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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 Km^2 (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 Adebiyi, 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 ethnomedically 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 a 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 x 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
K. senegalensis (water extract)	Leaf	++	+++	+++	++	++	+	++	+	-
	Stem-bark	+	++	+	++	++	+	+	++	+++
	Root	+++	++	+++	+++	++	+	+	+	-
K. senegalensis (methanolic extract)	Leaf	+++	+++	+++	++	++	+	++	-	-
	Stem-bark	++	+++	++	+++	+	+	+++	+++	++
	Root	++	++	+++	++	++	+	++	+	-

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

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

	5 mg/ml			10 mg/ml			20 mg/ml			Control	
Time (min)	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 tryanothione reductase that is very sensitive to alteration in redox balance. Phyto-chemicals 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-trypanosome activity (Tarus et al., 2002). While Nok et al. (1992) reported Tri-*n*-Butyltin Oxide to have activity against *T. brucei* and azaanthra-quinone 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	Parasites still alive
30	-	-	-	-	-	-	++	-	+	than 1 min	after 2 h
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 LD_{50} and ED_{50}.

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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