypanocidal activity, mando road, *in vitro*, water, methanolic.

INTRODUCTION

It is variously estimated that some 45 - 50 million cattle live under trypanosomiasis risk, in a tsetse-infested area of some 8 - 10 million Km² (Budd, 1999; Gilbert et al., 2001). *Trypanosoma evansi* is a species belonging to the subgroup *Trypanozoon* causing infection in camels called "Surra" and does also affect domestic and wild animals (Franke et al., 1994) and in a human in India where the parasite was found to have survived and proliferated for at least 5 months (Joshi et al., 2005). It is transmitted mechanically by biting flies of the genera *Tabanus*, *Lyperosia*, *Stomoxys* and *Atylotus* (Brun et al., 1998) displaying typical signs such as fever, anemia, weight loss, edema, lymphadenopathy and sudden death (Brun et al., 1998; Aquino et al., 1999) and it is the most important single cause of economic losses in camel rearing areas, causing a morbidity of up to 30.0% and mortality of around 3.0% (Pacholek et al., 2001). Surra has attracted international attention in recent years with the hosting of an international symposium on strategies

for research and control of the disease (Obihiro, 1998).

The chemotherapy of trypanosomiasis is beset by several problems associated with the treatment range from limited repertoire to protracted treatment protocols (Tropical Disease Research 7th Programme Report, 1984; Gutteridge, 1985). These factors couple with the attention been paid to *T. evansi* right now emphasise the need for research into better and cheaper sources of trypanocides.

Finding healing powers in plants is an old idea and disease management in Nigeria history also provides evidence of the relationship of plants and medicine (Raghevendra et al., 2006; Ayandele and Adebisi, 2007). The exploitation of certain herbs and other plant materials said to be traditionally used in the control of trypanosomiasis have increased (Asuzu and Chineme, 1990), providing better and cheaper alternatives (Freiburghans et al., 1996; Nok, 2005). The plant, *Khaya senegalensis* (Desr.) A. Juss (Arbonnier, 2004), is a dry zone Mahogany belonging to the family Meliaceae, that is easily recognised by its round evergreen crown of dark shiny foliage pinnate leaves and characteristic round capsules (Keay et al., 1989). *K. senegalensis* is highly

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reputed for its numerous medicinal uses (Arbonnier, 2004), it has been used ethnomedicinally as a remedy for several human and animal ailments (Deeniand and Sadiq, 2002), active *in vitro* against *T. brucei brucei* (Wurochekke and Nok, 2004), *T. congolense* (Atawodi et al., 2003; Atawodi, 2005), helminthiasis (Fajimi and Taiwo, 2005), it possess antiviral, antifungal and bacteriocidal properties (Abdelgaliel and Nakatani, 2003), and showing a moderate to high efficacy against *Haemonchus Cooperia*, *Oesophagostomum* and *Trichostrongylus* spp. (Chiezey et al., 2005). The aqueous extract is taken against diarrhoea, gynaecological disturbances, digestive disorders and nervous confusions (Nacoulma-Ouedraogo, 1996), as an antipyretic, anti-malarial and fodder for cattle (Arbonnier, 2004) and the dried stem-bark is used externally for the treatment of leprosy, dermatoses, sores and ulcers in adults (Le Grand, 1989). Here we report on some trypanocidal effects of *K. senegalensis* extracts on *T. evansi*.

MATERIALS AND METHODS

Plant materials/collection

The plants parts screened were obtained from Nasarawa State, Nigeria. The plant was taken to the herbarium unit of Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria and a voucher specimen number 900181 was deposited there.

Plant extraction

The plant parts were dried at room temperature for two weeks and pound into fine powder using pestle and mortar. The powdered material weighing 200 g was packed into a Soxhlet extractor and extracted exhaustively and successively with water and methanol. The various extracts were respectively, concentrated *in vacuo* at 40°C using a rota vapor after which 46 g of water and 37.5 g of methanol extracts were respectively, realised. The solvent free extracts were stored at 4°C till needed.

