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Full Length Research Paper

Phytochemical screening and evaluation of some medicinal plants for their *in vitro* activities on *Trypanosoma evansi*

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In an attempt to search for new eco-friendly trypanocidal drugs, water and methanol extracts were prepared from three medicinal plants used by herbalists in Nigeria for the treatment of malaria and other ailments. The different portions of the extracts were incubated at various concentrations, 2, 4, 8, 10 mg/ml with *Trypanosoma evansi*. The results revealed that *Khaya senegalensis* and *Anonna senegalensis* were able to immobilize the parasites at 10 mg/ml while *Prosopis africana* did not show any activity. Phytochemical profile of the plants showed the presence of alkaloids, flavonoids, tannins, saponnins and cardiac glycosides. The results obtained with these crude extracts showed that these plants are potential sources of trypanocidal drugs/chemical leads.

Key words: Antitrypanosomal activity, *Khaya senegalensis, Anonna senegalensis, Prosopis africana, Trypanosoma evansi*.

INTRODUCTION

Trypanosomiasis continues to cause morbidity and mortality on a large scale in Africa. *Trypanosoma evansi* is a species belonging to the subgroup Trypanozoon and is the causative agent of camel trypanosomiasis. Trypanosomasis due to *T. evansi* affects many species of domestic and wild animals (Franke et al., 1994). Surra is wide spread in different parts of the world and poses a major constraint to camel productivity (Elamin et al., 1999). Prevalence has been reported in Nigeria, Chad, Mauritania, Niger (Losos, 1980). Infection by *T. evansi* is characterised by marked elevation of fever, anaemia, marked depression, dullness, loss of condition and often death (Rami et al., 2003). Camel productivity is very important in arid areas, they serve as source of meat, milk, transport and draught power (Elamin et al., 1999). The widespread occurrence of *T. evansi* is largely due to its mechanical spread by the bites of haematophagous flies e.g. *Tabanus* (Luckins, 1998). Plants have been reported to be the basis of traditional treatment for various types of ailments (Adewumi et al., 2001; Tagboto and Townson, 2001; Aderbauer et al., 2008).

For the present investigation the *in vitro* activity of three medicinal plants which has been reported in literature to have antitrypanosomal activity was evaluated using *T. evansi*.

MATERIALS AND METHODS

Plant materials/collection

The plants screened were obtained from Nasarawa, Kogi and the Federal Capital Territory, Abuja Nigeria in May 2006. The plants were taken to the herbarium unit of Biological Science Department, Ahmadu Bello University, Zaria, Nigeria where they were identified and given voucher numbers (*Khaya senegalensis* v/no 900181, *Annona senegalensis* v/no 190 and *Prosopis africana* v/no 6908) .

Plant extraction

The plant parts were dried at room temperature for two weeks and pulverized in to powder using an electric blender (Kenwood®). Extracts 100 g of the powdered plant parts were macerated in 10 times the amount of distilled water for 24 h at room temperature, other portions were Soxhlet extracted in methanol. The filterate obtained was concentrated on a water bath set at 35°C. The solvent free extracts were stored at 4°C till needed.

Parasite

T. evansi was obtained from the Faculty of Veterinary Medicine,

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Department of Veterianary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria. The parasite were inoculated in to 3 rats. The animals were then 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 parasitemia using the Herbert and Lumsden (1976) method.

Trypanocidal drug

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

In vitro screening of plants

Two (2%) of each of the crude extract was prepared and serially diluted to give 10, 8, 4 and 2 mg/ml using 0.5% Dimethylsulfoxide (DMSO). Aliquots of 10 µl of the extract were incubated with 40 µl of parasitized blood (harvested at peak parasitemia in rats) in 96 well microtiter plates at different concentrations at 37°C. The control consisted of 10 µl of 0.5% Dimethylsulfoxide (DMSO) incubated with the parasitized blood, and the blood was examined at 5 min interval using an olympus microscope (x40) objective. Assay with commercial drug (Samorenil® Animal Care) at a concentration of 200 µg were also performed to have a reference value. The inhibitory concentration, IC was the concentration at which no motile cells were seen moving in comparison to the control cultures.

Phytochemical screening

Standard protocols to identify the constituents as described by Sofowora (1993), Trease and Evans (1989) and Harbone (1973) were carried out.

