**Original Article**

**TJPS**
**The Thai Journal of Pharmaceutical Sciences**
**39 (4), October-December 2015: 156-160**

# Evaluation of Methanol Root Extract of *Securidaca longipedunculata* for Antitrypanosomal Activity *in vitro* and *in vivo*

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**Abstract**

*Securidaca longipedunculata* is a medicinal plant used in different parts of Africa for treatment of various ailments including sleeping sickness in Northern Nigeria. On this basis, the methanol root extract of *S. longipedunculata* was investigated for antitrypanosomal effect to determine a possible rationale for this purpose. Acute toxicity was investigated orally and intraperitoneally (IP) to determine the $LD_{50}$. Trypanocidal activity was evaluated *in vitro* and *in vivo*. The extract was given orally for nine days at the doses of 200, 300 and 400 mg/kg. Then, the effects on haematology, Alanine Aminotransferase (ALT), Aspartate aminotransferase (AST) and weight gain were investigated. The minimum inhibitory concentrations (MICs) of the extract and diminazene aceturate (DA), a standard trypanocidal drug, were 5 µg/ml and 2.1 µg/ml, respectively. For *In vivo* study, the extract was well tolerated orally up to 5000 mg/kg, therefore oral $LD_{50}$ was not determined. Meanwhile, the IP $LD_{50}$ was 3.3 mg/kg. The extract significantly decreased parasitaemia at the dose of 400 mg/kg for six days of post-treatment. There was also significant decrease in parasitaemia on day 9 post-treatment in all the extract treated groups. However, parasitaemia cleared by day 3 post-treatment in the DA-treated group. The extract at all doses significantly increased packed cell volume (PCV) on day 7 and 14 post-infection. The extract also significantly improved weight gain at all doses used on day 7 and 14. The serum levels of ALT and AST were significantly decreased at the extract dose of 400 mg/kg. Our study suggests promising trypanocidal activity especially *in vitro*, giving some level of credence to the traditional use of the plant in Northern Nigeria for the treatment of sleeping sickness.

*Keywords: Securidaca longipedunculata*, Trypanosomosis, Diminazene, *In vitro, In vivo.*

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*Received: 29 April 2015*
*Revised: 11 September 2015*
*Accepted: 21 September 2015*

*Academic Editor: Wanchai De-Eknamkul*

## Introduction

Animal trypanosomosis is a disease caused by tsetse-fly-transmitted *Trypanosoma congolense, T. vivax, or T. brucei brucei*, or mixed infection by any of these trypanosome species [1, 2]. In humans, this is known as sleeping sickness. Animal trypanosomosis is most important in cattle but can cause serious losses in pigs, camels, goats, sheep and dogs. Infection of cattle by any of these trypanosomes results in acute, sub-acute or chronic disease characterized by intermittent fever,

anaemia, occasional diarrhoea, and rapid loss of condition and often terminates in death if not treated [2]. Since effective vaccine against trypanosomosis is not yet available, combating trypanosomosis depends on chemotherapy, the use of nets and fumigation of the environment with insecticides. The trypanocides currently employed are: homidium salts (Ethi-dium-Novidium); quinapyramine sulfate (Antrycide); diminazene aceturate (Berenil); isometamidium (Samorin-Trypamidium) and suramin sodium. Resistance to these trypanocides is wide spread leading to a high rate of relapse in treated animals. Furthermore, most of these agents have very narrow safety margin, therefore toxicity is a serious concern. It therefore becomes imperative to search for new remedies for treatment of trypanosomosis to augment the already existing ones.

Herbal remedies have been used over the years for treatment of human and animal trypanosomosis in Nigeria and many other parts of Africa hence the evaluation of effectiveness and safety of their use. *Securidaca longipedunculata* (violet tree) is one of such plants. Different medicinal uses have been attributed to this plant. The roots of the tree can be used for treatment of human ailments such as coughs, chest complaints, toothaches, gout, fevers, constipation, diabetes and microbial infections. It also possesses anti-inflammatory properties that help to reduce arthritic pains [3]. The roots are also used for treatment of sleeping sickness in Northern Nigeria [4]. A combination of both the methanol extract and the methyl salicylate component from the roots of the plant create a poison that is used for multiple purposes. This poison is used on arrows to hunt games in West Africa [5].

