African Journal of Biotechnology Vol. 8 (10), pp. 2047-2049, 18 May, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Short Communication

In vitro trypanocidal effect of methanolic extract of *Sclerocarya birrea, Commiphora kerstingii* and *Khaya senegalensis*

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Accepted 16 February, 2009

The anti-trypanosomal activity of methanolic extract of *Sclerocarya birrea*, *Commiphora kerstingii* and *Khaya senegalensis* were evaluated against *Trypanosoma brucei brucei in vitro* at concentrations of 2 and 4 mg/ml. Susceptibility of the organism was determined in culture medium containing 5% dextrose and 0.9% saline solution alone as control and 2 and 4 mg/ml of these plants extracts in the same solution. Complete mortality of the organism was observed at almost all the concentrations within 30 min; the organism however survived for almost 3 h in the control test tube. The result suggests that *S. birrea*, *C. kerstingii* and *K. sengalensis* extracts may possess some trypanocidal principles which may require further elucidation.

Key words: Trypanosomosis, trypanosome, medicinal plant.

INTRODUCTION

African trypanosomosis is a wasting disease of animals and man. It is caused by haemoprotozoan *Trypanosoma* species. In Africa, the most important *Trypanosoma* species are transmitted by the tsetse fly of the genus *Glosina* (Ooijen, 1993). It occurs across more than a third of Africa, and almost all animal species, except poultry, are affected. Approximately 20% of Africa's 173 million cattle are at risk of infection (Adeniji, 1993). In addition, 36 out 52 African countries are endemic for sleeping sickness, with 55 million people at risk of contracting the infection (Cattand, 1995). The search for vaccination against African trypanosomosis remains elusive and effective treatment is beset with problems of drug resistance and toxicity (Onyeyili and Egwu, 1995; Gutteridge, 1985; Aldhous, 1994).

African trypanosomosis is one of the tropical diseases in which new and better drugs are needed. The current methods of controlling the disease include the use of trypanotolerant cattle, vector control and drug therapy. Four drugs (suramin, pentamidine, melarsoprol and eflornithine) are currently available to treat trypanosomosis (Kuzoe, 1993), with only melarsoprol and eflornithine being effective against the meningoencephalitis that develops in the late stages of the disease. In addition to emergency cases of drug resistance, all four drugs require lengthy, paranteral administration and all but eflornithine have severe toxic side effects (Onyeyili and Egwu, 1995; Gutterridge, 1985) thus, making the search for the development of more effective and safer trypanocidal agents a necessity.

Plants have always been among the common sources of medicaments. In Africa, traditional medicine in the form of herbal treatment has a long tradition and still holds a strong position in medical and veterinary care (Felerman, 1981). Several reports on the evaluation of different chemicals/drugs for trypanocidal activity have appeared (Bodley et al., 1995) just as interesting reports on the antitrypanosomal effects of plant extracts and plant derivatives (Freiburghaus et al., 1996, 1997, 1998; Sepulveda–Boza et al., 1995; Nok et al., 1993; Asuzu and Chinerne, 1990; Atawodi et al., 2003; Mikail and Ajagbonna, 2007). This publication, present report on systematic *in vitro* assessment of methanolic extracts of three plants namely *Sclerocarya birrea*, *Commiphora* kerstingii and *Khaya senegalensis* for their trypanocidal activity using *Trypanosoma brucei brucei* as test organism.

Table 1. *In vitro* trypanocidal efficacy of different concentrations of *S. birrea, C. kerstingii* and *K. senegalensis* against *T. brucei brucei*.

Different conc. of plant extracts	Survival of trypanosomes in minutes								
	0	30	60	90	120	150	180	210	240
S. birrea (leaves) 2 mg/ml	++++	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
S. birrea (leaves) 4 mg/ml	++++	+	-ve	-ve	-ve	-ve	-ve	-ve	-ve
S. birrea (stem bark) 2 mg/ml	++++	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
S. birrea (steam bark) 4 mg/ml	++++	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
K. senegalensis (stem bark) 2 mg/ml	++++	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
K. senegalensis (stem bark) 4 mg/ml	++++	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
C. kerstingii (stem bark) 2 mg/ml	++++	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
C. kerstingii (stem bark) 4 mg/ml	++++	+	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Control	++++	++++	+++	+++	++	++	+	-ve	-ve

++++ Strong parasite presence, ++ moderate parasite presence, +minimal parasite presence, -ve no parasite presence.