Phytochemical screening

Standard protocols to identify the constituents as described by Sofowora (1993), Trease and Evans (1989) and Harbone (1973) were carried out. Test for alkaloids, carbohydrates, tannin, saponin, flavonoids, terpenes, sugars, cardiac glycosides and phlobatannins were carried out on all the extracts.

Trypanosome

T. evansi isolated from a camel in Kano, Nigeria, was obtained from the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The parasites were inoculated in to 3 rats at 1×10^5 trypanosomes through the intraperitoneal route using 0.2 ml of blood diluted in phosphate saline glucose. The animals were transported to our laboratory at College of Agriculture and Animal Science, Division for Agricultural Colleges, Ahmadu Bello University, Mando, Kaduna. They were monitored daily for parasitaemia using the Herbert and Lumsden (1976) method.

Trypanocidal drug

Commercial diminazene aceturate (Samorenil® Animal Care) was used to validate the test and to give reference values.

In vitro screening of *K. senegalensis* with *T. evansi*

Two percent of each of the water and methanol leaf, stem bark and root of the crude extracts was prepared and serially diluted to 20, 10 and 5 mg/ml using 0.5% dimethylsulfoxide (DMSO). Aliquots of 10 µl of the extract were incubated with 40 µl of infected blood (harvested at day 6 of peak parasitaemia in rats), in 96-well microtitre plates at 37°C. Two control groups were set up for this work, the first is the untreated control where 10 µl of 0.5% DMSO was incubated with the parasitized blood and the blood was examined at 5 min interval using an Olympus® microscope (x40) objective. While the second was assay with a commercial drug (Samorenil® Animal Care) at a concentration of 200 µg were also performed to have a reference value and this is the treated control. The inhibitory concentration, IC was the concentration at which no motile cells were seen moving in comparison to the control cultures.

Hematology

The morphology of red blood cells will be checked under the microscope at x40 and x100 using an Olympus® microscope so as to determine if the extracts have any effect on the integrity of the red blood cells.

RESULTS AND DISCUSSION

Water and methanol extracts from *K. senegalensis* were tested for their *in vitro* antitrypanosomal activity on *T. evansi*. The result reveals that *K. senegalensis* (water extract of leaf, stem bark and root) at 5 and 10 mg/ml had no effect on the parasite, but the water extract (leaf, stem bark and root) showed significant activity at 20 mg/ml after 25 min of incubation. The methanol extract showed little to no activity at 5 and 10 mg/ml (leaf, stem bark and root) and 20 mg/ml (leaf), whereas at 20 mg/ml the methanolic stem bark and root extracts showed significant activity by inhibiting parasite motility after 30 min of incubation (Tables 1, 2 and 3). Plants are known to contain a myriad of complex chemical compounds which could be beneficial to human and animal health (Edeoga et al., 2005). The complete elimination of motility or reduction in motility of parasites when compared to the control could be taken as indices of activity (Atawodi et al., 2003; Mbaya et al., 2007). Our results agree with Wurochekke and Nok, 2004; Hoet et al., 2004; Atawodi, 2005; Ogbadoyi et al., 2007), who reported the trypanocidal activity of some medicinal plants on trypanosomes *in vitro*. 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/immobilise 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

Table 1. Phytochemical screening of *K. senegalensis*.

Plant	Portion of extract	Alkaloids	Carbohydrates	Tannin	Saponin	Flavonoids	Terpenes	Sugars	Cardiac glycosides	Phlobatannins
<i>K. senegalensis</i> (water extract)	Leaf	++	+++	+++	++	++	+	++	+	-
	Stem-bark	+	++	+	++	++	+	+	++	+++
	Root	+++	++	+++	+++	++	+	+	+	-
<i>K. senegalensis</i> (methanolic extract)	Leaf	+++	+++	+++	++	++	+	++	-	-
	Stem-bark	++	+++	++	+++	+	+	+++	+++	++
	Root	++	++	+++	++	++	+	++	+	-

+++ = highly present; ++ = moderately present; + = faintly present; - = absent.