On the background of various medicinal uses of this plant especially in the treatment of sleeping sickness, we therefore evaluated the safety and antitrypanosomal effect of methanol root extract of *Securidaca longipedunculata* (*Polygalaceae*) *in vitro* and *in vivo.*

## Materials and Methods

## Plant material

Fresh roots of S. *longipedunculata* were obtained from Nsukka local government area of Enugu state and were identified as *S. longipedunculata* by a plant taxonomist at the Biodiversity Conservation Program Centre (BDCP) Nsukka. They were chopped into smaller pieces using machetes and air-dried under shed at room temperature. The dried plant material was pulverized using hammer mill. About 300 g of pulverized plant material was extracted in 70 % methanol for 48 h and filtered using Whatmann no. 1 filter paper. The filtrate was concentrated to dryness using rotary evaporator and referred to as the extract. The extract was stored at 4˚C [6].

## Animals

Sixty five albino rats of both sexes were used in this study. They were housed in stainless steel cages. Food and water were provided *ad libitum* except where fasting was necessary. They were acclimatized for 7 days before the experiments. The animal experimental protocol was approved by the Experimental Animal Ethics Committee of the Faculty of Veterinary medicine, University of Nigeria, Nsukka and in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC).

## Trypanosomes

The *T. b. brucei* used in this study was originally isolated from a mongrel dog presented at the Veterinary Teaching Hospital University of Nigeria, Nsukka. Morphological identification was as described by Soulsby (1982) [7], Rickman and Robson (1970) [8]. These parasites were maintained in mice from which experimental rats were infected.

## Acute toxicity studies

The up and down method (OECD guideline, 2001) [9] was used to determine the $LD_{50}$ of the extract. Three groups of rats (A – C) of 5 rats per group were dosed orally at the doses of 1250, 2500 and 5000 mg/kg, respectively. They were monitored for 72 h for signs of acute toxicity, and then 14 days after which the experiment was terminated. For intraperitoneal acute toxicity, 4 groups of rats (D – G) of five rats per group were dosed with 3.9, 15.6, 62.5 and 125 mg/kg respectively. They were monitored for 72 h for signs of acute toxicity, then for 14 days after which the experiment was terminated.

## Assessment of antitrypanosomal activity

## In vitro antitrypanosomal assay

This was performed according to the method of Atawodi *et al*., (2003) [4] with some modifications. An extract concentration of 3.1 mg/ml was prepared and 100 µl of this was pipetted into a microtiter well. Serial double dilution of the extract was carried out to produce 7 different concentrations of the extract using phosphate saline glucose (PSG) as the nutrient medium for the trypanosomes. Same procedure was repeated for diminazene aceturate (DA) (Trypazene®, Pantex Holland). As control 50 µl of PSG was pipetted into another free well. Trypanosome-infected blood at 40 µl containing 2 x $10^5$ trypanosomes suspended in PSG was pipetted into each well containing the extract and PSG and the final concentrations of the extract in each well was determined. The set up was incubated for 1 h at 37˚C. For assessment of antitrypanosomal activity 5 µl of each sample was taken every 10 min interval and placed on a microscope slide covered with a cover slip and viewed under the microscope for trypanosome motility.

## In vivo antitrypanosomal assay

Thirty rats were grouped into 6 groups (A – F) of 5 each. Group A served as a standard control so was not infected with trypanosomes nor treated with any drug. Group B was intraperitoneally (IP) infected with trypanosomes but not treated with any drug. Group C was infected with trypanosomes and treated with a single dose

of 3.5 mg/kg DA, a standard trypanocide, while groups D – F were all infected with trypanosomes and treated with 200, 300 and 400 mg/kg of extract *per os* respectively for 9 days. One million trypanosomes suspended in 0.2 ml phosphate buffered saline (PBS) were used in infecting each rat.

After infection with trypanosomes, establishment of parasitaemia was monitored every other day using the wet mount method. Parasitaemia was confirmed on day 5 post-infection and treatment started the same day. Antitrypanosomal effect was evaluated by daily monitoring of parasitaemia using tail-tip blood and performing wet mount. Parasitaemia was quantified using the rapid matching method [10]. Changes in body weight were measured.

