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Full Length Research Paper

Ex vivo trypanostatic effect of stem-bark extracts of *Securidaca longipedunculata* (Fres. Holl) against *Trypanosoma brucei brucei*

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Current treatment of trypanosomosis in sub-Saharan Africa is associated with widespread inefficiency. There is therefore the need to find more effective drugs against the disease from promising traditional medicinal herbs. This work is aimed at evaluating *ex vivo* anti-trypanosomal effect of stem-bark extracts of *S. longipedunculata* against *T. brucei brucei*. One hundred microlitre of crude methanol, ethyl acetate and aqueous methanol extracts of *S. longipedunculata* at concentrations of 3 and 6 mg/ml each were mixed with 50 µl of blood containing 8.1 × 10^6 trypanosomes and incubated at 37°C for 90 min. Similarly, diminazene aceturate (10 µg/ml), physiological saline solution (50 µl) and blood (100 µl) containing trypanosomes only served as treated, negative and untreated controls, respectively. Motility of the parasite was monitored under light microscope (×400) at 5 min interval throughout the 90 min observation period. All experiments were done in duplicate. The mixtures were subsequently inoculated into rats that were not previously infected with trypanosomes. Phytochemical screening of the extracts revealed the presence of carbohydrates, cardiac glycosides, saponins, steroid, triterpenes, flavonoids and tannins. However, aqueous and ethyl acetate fractions were devoid of flavonoids. The crude methanol immobilized the parasites within 75 min, while ethyl acetate and aqueous extracts induced slight reduction in motility of the parasite at 90 min of incubation. However, inoculated rats developed infection and succumbed to the infection. It is concluded that the stem-bark of the plant possesses trypanostatic, but not trypanocidal, activity against the parasite.

Key words: Antitrypanosomal, drug incubation infectivity test, *in vitro*, phytochemical screening, stem-bark.

INTRODUCTION

African trypanosomosis is a disease of humans and livestock caused by several species of flagellated single-celled protozoan parasites belonging to the genus *Trypanosoma* (Kuriasko et al., 2012). Trypanosomosis is

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an important constraint to livestock development in sub-Saharan Africa with estimated direct annual economic loss exceeding US$1 billion (Simukoko et al., 2007). In animals, trypanosomosis is characterized by pyrexia, anaemia, loss of condition, reduced productivity, cachexia and mortality (Losos and Ikede, 1972; Anosa, 1983; Murray et al., 1983; Nwosu and Ikeme, 1992; Igbokwe, 1994). The disease is ranked among the top 10 global cattle diseases affecting livestock production in sub-Saharan Africa (Perry et al., 2002). The scarcity of modern effective drugs for the treatment and management of trypanosomosis, combined with their high cost have created a growing public interest in alternative natural drugs from botanicals (Etet and Mahomoodally, 2012).

In countries where trypanosomosis is endemic, plants have been used traditionally for centuries and are still widely used to treat this illness and other parasitic diseases (Tagboto and Townson, 2001). *Securidaca longipedunculata* (Polygalaceae) is a small tree of up to 6 to 9 m high with a pale grey, smooth bark and oblong, more less hairless alternate leaves of varying size and shape and crowded towards the stem tips (Van Wyk et al., 2009). Root, stem-bark, leaves of the plant and whole plant are used for medicinal purposes by local folk (Nadembega et al., 2011; Wanzala et al., 2012; Borokini et al., 2013; Mustapha, 2013). The plant is widely used in African traditional medicine as a general remedy for cough, malaria, backache, venereal disease, snakebite, erectile dysfunction and tuberculosis (Watt and Breyer-Brandwijik, 1962; Atawodi et al., 2002; Ajali and Chukwurah, 2004; Adebiyi et al., 2006; Marion Meyer et al., 2008). *S. longipedunculata* is known as violet tree, fibre tree or Rhodesian violet in English, and popularly known as *Uwar magunguna* (mother of all medicine) in Hausa speaking communities of Northern Nigeria (Ajali and Chukwurah, 2004; Adebiyi et al., 2006; Orwa et al., 2009). The aim of this study is to determine the anti-trypanosomal effect of the stem-bark extracts of *S. longipedunculata* against *T. brucei brucei*.

