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In vivo antitrypanosomal effects of stem-bark extracts of *Securidaca longipedunculata* in rats experimentally infected with *Trypanosoma brucei brucei*

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Abstract

The efficacy of stem-bark extracts of *Securidaca longipedunculata* against *Trypanosoma brucei brucei* infected rats was investigated. For curative study, forty adult Wistar rats of both sexes were randomly divided into 8 groups of 5 rats each. Each rat was infected with 10^6 cells of trypanosomes per ml of blood intraperitoneally (*ip*). Rats in groups 1 and 2 received the crude methanol extract (CME) at 0.7 and 0.35 mg/kg, respectively. Similarly, rats in groups 3 and 4 received ethyl acetate fraction (EAF) at 0.7 and 0.35 mg/kg, respectively; while 5 and 6 were treated with 0.9 and 0.45 mg/kg of aqueous methanol fraction (AMF), respectively. Rats in groups 7 and 8 were treated with diminazene aceturate (3.5 mg/kg) and phosphate buffered saline, PBS (2 ml/kg), respectively. Four rats (group 9) were neither infected nor treated and served as neutral control. In the prophylactic studies, 25 rats of both sexes were randomly divided into V groups of 5 rats each. Rats in groups I, II, and III were pre-treated with CME at 0.7 mg/kg *i.p.* for 3, 5 and 7 days, respectively; while group IV received PBS for 7 days and served as negative control. The rats were then individually infected with 10^6 parasites per ml of blood on days 3, 5 and 7 for groups I, II and III, respectively. Rats in group V were neither treated nor infected and served as neutral control. CME of *S. longipedunculata* suppressed level of parasitaemia and prolonged the survival period of rats when compared to other groups (P<0.05). Pre-treatment of animals with CME before challenge with the parasite could not prevent infection. Thus, stem-bark extract of *S. longipedunculata* exhibited some levels of curative antitrypanosomal effect against *T. brucei brucei* infection in rats despite its low margin of safety.

Keywords: Curative effect, High toxicity, *In vivo*, Phytochemical screening, Prophylactic effect

Introduction

Tsetse-transmitted trypanosomosis is an important constraint to livestock development in sub-Saharan Africa with estimated direct annual losses to producers and consumers exceeding US$1 billion (Kristjanson *et al*., 1999; Simukoko *et al*., 2007). 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 has created a growing public interest in the pursuit of alternative natural drugs from botanicals (Etet & Mahomoodally, 2012). Phytotherapy is the oldest form of therapeutic treatment world-wide with the use of over 21, 000 plant species as herbal medicine (Efferth, 2010).

Natural products are important sources of lead compounds in the development of new drugs (Kayser *et al*., 2003). However, the great potential of plants as lead to the discovery of newer antitrypanocidal drug is still at its lowest ebb (Adams *et al*., 2013). Many existing drugs were derived from natural compounds (Newman & Cragg, 2012). There are only three available trypanocidal drugs for the management of trypanosomosis in ruminants. Diminazene aceturate with only therapeutic activity, homidium as well as isometamidium both with therapeutic and prophylactic activities (Tauheed *et al*., 2016a) Therefore, screening natural products may provide a link to the discovery of a new compounds with unique structure of high activity and selectivity.

Securidaca longipedunculata (Polygalaceae) also known as violet tree, fibre tree or Rhodesian violet in English, and popularly known as *uwar magunguna* (mother of all medicines) in Hausa speaking communities of northern Nigeria (Tauheed *et al*., 2016b). It is a small tree of up to 6-9 m high with a pale grey, smooth bark and oblong, more or less hairless alternate leaves of varying size and shape and crowded towards the stem tips (Van Wyk *et al*., 2009). Whole plant, root, stem-bark and leaves of the plant are used for medicinal purposes in folkloric medicine. The plant is widely used in African traditional medicine as a general remedy for cough, malaria, backache, venereal disease, snakebite, erectile dysfunction and tuberculosis (Mongalo *et al*., 2015). The aim of this study was to determine the anti-trypanosomal effect of stem-bark extracts of *Securidaca longipedunculata* against *T. brucei brucei* experimental infection in rats.