## Effect of the extract on haematological parameters

Haematological parameters like packed cell volume (PCV) (Microhaematocrit method) and total leukocyte count [11] were determined on day 0, 7 and 14 of the experiment.

## Effect of the extract on liver enzymes markers

Serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and serum alkaline phosphatase [12, 13] were determined on day 14 of the experiment using Randox$^{(R)}$ kits.

## Statistical analysis

Data obtained from parasitaemia, PCV, and liver enzyme markers were analysed using one way analysis of variance while data obtained from total leukocyte count was analysed using repeated measure ANOVA. Variant means were separated post-hoc using the least significant difference (LSD) method and significance was accepted at $P<0.05$.

## Plant extraction

The methanol extract of *S. longipedunculata* was yellowish-brown in colour, pasty in consistency and has a minty smell. It was also very soluble in water. Percentage yield of the extract was 15 % w/w.

## Acute toxicity

Rats tolerated the extract up to 5000 mg/kg orally; therefore $LD_{50}$ was not determined for oral administration (Table 1). However $LD_{50}$ of 3.3 mg/kg was determined for intraperitoneal route (Table 2). Rats showed signs of depression before death

## In vitro antitrypanosomal assay

The result of the *in vitro* antitrypanosomal effect of the extract is presented in table 3. It shows that the extract has a minimum inhibitory concentration (MIC) of 5 µg/ml. Concentrations below 5 µg/ml progressively weakened the trypanosomes but was not able to eliminate motility, but at 1µg/ml downwards the trypanosome organisms were very active. The *in vitro* trypanocidal effect of DA is presented in table 4 and shows that DA has an MIC of 2.1 µg/ml.

## In vivo antitrypanosomal assay

The *in vivo* antitrypanosomal effect of the extract as shown in figure 1 shows that the extract at 400mg/kg caused significant ($p<0.05$) reduction in parasitaemia on day 6 of treatment compared with the infected untreated control, but there was no significant difference within the extract groups. More so, on day 9 of treatment all the extract treated groups showed significant reduction in parasitaemia compared with the infected untreated group, but 400 mg/kg was significantly ($p<0.05$) lower than 300 mg/kg. There was total clearance of parasitaemia on day 3 post-treatment in the DA treated group.

## Effect of the extract on body weight of rats infected with T.b. brucei

The weight gain in the normal control was significantly ($p<0.05$) higher than all the infected groups on days 7 and 14.The infected untreated and all the extract-treated groups gained significantly ($p<0.05$) more weight than DA on day 7, but by day 14 DA and all the extract-treated groups have gained significantly ($p<0.05$) more weight than the infected untreated. Among all the infected groups the weight gain in the 200 mg/kg was significantly ($p<0.05$) higher on both days 7 and 14 (Figure 2).

## Effect of the extract on some haematological parameters of rats infected with *T.b. brucei*

There was no significant difference in the PCV of all the experimental groups on day 0. But by day seven, the PCV of the normal control was significantly ($p<0.05$) higher than all the infected groups, except the 400 mg/kg extract-treated group. Furthermore, the PCV of all the extract-treated groups were significantly ($p<0.05$) higher than the infected untreated group on day 7 and 14. There was no significant difference in the PCV of the DA group and the infected untreated group on day 7, but the PCV of the DA group was significantly higher on day 14. There was no significant difference in PCV among the extract-treated groups on days 0, 7 and 14 (Figure 3). The extract at all doses used did not significantly improve the total WBC count on both days 7 and 14 compared with infected untreated. However, the WBC count of the DA group was significantly ($p<0.05$) higher than all the other groups on day 14. (Figure 4).

## Effect of the extract on liver enzyme markers of Rats infected with T.b. brucei

There was no significant difference in the ALP among the groups. The serum levels of ALT and AST were significantly ($p<0.05$) lower in the normal control, DA and 400 mg/kg extract group than the infected untreated, 200 mg/kg and 300 mg/kg extract groups. There was no significant difference in AST between the normal control and the 400 mg/kg group, but the 400 mg/kg extract group was significantly ($p<0.05$) lower than DA and other extract-treated groups (Table 3).

## Effect of the extract on survivability of rats infected with *T b. brucei*

There was no mortality in the uninfected untreated group and the DA groups. All the infected untreated group members died by 16th day post-infection, the 200 and 300 mg/kg extract treated groups died by day 18 and 19 respectively. There was no mortality in the 400 mg/kg extract-treated group till day 30 when the experiment was terminated, though they were still parasitemic (Table 6).