MATERIALS AND METHODS

Plant collection and identification

Fresh stem-bark of *S. longipedunculata* was collected in Zaria, Nigeria. The plant was identified in the Herbarium, Department of Biological Science, Ahmadu Bello University (A.B.U.), Zaria, Nigeria where a voucher number specimen of 900213 was allotted. The identified stem-bark was dried in an open air in the laboratory and the dried sample was kept in polythene bags until required for preparation of the extract.

Plant extraction, concentration and fractionation

Seven hundred and ninety grams of the dried powder of the plant was extracted with absolute methanol in a soxhlet extractor. The liquid extract was concentrated to dryness over a water-bath.

Seventy-three grams of the crude methanol extract was dissolved in 300 ml of distilled water and the solution was transferred to 1 L separating funnel. The mixture was partitioned twice its volume with ethyl acetate. The lower denser aqueous fraction was collected into a separate conical flask and upper portion (ethyl acetate fraction) was dispensed into a clean conical flask. The aqueous portion of the extract was further partitioned with ethyl acetate and similar fractions were combined. The fractions were concentrated to dryness over a water-bath.

Phytochemical screening

The extract and fractions of *S. longipedunculata* were evaluated for the presence of carbohydrates, anthraquinones, flavonoids, tannins, alkaloid, saponins, cardiac glycosides, steroids and triterpenes using standard procedures (Trease and Evans, 1983).

Experimental animals

Twenty Wistar rats of both sexes weighing between 170 and 190 g were obtained from the Animal House, Department of Physiology, Faculty of Medicine, A.B.U., Zaria, and were allowed to acclimatize for two weeks in the laboratory at the Department of Veterinary Pharmacology and Toxicology, A.B.U., Zaria. They were housed in clean plastic cages with wood shavings as beddings. The beddings were changed twice in a week. The rats were fed on standard rat feed and given access to clean water *ad libitum*. The animal experiment was approved by A.B.U., Zaria Animal Welfare Committee (Vet-Med/15632/10-11).

Test organism

Trypanosoma brucei brucei was obtained from the Department of Veterinary Parasitology and Entomology, A.B.U., Zaria. The parasite was maintained in rats by continuous passage. Each cycle of passage was done when parasitaemia was in the range of 35 to 40 parasites per field, which corresponded to an interval of 6 days post-infection. For several passages, about 3 ml of blood was obtained from an infected rat by cardiac puncture after light chloroform anaesthesia into 5 ml syringe and emptied into a vial containing 9 ml of physiological saline solution (PSS). About 1×10^6 trypanosomes contained in 0.2 ml was used to infect a trypanosome-free rat by i.p route (Figure 1).

In vitro screening

In vitro antitrypanosomal screening test was done in duplicate using the rapid matching method (Herbert and Lumsden, 1976) and the drug incubation infectivity test (Vincent et al., 2008). Briefly in the rapid matching method, 100 µl of blood containing 8.1×10^6 parasites was individually mixed with 50 µl of 3 and 6 mg/kg solutions of the crude methanol extract, ethyl acetate and aqueous methanol fractions in a 48-microtitre plate. The mixture was rocked gently and then incubated at 37°C for 5 min. Similarly, 100 µl of blood containing 8.1×10^6 trypanosome was mixed with diminazene aceturate 10 µg/ml and PSS (50 µl); and blood (100 µl) containing trypanosome only served as treated, negative and untreated controls, respectively. At the end of the incubation period, 2 µl of individual test mixtures were separately observed under a light microscope at ×400 magnification. The parasites were observed every 5 min for a total of 90 min. A reduction or complete cessation of motility of parasites in the treated blood samples when compared to that of untreated control was taken as a measure of trypanocidal activity.