Materials and Methods
Plant collection and identification
Fresh stem-bark of *Securidaca longipedunculata* was collected from Zaria, Nigeria. The plant was identified in the Herbarium, Department of Biological Sciences, Ahmadu Bello University (A.B.U.), Zaria, Nigeria where a voucher number specimen of 900213 was assigned. 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 (790) g of the pulverised stem-bark of *S. longipedunculata* was extracted with absolute methanol in a soxhlet extractor. The liquid extract was concentrated to

dryness over a water-bath at 60^0C. About 73 g of the crude methanol extract was dissolved in 300 ml of distilled water. The solution was transferred to 1 L separating funnel and partitioned with 600 ml of ethyl acetate for 8 hours. 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 process was repeated two more times and similar fractions were pooled together. The fractions were concentrated to dryness over a water-bath at 50^0C and 70^0C for ethyl acetate and aqueous methanol fractions, respectively.

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

Experimental animals
Adult male and female Wistar rats weighing between 170 to 190 g were obtained from the animal house, Department of Physiology, Faculty of Medicine, Ahmadu Bello University (A.B.U.), Zaria, Nigeria. They were allowed to acclimatize for 2 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 bedding, which was changed twice a week. The rats were fed standard rat feed and given access to clean water *ad libitum*. The approval for the use of animal was obtained from the Ethical Committee on Animal Use and Care, A.B.U., Zaria, Nigeria.

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 – 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 phosphate buffered saline (PBS). About 1×10^6 cells trypanosomes contained in 0.2 ml was used to infect a trypanosome-free rat by *i.p.* route.

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 & Lumsden (1976).

Determination of median lethal dose 50 (LD_{50})
The median lethal dose (LD_{50}) was determined by the method of Lorke (1983). In the initial phase, 9 rats of both sexes were randomly divided into three groups of 3 rats each. Rats in groups 1, 2 and 3 were treated *i.p.* with crude methanol extract at 10, 100 and 1000 mg/kg, respectively. The rats were then observed over 48 hours for signs of toxicity and mortality. The procedure was repeated for ethyl acetate and aqueous methanol fractions. In the second phase of the study, 3 rats for each extract were assigned into 3 groups of 1 rat each. The animals were individually administered 2, 6, and 8 mg/kg of crude methanol and ethyl acetate fraction, while animals for aqueous methanol fraction were administered 6, 8, and 10 mg/kg. The LD_{50} was then computed as geometric mean of highest dose that did not kill the rat and lowest dose that killed the rat.

In vivo screening
A total of 44 male and female Wistar rats were used for *in vivo* study. Forty of them were infected *i.p.* each with 10^6 *T. brucei brucei* and then observed daily for development of parasitaemia. The forty trypanosome-infected rats were divided into 8 groups of 5 rats each. Rats in groups 1 and 2 received the crude methanol extract of *S. longipedunculata* at 0.7 and 0.35 mg/kg, respectively for 7 consecutive days, while rats in groups 3 and 4 were given ethyl acetate fraction of the extract at 0.7 and 0.35 mg/kg, respectively for 4 consecutive days. Similarly, rats in groups 5 and 6 received 0.9 and 0.45 mg/kg of aqueous methanol fraction of the extract, respectively for 5 consecutive days, while groups 7 and 8 rats were treated with diminazene aceturate (3.5 mg/kg) once and PBS (2 ml/kg) for 4 consecutive days, and served as treated and untreated control groups, respectively. The remaining four rats (group 9) were neither infected with trypanosomes nor treated, and served as neutral control animals. The day of commencement of treatment was designated day 0. The levels of parasitaemia and survival period were observed.

In vivo prophylaxis study

Twenty-five rats of both sexes were randomly divided into five groups of 5 rats each. Rats in groups I, II, and III were treated with crude methanol extract of SL at 0.7 mg/kg *i.p.* once daily for 3, 5, and 7 days for groups 1, 2 and 3, respectively, while rats in group IV were administered PBS (2 ml/kg) for 7 days and served as untreated control. All the rats were then individually challenged with 10^6 *T. brucei brucei i.p.* and observed for parasitaemia. Rats in group V were neither infected with trypanosomes nor treated and served as neutral control.