Table 2. *K. senegalensis* (water extract) activity on *T. evansi*.

Time (min)	5 mg/ml			10 mg/ml			20 mg/ml			Control	
	Bark	Leaf	Root	Bark	Leaf	Root	Bark	Leaf	Root	Infected treated diminazene	Infected untreated
5	-	-	-	-	-	-	-	-	-	All parasites dead in less than 1 min.	Parasites still alive after 2 h.
10	-	-	-	-	-	-	-	-	-		
15	-	-	-	-	-	-	-	-	-		
20	-	-	-	-	-	-	-	-	-		
25	-	-	-	-	-	-	++	+	+		
30	-	-	-	-	-	-	+++	++	+		
35	-	-	-	-	-	-	+++	++	+		
40	-	-	-	-	-	-	+++	++	++		
45	-	-	-	-	-	+	+++	++	++		
60	-	-	-	-	-	+	+++	++	++		

+++ = extract highly active; ++ = extract moderately active; + = extract faintly active; - = extract activity absent.

activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defences against oxidative stress. The radicals generated by these natural products cause peroxidative damage to trypanothione reductase that is very

sensitive to alteration in redox balance. 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 (Tyler, 1999). Some literatures have reported that some flavonoids had anti-trypansomae activity (Tarus et al., 2002). While Nok et al. (1992) reported Tri-*n*-Butyltin Oxide to have activity against *T. brucei* and azaanthraquinone against *T. congolense* (Nok, 2002). From

Table 3. *K. senegalensis* (methanol extract) activity on *T. evansi*.

Time (min)	5 mg/ml			10 mg/ml			20 mg/ml			Control	
	Bark	Leaf	Root	Bark	Leaf	Root	Bark	Leaf	Root	Infected treated diminazene	Infected untreated
5	-	-	-	-	-	-	-	-	-		
10	-	-	-	-	-	-	-	-	-		
15	-	-	-	-	-	-	-	-	-		
20	-	-	-	-	-	-	-	-	-		
25	-	-	-	-	-	-	+	-	+	All parasites dead in less than 1 min	Parasites still alive after 2 h
30	-	-	-	-	-	-	++	-	+		
35	-	-	-	+	-	-	++	-	+		
40	+	-	-	+	-	-	++	+	+		
45	+	-	-	++	-	-	++	+	+		
60	+	-	-	++	-	-	++	+	+		

+++ = extract highly active; ++ = extract moderately active; + = extract faintly active; - = extract activity absent.

our work trypanocidal activity was observed with both extracts at the highest concentration of 20 mg/ml, with the most activity seen in the water extracts. That there is a difference in degree of activity among the extracts at same dosage level could be due to the medium used in the extraction procedure since water and methanol are of different polarity. Phytochemical screening have shown the presence of flavonoids, alkaloids, glycosides, sugars and others (Table 1), at this stage we can not say which could be responsible until they are tested *in vivo* and a column chromatography carried out. Currently we are carrying out the *in vivo* experiment in Wistar rats to confirm its activity and the toxicology of these extracts are also been assessed with a view to finding the LD₅₀ and ED₅₀.

Conclusion

Water and methanol extracts of *K. senegalensis* possess antitrypanosomal activity and could provide therapeutic agents for treatment of African trypanosomiasis, a disease that has continued to be of economic and health importance in many African countries (WHO, 1975; Welburn et al., 2001).

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REFERENCES

Abdelgaleil SA, Nakatani M (2003). Antifeeding activity of limonoids from *Khaya senegalensis*. J. Appl. Entomol., 127: 236-239.