## Discussion

The methanol root extract of *Securidaca longipedunculata* was well tolerated by rats when given orally up to 5000 mg/kg and this agrees with the work of Haruna *et al*., (2013) [14] who gave a maximum dose of 3000 mg/kg orally and reported that rats tolerated it, but disagrees with the report of Auwal *et al*., (2012) [15] who reported an oral $LD_{50}$ of 771 mg/kg with the aqueous root bark extract. So it is possible that the aqueous root bark extract is more toxic than the methanol root extract, possibly because the toxic principles are more concentrated in aqueous root bark extract than the methanol root extract. However this work went further to investigate the intraperitoneal acute toxicity and found that the extract was very toxic when administered intraperitoneally with $LD_{50}$ of 3.3 mg/kg. This sharp difference in toxicity when administered orally and intraperitoneally suggests that the extract contains potent toxic principles, but which when given orally either undergoes extensive biotransformation and detoxification in the liver through first-pass effect or was not well absorbed from the gastrointestinal tract, thereby drastically reducing its toxicity [16,17]. However, when administered intraperitoneally toxic principles are released directly into the systemic circulation thereby producing acute toxic effect.

The *in vitro* study showed that the extract contains potent trypanocidal principles with an MIC of 5µg/ml 20 min post-incubation and this is very comparable with the effect of DA which is a potent standard trypanocidal drug. This prompted more investigation *in vivo*. *In vivo* there was antitrypanosomal effect at all the doses of the extract used, but the effect was more at the highest dose of 400 mg/kg. The degree of antitrypanosomal effect *in vivo* did not correspond with the *in vitro* effect suggesting that there may be first-pass effect in the liver or very low absorption from the gastrointestinal tract after oral administration leading to extensive biotransformation, degradation and very low bioavailability of active antitrypanosomal principle [18]. Therefore it is suggested that parentheral route of administration may be the best route of administration of this extract. However toxicity concerns limited this suggestion because the $LD_{50}$ of 3.3 mg/kg could not be exceeded and doses below this were not trypanocidal when investigated. The antitrypanosomal effect of the extract may be attributed to some of its phytochemical constituents which include flavonoids, methylsalicilates, terpenoids, steroids and volatile oils [19, 20].

The effect of the extract on weight gain showed that the lowest dose of 200 mg/kg led to higher weight gain and this could be by the suppression of anorexia associated with trypanosomosis [21]. There was also significant improvement of packed cell volume in all the extract treated groups with the greatest effect noticed in the lowest dose of 200 mg/kg. This effect suggests that the extract could have erythrogenetic effect in *T brucei brucei* infected rats. However there was no significant improvement of total WBC in all the extract-treated rats and this could be attributed to the short period of the experiment. Trypanosomes are known to produce hepatocellular damages which manifests as increase in the level of some liver enzymes in the serum [22]. The extract at 400 mg/kg showed protective effect on the liver by decreasing significantly the serum levels of AST and ALT. The extract also enhanced the survivability of rats infected with *T brucei brucei* probably due to improved haematocrit, improved appetite and decreased parasitaemia and in the 400 mg/kg extract-treated group improvement of liver function. In conclusion, though the extract did not clear the parasitaemia at all doses used in this study, it ameliorated some of the parasite-induced changes that can lead to morbidity and mortality associated with trypanosomosis.

Our findings therefore showed that the methanol root extract of *S. longipedunculata* possesses excellent trypanocidal properties *in vitro* and some degree of antitrypanosomal effect *in vivo* thereby giving some level of credence to its traditional use in northern part of Nigeria for the treatment of sleeping sickness. Based on the findings of this study we recommend the following: that effort should be made to separate the toxic principle from the antitrypanosomal principle if they are different so as to allow pharmaceutical dosage form modification. That further work should be geared towards isolation and characterization of the antitrypanosomal principle..

## References

[1] Anosa VO. Haematological and biochemical changes in human and animal trypanosomosis. Part I. Rev Elev Med Vet Pays Trop 1988; 41: 65 - 78.