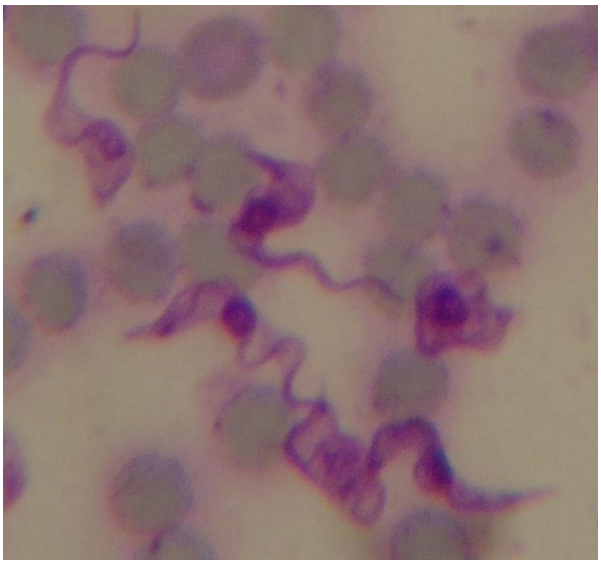

Figure 1. Photomicrograph of thin blood smear of *T. brucei brucei* used for the study ×100.

Drug incubation infectivity test (DIIT)

About 0.2 ml of the concentration of extract that was observed to have immobilized the parasites was immediately taken into a clean 1 ml syringe and inoculated into 2 rats each not previously infected with trypanosome. Those extracts and concentrations that neither reduced the motility of nor immobilized the parasites at the end of 90 min observation were also inoculated i.p into 2 rats each not previously exposed to trypanosomal infection. The rats were observed daily for development of parasitaemia for 3 weeks.

Determination of parasitaemia in experimental rats

Parasitaemia was monitored in blood obtained from the tail of infected rats. The number of parasites per ml of blood was determined microscopically at ×400 magnification using the "Rapid Matching" method of Herbert and Lumsden (1976).

Data analysis

Data for pre-patent periods were expressed as mean (±SEM) and were subjected to analysis of variance followed by Tukey post hoc using GraphPad Prism version 4. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Plant yield

Nine hundred and eighty-six gram (986 g) of the pulverized stem-bark of *S. longipedunculata* (SL) gave 109.49 g (13.9%) of crude methanol extract. Upon partitioning with different solvents, the crude methanol yielded 4.83 g (6.7%) and 18.44 g (25.4%) of ethyl acetate and aqueous methanol fractions, respectively.

Phytochemical screening

Preliminary phytochemical screening of the extract and fractions revealed the presence of carbohydrates, cardiac glycosides, saponons, steroids, triterpenes, flavonoids and tannins. However, the ethyl acetate and aqueous methanol fractions did not contain flavonoids.

In vitro study

Complete cessation of motility was observed in trypanosomes that were treated with crude methanol extract of SL at concentrations of 3 and 6 mg/ml at 75 min of observation (Table 1). The ethyl acetate and aqueous methanol fractions at concentrations of 3 and 6 mg/ml slightly reduced parasites motility at 90 min. However, trypanosomes that were treated with diminazene aceturate (10 µg/ml) were immobilised within 15 min of treatment.

Drug incubation infectivity test

The prepatent period of rats in the crude methanol treated groups was 8 days compared to 6 days recorded in the physiological saline solution and untreated groups. The difference was not statistically significant. Furthermore, rats treated with the ethyl acetate and aqueous methanol fractions were also patent on day 6 post-infection. However, the diminazene aceturate treated rats remained aparasitaemic for 21 days. The differences in the time course of the disease between the crude methanol extract, fractions, physiological saline solution and untreated groups were not statistically significant

DISCUSSION

Animal trypanosomosis remains one of the most important haemoparasitic diseases bedeviling sub-Saharan African. It is a constraint to improved livestock production in the region. *In vitro* evaluation of plant extract for antitrypanosomal effect is a rapid and cheap test for evaluating the efficacy of plant against trypanosomes. Some plants had been reported to exhibit *in vitro* trypanocidal effect measured by cessation of motility of the parasites (Habila et al., 2011) and minimum inhibitory concentration (Freiburghaus et al., 1996; Aderbauer et al., 2008), while some were reported to exhibit trypanostatic effect measured by reduction in motility of the parasites (Atawodi, 2005). The result of this study showed that crude methanol extract of *S. longipedunculata* was able to immobilize the parasite completely within 75 min observation period. Furthermore, the ethyl acetate and aqueous methanol showed slightly reduced parasite motility. Since

Table 1. *Ex vivo* effect of extract and fractions of *S. longipedunculata* on the motility of *T.rypanosoma brucei brucei.*

Treatment	Concentrations (mg/ml)	Immobilization of parasite motility (min)
Crude methanol extract	3	75
	6	75
Ethyl acetate fraction	3	90*
	6	90*
Aqueous Methanol fraction	3	90*
	6	90*
Diminazene aceturate	0.01	15
Physiological saline solution	50 µl	No effect

*= Slight reduction in motility.