MATERIALS AND METHODS

Plant materials and extract preparation

Plants were collected from Zaria, Kaduna State, Nigeria. The Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria confirmed the identities of the plants. Stem bark of all the three plants were harvested. The leaves of *S. birrea* were also harvested in addition to its stem bark. The harvested plant parts were dried in open air in the laboratory (to avoid heat destruction of the active components). Dried materials were pounded in laboratory mortar into small particles. Fifty grams (50 g) of the pounded dried plants materials were weighed and extracted by maceration for 72 h in 100% methanol. The methanolic extracts were filtered and evaporated to dryness *in vacuo* and stored in capped bottles inside the refrigerator at 4°C until required.

Trypanosome stock

T. brucei brucei obtained from protozoalogy Department of Faculty of Veterinary Medicine Ahmadu Bello University Zaria was used for this study. The organisms were maintained by serial passages in rats.

In vitro anti trypanosomal activity

Solutions of 4 and 2 mg/ml were prepared in 5% dextrose and 0.9% saline solution from the different plant extracts, 2 ml each of the solutions was pipette into different glass test tubes. All the crude extracts were freshly prepared, control glass test tube without plant extract was included.

Anti-coagulated blood was collected from the infected rats. Serial dilutions of infected rat blood were made using phosphate glucose buffered saline solution. 0.5 ml of the blood was added to each of the glass test tubes. The parasitic load of the diluted blood was estimated to be 5 x 10^5 parasites/ml (Murray et al., 1983). The glass test tubes were closed with the aid of rubber stoppers. The solution was allowed to stand at room temperature (25°C) for about 3 - 5 h. During this period, the motility or lack of motility of the parasites in the solution was checked at 30 min intervals using light microscopy (x 40 objective lens), about 2 µl of test mixtures were placed on separate microscope slides and covered with cover slips and the parasites observed.

RESULTS

Methanolic extracts from three plants harvested from Zaria, Kaduna State, Nigeria were analyzed for their *in vitro* trypanocidal activity against *T. brucei brucei* at effective concentrations of 4 and 2 mg/ml complete elimination of motility of parasites when compared to control were taken as indices of trypanocidal effects.

Three plants, namely, *S. birrea*, *C. kestingii* and *K. Senegalerisis* caused complete cessation of motility of *T. b. brucei* within 30 min, though minimal parasite motility were observed in *S. birrea* leaves extract and *C. kerstingii* stem bark extracts at concentration of 4 mg/ml which was completely absent within 60 min (Table 1). The organism however survived for almost 3 h in the control glass test tube without the plant extract.

The plants, at different concentrations used in this study showed considerable trypanocidal activity. This finding is in line with earlier reports (Freiburghaus et al., 1996, 1997, 1998; Nok et al., 1993; Asuzu and Chineme, 1990; Atawodi et al., 2003; Mikail and Ajagbonna, 2007) that clearly indicated that plants of different families could possess potent trypanocidal activity. In fact, natural products with trypanocidal activity and belonging to a variety of phytochemical classes have been identified (Hopp et al., 1976; Oliver–Bever, 1986; Sepulveda-Boza and Cassels, 1996).

This investigation did not involve structure elucidation. It may be difficult to extrapolate this *in vitro* result to mean efficacy *in vivo* because discrepancies between *in vitro* and *in vivo* correlations due to metabolic processes which occur in multicellular organisms are well known (Fans worth and Moris, 1976). Nevertheless, and for practical purposes bioactive screening *in vitro* remains a useful method for preselection of plant for anti-trypanosomal activity (Freiburghaus et al., 1996). Therefore, plants found to be active in this report must be tasted *in vivo* before a definite statement can be made on their trypanocidal potentials.

Also previous workers (Freiburghaus et al., 1997) have shown that the mean MIC value of common trypanocidal drugs is 10.7 mg/ml and that agent with MIC value between 5 – 20 mg/ml could be regarded as very active. In this study, *S. birrea*, *C. kerstingii* and *K. senegalensis* were found to be active at 2 and 4 mg/ml, this is comparable to the value reported for standard trypanocidal drug.

It is difficult to speculate the mechanism by which these extracts exhibit their trypanocidal action. However, accumulated evidence (Supulveda–Boza and Cassels, 1996) suggest 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 trypanothione reductase that is very sensitive to alterations in redox balance. It is also known that some agents act by binding with the kinetoplast DNA of the parasite.

The trypanocidal principles of the plants tested in this study is unknown, until further studies are carried out.

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