[2] Seifert HSH. Vector-borne diseases. *Trop Anim Hlth*. 2nd edition. Kluwer academic publishers London, 1996; 152 – 169.

[3] Ojewole, J. A. O. "Analgesic, Anti-inflammatory and Hypoglycaemic Effects of *Securidaca longipedunculata* (Fresen.) [Polygalaceae] Root-bark Aqueous Extract." Inflammopharmacology 16.(4) (2008): 174-181.

[4] Atawodi SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman M, Galadima M. *In vitro* trypanocidal effect of methanol extract of some Nigerian savannah plants. *Afr J Biotechnol* 2003; 2: 317 - 321.

[5]Ndou, Avhurengwi Phillemon. "*Securidaca longepedunculata*." *Securidaca* South African National Biodiversity Institute, Aug. 2006. Web. 20 Nov. 2013.

[6] Eke IG, Anaga AO. Evaluation of anti-diarrheic properties of the aqueous methanolic extract of *Palisota*

*hirsuta* leaves and its fractions using *in vivo* models. *Afri J Pharm Pharmacol* 2014;8: 327 – 332.

[7] Soulsby EJL. *Helminthes arthropods and protozoa of domesticated animals.* 7th ed. London: Bailliere Tindall; 1982.

[8] Rickman LR, Robson J. The blood incubation infectivity test: a simple test which may distinguish *Trypanosoma brucei* from *T. rhodesiense*. *Bull Wld Hlth Org* 1970; 42: 650 – 651.

[9] OECD. OECD Guidelines for the Testing of Chemicals, OECD 425. Acute Oral Toxicity-Modified Up and Down Procedure. OECD Paris. 2001

[10] Herbert WJ, Lumsden WH. *Trypanosoma brucei*: "a rapid matching method" for estimating the host's parasitemia. *Exp Parasitol* 1976; 40: 427 431.

[11] Schalm OW, Jain NC, Caroll EJ. *Veterinary haematology* 3rd edition. Hea and Febiger Philadelphia 1975; 1919 – 1925.

[12] Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Amer J Clin Path* 1957; 28: 56 – 62.

[13] Toro, G. and Ackermann, P.G.: *Practical Clinical Chemistry*. 1st ed., Little Brown and Co. Inc., Boston, USA, 1975.

[14] Haruna YI, Elinge C, MI, Peni IJ, Dauda, D, Aiki, F. *In vivo* trypanocidal effect of aqueous root extracts of *Securidaca longepedunculata* and its phytochemical analysis. *Afr J Pharm Pharmacol* 2013; 7 (43): 2838 – 2842.

[15] Auwal SM, Atiku, MK, Wudil AM, Sule MS. Phytochemical composition and acute toxicity evaluation of aqueous root bark extract of S*ecuridaca longipedunculata*. *Bayero J Pure Appl Sci* 2012; 5(2): 67 – 72.

[16] Wrobel JT, Matuszewska M, Szychowski J, Bertazzo A, Traldi P, Costa CV, Allegri G. indole alkaloids and other constituents from the plant *Securidaca longipedunculata*. *Adv Exp Med Biol* 1996; 398: 685 – 689.

[17] Aliu YO. *Veterinary pharmacology*, 1st edition. Tamza pub co Ltd Zaria 2007; 439 – 443.

[18] Khan CM. *The Merck veterinary manual* 9th edition 2005.

[19] El-Olemy MM, Almuhtadi FJ, Afifi AA. *Experimental phytochemistry*: A laboratory manual. King Saud University press, Saudi Arabia 1994: 21 – 61.

[20] Steenkamp V, Nkwane O, Van Tonder J, Dinsmore A, Gulumian M (2013). Evaluation of Phenolic and flavonoid contents and radical scavenging of three southern African medicinal plants. *Afr J Pharm Pharmacol* 2013; 7(13):703 - 709.

[21] Radostits OM, Blood DC, Gay CC. Veterinary Medicine. A text book of the diseases of cattle, sheep, pigs, goats and horses. 8th Ed. WB Saunders co. Ltd. London, Philadelphia, Toronto, Sydney, Tokyo. 1994.

[22] Taiwo VO, Olaniyi MO, Ogunsanmi AO. Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental *Trypanosoma congolense* and *T. brucei* infections in sheep. *Israel J Vet Med* 2003; 58: 4.