RESULTS AND DISCUSSION

Water and methanolic extracts from *P. africana, K. senegalensis* and *A. senegalensis* were tested individually for their *In vitro* antitrypanosomal activity on *T. evansi*. The results revealed that water and methanolic extracts of *P. africana* had no effect on the parasites even at 10 mg/ml after 1 h of incubation (Table 1). *A. senegalensis* and *K. senegalensis* (water and methanolic extracts of leaf, stem bark and roots) both showed significant activity on the parasites by inhibiting their motility after 35 min of incubation at 10 mg/ml the activity was dose dependent, though *K. senegalensis* when compared to *A. senegalensis* extract showed more activity by immobilizing the parasites. Plants are known to contain a myriad of complex chemical compounds which could be beneficial health wise to humans and animals (Edeoga et al., 2005). Atawodi et al. (2003) reported that the complete elimination of motility or reduction in motility of parasites when compared to the control could be taken as indices of activity. Among the three plants that were screened for anti-trypanosomal activity against *T. evansi* only *K. senegalensis* and *A. senegalensis* showed activity on the parasites. Our results agrees with Igweh and Onabanjo, 1989; Freigburghaus et al. 1996; Adewumi et al. 2001; Atawodi et al. 2003; Hoet et al., 2004; Ogbadoyi et al. 2007, who reported the trypanocidal activity of some medicinal plants on trypanosomes *in vitro,* however the result is not consistent with Atawodi et al. (2003) who reported that *P. africana* exclusively eliminated motility in *T. congolensis* while *A. senegalensis* showed a slight effect on parasite motility. The differences could be attributable to variation in the phytochemical constituents of the plants, time of harvest of plants and geographical area. The phytochemical profile of the *P. africana, K. senegalensis* and *A. senegalensis* are shown in (Table 2). The results revealed that leaf, stem bark and root extracts had alkaloids, carbohydrates, tannins, saponins, flavonoids and terpenes all presents, however their presence differ in quantity (not shown). Though the extracts contain different types of phytochemicals (alka-loids, flavonoids, phenolics, cardiac glycosides), Hoet et al. (2004) reported that some flavonoids found in some medicinal plants showed trypanocidal activity, while alkaloids also have been similarly reported. Azaanthraquinone was reported (Nok, 2002) to have antitrypanosomal activity by potentially inhibiting its respiration, he suggest that target of azaanthraquinone mediated killing of the parasite was associated to the Co Q redox site. The mechanism by which the extracts immobilize the parasites needs further investigation. However, (Sepulveda and Cassels, 1996) suggest that many natural products exhibit their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. Atawodi et al. (2003) suggested that some agents act by binding with the kinetoplast DNA of the parasite. Further investtigation is ongoing as regards to identifying the phytochemical responsible for the action.

Conclusion

K. senegalensis, A. senegalensis and *P. africana* which are medicnal plants used for various ailments has been reported. The study has shown that water and methanolic extracts of *K. senegalensis* and *A. senegalensis* possess *in vitro* trypanocidal effects on *T. evansi,* which has never been reported. However, the activity was dose dependent, the results are promising. Further work needs to be carried out to ascertain their *in vivo* activities. The study thus provide further evidence on the traditional usage of these plants in treating diseases.

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Table 1. Antitrypanosomal activity of the medicinal plants.

Plant name	Family	Parts screened	Solvent used for extraction	Portions of extract used	Concentration (mg/ml)	Activity on *T. evansi*	Time (min)
P. africana	Leguminosae mimosidae	Leaf, stem bark, roots	H_2O/ MeOH	Leaf	2	Negative	>60
				Stem bark	2	Negative	>60
				Root	2	Negative	>60
				Leaf	4	Negative	>60
				Stem bark	4	Negative	>60
				Root	4	Negative	>60
				Leaf	8	Negative	>60
				Stem bark	8	Negative	>60
				Root	8	Negative	>60
				Leaf	10	Negative	>60
				Stem bark	10	Negative	>60
				Root	10	Negative	>60
K. senegalensis	Meliaceae	Leaf, Stem bark, roots	H_2O/MeOH	Leaf	2	Negative	>60
				Stem bark	2	Negative	>60
				Root	2	Negative	>60
				Leaf	4	Positive	>60
				Stem bark	4	Positive	>60
				Root	4	Positive	>60
				Leaf	8	Positive	>57
				Stem bark	8	Positive	>57
				Root	8	Positive	>57
				Leaf	10	Positive	35
				Stem bark	10	Positive	30
				Root	10	Positive	39
A. senegalensis	Annonaceae	Leaf, stem bark, roots	H_2O/MeOH	Leaf	2	Negative	>60
				Stem bark	2	Negative	>60
				Root	2	Negative	>60
				Leaf	4	Positive	>60
				Stem bark	4	Positive	>60
				Root	4	Positive	>60
				Leaf	8	Positive	>55
				Stem bark	8	Positive	>55
				Root	8	Positive	>55
				Leaf	10	Positive	40
				Stem bark	10	Positive	45
				Root	10	Positive	50
		Samorenil® (Animal Care)	-	-	200 µg	Negative	25 min
		Control (DMSO)	-	-		-	Parasites very active even after 3h

Table 2. Phytochemical screening of the medicinal plants.

Plant	Portion of extract	Alkaloids	Carbohydrates	Tannin	Saponin	Flavonoids	Terpenes	Sugars	Cardiac glycosides	Phlobatannins
P. africana	Leaf	+	++	++	+	+	+	+	+	-
	Stem bark	+	+	++	++	+	+	+	+	-
	Root	+	+	++	+	+	+	+	+	-
A. senegalensis	Leaf	+	+	+	+	+	+	+	+	-
	Stem bark	+	+	+	+	+	-	+	+	-
	Root	+	+	+	++	+	+	+	+	-
K. senegalensis	Leaf	++	+++	+++	++	++	+	++	+	-
	Stem bark	+	++	+	++	++	+	+	++	+++
	Root	+++	++	+++	+++	++	+	+	+	-

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

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