Data analysis
Data obtained were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used, followed by Tukey's post-hoc test for multiple comparisons of groups. Values of $P < 0.05$ were considered significant. Line graph and bar chart were used to present the effects of treatment with the extracts on the level of parasitaemia and survival period, respectively.

Results
Phytochemical screening
Preliminary phytochemical screening of the extract and fractions revealed the presence of carbohydrates, cardiac glycosides, saponins, steroids, triterpenes and tannins. However, crude methanol extract contains flavonoids in addition.

Median lethal dose (LD_{50})
All rats administered 10, 100 and 1000 mg/kg of extract and fractions of *S. longipedunculata* died within 72 hours of observation in the first phase. In the second phase, mortality was recorded at 8 mg/kg for crude methanol extract and ethyl acetate fraction; while 6 mg/kg was observed as non lethal dose. However, 10 mg/kg and 8 mg/kg were observed as lethal and non lethal doses of aqueous methanol fraction in the second phase of LD_{50} determination. Thus, LD_{50} was computed as the geometric mean of lowest dose that produced mortality and highest dose with no lethal effect.
For crude methanol extract and ethyl acetate fraction, $LD_{50} = \sqrt{8 \times 6} = 6.9 \approx 7$ mg/kg
For aqueous methanol fraction, $LD_{50} = \sqrt{10 \times 8} = 8.94 \approx 9$ mg/kg

Effect of treatment on the level of parasitaemia in infected rats
The pattern of parasitaemia is shown in figure 1. Parasitaemia was observed in all the infected groups

4 days after inoculation. The level of parasitaemia increased progressively in rats treated with ethyl acetate fraction, EA (0.7 mg/kg) and PBS (2 ml/kg). However, animals that received crude methanol extract (CM) at 0.7 and 0.35 mg/kg and EA at 0.35 mg/kg showed undulating parasitaemia. Also, rats that were treated with aqueous methanol fraction (AM) initially showed low level of undulating parasitaemia at day 2 of treatment but later showed a progressive increase in the level of parasitaemia at day 3 post-treatment. The parasites were not seen in the blood of rats treated with diminazene aceturate (3.5 mg/kg) 24 hours after treatment and remained aparasitic for 3 weeks. There was significant (P<0.05) reduction in the level of parasites in CM (0.35 mg/kg) 3 days after treatment when compared with animals in PBS group. No significant (P>0.05) difference was observed in the level of parasitaemia in the remaining groups when compared to animals in the PBS treated group.

Effect of treatment on survival period
The effect of treatment with *S. longipedunculata* extracts on the survival period of rats infected with *T. brucei brucei* is shown in figure 2. The animals in CM (0.7 mg/kg) treated group survived longer than animals treated with CM (0.35 mg/kg), ethyl acetate, aqueous methanol and PBS treated group. Mortality of animals in extract treated groups started on day 3 post-administration while PBS group began on day 2 of commencement of treatment. Only 2 animals in CM (0.7 mg/kg) group completed dosage regimen of daily treatment for 7 days. None of the animals in DA and NC died throughout the period of observation. Only one animal in PBS group lived for 4 days during treatment. Overall, CM (0.7 mg/kg) and (0.35 mg/kg) animals lived longer (P>0.05) than other extract treated groups.

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. The values of *i.p.* LD_{50} obtained in this study is lower compared to the 20 mg/kg LD_{50} and 37 mg/kg reported in mice (Adeyemi *et al.*, 2010) and rats (Dapar *et al.*, 2007),
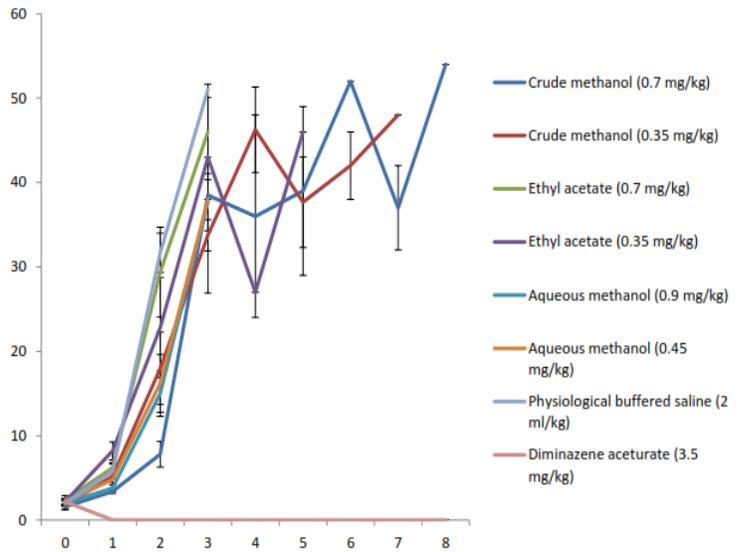