- Arbonnier M (2004). Trees, Shrubs and Lianas of West African dry zones. Magraf Publishers CIRAD, GMBH, MNHN.
- Asuzu IU, Chineme CN (1990). Effects of *Morinda lucida* leaf extract on *Trypanosoma brucei brucei* infection in mice. J. Ethnopharm., 30: 307-313.
- Atawodi SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman M, Galadima M (2003). In vitro trypanocidal effect of methanolic extract of some Nigerian Savannah plants. Afr. J. Biotechnol., 2(9): 312-321.
- Atawodi SE (2005). Comparative *in vitro* trypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian Savannah plants. Afr. J. Biotechnol., 4(2): 177-182.
- Aquino LP, Machado RZ, Alessi AC, Marques LC, de Castro MB, Malheiros EB (1999). Clinical, parasitological and immunological aspects of experimental infection with *Trypanosoma evansi* in dogs. Mem. Inst. Oswaldo Cruz., 94: 255-260.
- Ayandele AA, Adebisi AO (2007). The phytochemical analysis and antimicrobial screening of *Olox subscorpioidea*. Afr. J. Biotechnol. 6(7): 868-870.
- Brun R, Hecker H, Lun ZR (1998). *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). Vet. Parasitol., 79: 95-107.
- Budd L (1999). DFID-funded Tsetse and Trypanosome Research since 1980. Vol. 2: Economic Analysis. Department for International Development (DFID), Aylesford, Kent, p. 123.
- Chiezey NP, Gefu JO, Jagun AG, Abdu PA, Alawa CBI, Magaji SO, Adeyinka IA, Eaduvie LO (2000). Evaluation of some Nigerian for anthelmintic activity in young cattle. Proceedings of an international workshop on Ethnovet. Practices held between 14-18 August, Kaduna. Published by the National Animal Production Research Institute, Ahmadu Bello University, Zaria, Nigeria.
- Deeniand YY, Sadiq NM (2002). Antimicrobial properties and phytochemical constituents of the Leaves of African mistletoe (*Tapinanthus dodneifoliis* (DC) Danser) (Loranthaceae): an ethnomedicinal plant of Hausaland, Northern Nigeria. J. Ethnopharmacol., 83(3): 235-240.
- Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical Constituents of some Medicinal Plants. Afr. J. Biotech., 4(7): 685-688.
- Fajimi AK, Taiwo AA (2005). Herbal remedies in animal parasitic diseases in Nigeria: a review. Afr. J. Biotechnol., 4(4): 303-307.
- Franke CR, Greiner M, Mehlitz D (1994). Investigating on Naturally *T. evansi* infection in horses, cattle, dogs and capybaras (*Hydrochaeris hydrochaeris*) in Pantanal of the Pocone (Mal Grosso, Brazil), Acta Trop., 55: 159-169.
- Freiburghaus F, Kaminsky R, Nkuna MHN, Brun R (1996). Evaluation of African medicinal plants for their *in vitro* trypanosomal activity. J. Ethnopharm., 55: 1-11.
- Gilbert M, Jenner C, Pender J, Rogers D, Slingenbergh J, Wint W