Table 2. Effect of extracts of *S. longipedunculata* on pre-patent period of parasite under drug incubation infectivity test.

Treatment	Concentrations (mg/ml)	Pre-patent period (Days)
Crude methanol extract	3	8
	6	8
Ethyl acetate fraction	3	6
	6	6
Aqueous Methanol fraction	3	6
	6	6
Physiological saline solution	50 µl	6

immobilization of parasite motility by extract is used as trypanocidal effect, the stem-bark of *S. longipedunculata* could be said to be trypanocidal in effect.

In the drug incubation infectivity test (DIIT) study, parasites that were immobilized by extract of *S. longipedunculata* were shown to be infective when inoculated into rats. It follows that DIIT can be used to evaluate or confirm the effect of a plant extract for anti-trypanosomal effect. In addition, the course of the disease in the animals was only slightly longer than that of the animals from *in vivo* study, indicating that the virulence of the pathogen was not affected. This is very important because hitherto cessation of parasite motility has been taken as trypanocidal effect (Freiburghaus et al., 1996; Aderbauer et al., 2008; Habila et al., 2011) while reduction in trypanosome motility is taken as trypanostatic (Atawodi, 2005). These conclusions should be made with caution when only *in vitro* test is used. It may be inferred that under *in vitro* conditions, there is initial stage of reversible parasite paralysis before clinical death of the parasite. It is possible that cessation of parasite motility in this study was at the point of paralysis of the parasite.

The trypanocidal effect observed in this study could be attributable to one or combination of secondary metabolites detected in the present study. Furthermore, flavonoids could be said to be the primary secondary metabolite in the stem-bark of *S. longipedunculata* with trypanocidal effect since only crude methanol extract (which contained flavonoids) was able to immobilize the parasite completely. Flavonoids found in higher plants have been reported to possess antimicrobial and antitrypanosomal activities (Cordell et al., 2001). Kobo et al. (2014) reported protective effect of flavonoids on erythrocytes of rats infected with *T. brucei brucei*. Since ethyl acetate and aqueous methanol extracts devoid of flavonoids exhibited slightly reduced motility, flavonoid could be said to be responsible for trypanocidal activity in this study.

Moreover, mild antitrypanosomal effect shown by ethyl acetate and aqueous methanol fractions of *S. longipedunculata* revealed that other bioactive metabolites detected in the extracts also possessed some degree of activity against *T. brucei brucei*. Trypanocidal activity of leaf extract of *Annona senegalensis* has been attributed to the presence of saponins and tannins (Ogbadoyi et al., 2007). Also, Atawodi et al. (2011) speculated that the antitrypanosomal activity of *Boswellia dalzielii* could be due to one or combination of flavonoids, saponins, alkaloids, tannins, resins, steroids and triterpens detected.

Therefore, antitrypanosomal potential observed in the present study could be due to one or combined effect of flavonoids, tannins, saponins, triterpens and or steroids detected. This finding further underpins the fact that secondary metabolites work synergistically to produce an effect. Crude extracts exhibited better bioactivity than individual component (Cock, 2011). Mahomoodally et al. (2010) observed that bioactive metabolites in plant extract worked catalytically and synergistically to produce an effect that surpassed the total activity of the individual constituents.

Since completely immobilized parasite in crude methanol extract of *S. longipedunculata* in the *in vitro* test became infective following inoculation into rats, it can be concluded that stem-bark of *S. longipedunculata* is trypanostatic, but not trypanocidal in action. Elucidating the mechanism of trypanostatic effect of plant extract can facilitate screening of medicinal plants for their trypanocidal potential.

Conflict of Interests

The authors have not declared any conflict of interests.

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