- (2001). The Programme against African Trypanosomiasis Information System (PAATIS) In: Black SN and Seed JR (eds) World Class Parasites: The African Trypanosoma, Kluwer Academic Publishers, Dordrecht. The Netherlands, 1: 11-24.
- Gutteridge WE (1985). Existing chemotherapy and its limitation. British Med. Bull. 41(2): 162-168.
- Harbone JB (1973). Phytochemical methods. London, Chapman and Hall Ltd., pp. 49-188.
- Herbert WJ, Lumsden WHR (1976). *Trypanosoma brucei*: A rapid "matching" method for estimating the host's parasitaemia. Exptl. Parasitol., 40: 427-431.
- Hoet S, Opperdoes F, Brun R, Adjakidje V, Quetin-Leclecq J (2004). *In vitro* antitypanosomal activity of ethnopharmacologically selected Beninese plants. J. Ethnopharm., 91: 37-42.
- Joshi PP, Shegokar VR, Powar RM, Herder S, Katti R, Salkar HR, Dani V, Bhargava A, Jannin J, Truc P (2005). Human trypanosomiasis caused by *Trypanosoma evansi* in India: the first case report. Am. J. Trop. Med. Hyg., 73(3): 491-495.
- Keay RW, Omochie CF, Stanfield J (1989). A Revised Version of Trees of Nigeria (1964). Clarendon Press, New York. pp. 339-340.
- Le Grand A (1989). Anti-infectious phytotherapy of the tree savannah, Senegal (West Africa) 3: A review of the phytochemical substances and antimicrobial activity of 43 species. J. Ethnopharm., 25: 315-338.
- Mbaya AW, Nwosu CO, Onyeyili PA (2007). Toxicity and anti-trypanosomal effects of ethnoc extract of *Butyrsperrum paradoxum* (Sapotaceae) stem bark in rats infected with *Trypanosoma brucei* and *Trypanosoma congolense*. J. Ethnopharm., 111: 526-530.
- Nacoulma-Ouédraogo OG (1996). Plantes medicinales et pratiques medicinales traditionnelles au Burkina Faso: cas du plateau central. Theses de doctorate s-sciences. Universite de Ouagadougou, tomes, 3: 508-515.
- Nok AJ, Esievo KAN, Auda A, Isaac AI, Emmanuel GC, Solomon OM (1992). Trypanocidal activity of an Organotin Compound (Tri-*n*-Butyltin Oxide) toward *Trypanosoma brucei*. J. Clin. Biochem. Nutr., 13: 81-85.
- Nok AJ (2002). Azaanthraquinone inhibits respiration and *In vitro* growth of long and slender blood stream forms of *Trypanosoma congolense*. Cell Biochem. Function, 20: 205-212.
- Nok AJ (2005). Effective measures for controlling trypanosomiasis. Expert Opinion. Pharmather. 6(10): 1-9.
- Obihiro (1998). Proceedings of RCMPI-Obihiro/OIE Paris International Symposium on Strategies for Research and Control of Surra *Trypanosoma evansi* infection. J. Protozool. Res., 8: 1-15.
- Ogbadoyi EO, Abdulganiyu AO, Adama TZ, Okogun JJ (2007). *In vivo* Trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *T. brucei brucei*. J. Ethnopharm., 112: 85-89.
- Pacholek X, Gamatic D, Franke SG, Tibayrene R (2001). Prevalence of *Trypanosoma evansi* trypanosomosis in young camels in West Niger. Revue. Elev. Med. Vet. Pays. Trop., 44: 177-182.
- Raghavendra MP, Satish S, Raveesha KA (2006). Phytochemical analysis and antibacterial activity of *Oxalis corniculata*, a known medicinal plant. J. Sci., 1(1): 72-78.
- Sofowora A (1993). Medicinal plants and Traditional Medicines in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.
- Sepulveda-Boza S, Cassels BK (1996). Plant metabolite active against *Trypanosoma cruzi*. Planta Med., 62: 98-105.
- Tarus PK, Machochi AK, Lanmgat-Thoruwa CC, Chabra SC (2002). Flavonoids from *Tephrosia aequilata*. Phytochemistry, 60: 375-379.
- Trease GE, Evans WC (1989). Pharmacognosy. 11th Edition, Bailliere Tindall Can. Macmillan Publishers. Tropical disease Research 7th Programme Report (1984). Chemotherapy of African trypanosomiasis. United Nations Development Programme/World Bank/WHO, Geneva. pp. 3-19.
- Tyler VE (1999). Phytomedicines: back to the future. J. Nat. Prod., 62: 1589-1592.
- Welburn SC, Coleman PG, Fevre E, Mandlin I (2001). Sleeping sickness-a tale of two diseases. Trends Parasitol., 17: 19-24.
- WHO (1975). Tropical Diseases Today: The Challenges and opportunities, World Health Organisation, Geneva, Switzerland.
- Wurochekke AU, Nok AJ (2004). *In vitro* antitypanosomal activity of some medicinal plants used in the treatment of trypanosomosis in Northern Nigeria. Afr. J. Biotechnol., 3(9): 481-483.