Figure 1: Effect of treatment with stem-bark extracts of *S. longipedunculata* on the level of parasitaemia in *T. brucei brucei*-infected Wistar rats
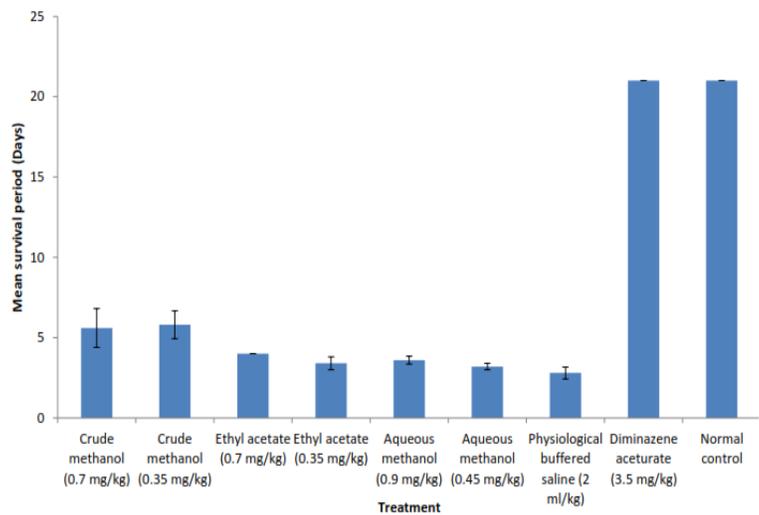

Figure 2: Effect of treatment with stem-bark extract of *S. longipedunculata* on mean survival period of Wistar rats

respectively of *S. longipedunculata* roots. This shows that stem-bark of *S. longipedunculata* is more toxic than the root. Our observation is in agreement with the finding of Cock (2011), who reported that chemical constituents of plant vary within the parts of the plant, and between plants with regard to location, age and stage of growth. Plants cultivated under different environmental conditions produce different phytochemical profiles or different amounts of individual components. Besides, different cultivars within species may produce different levels of bioactive components or other constituent which enhance or counteract their

medicinal activities (Juliani *et al.*, 2006). Therefore, it is possible that stem-bark of *S. longipedunculata* accumulates more toxic compounds resulting in lower values of LD_{50}. This may underscore the reason why root of *S. longipedunculata* have been reported to have more medicinal applications than other parts of the plant (Mongalo *et al.*, 2015). In previous studies with root of the plant, better efficacies against trypanosomosis were reported, probably due to higher doses ranging from 100 to 400 mg/kg (Adeyemi *et al.*, 2010; Okomolo *et al.*, 2011). Hence, had higher doses of stem-bark been used, probably by identifying and isolating toxic compound(s) contained in the plant, antitrypanosomal efficacy observed in the present study may have been enhanced. *Securidaca longipedunculata* has been used as ritual suicide (Neuwinger, 1996) and as a pesticide in stored grains (Steveson *et al.*, 2009). Furthermore, *ex vivo* study of the stem-bark of the plant only paralysed the parasite and was reported to be trypanostatic rather than trypanocidal (Tauheed *et al.*, 2016b). Therefore, the low LD_{50} resulted in low concentrations administered to the animals which may have resulted in low plasma concentration. The higher concentration of extract shows better efficacy than the extract with lower concentration since an increase in concentration results in a supplementary input of different active compounds (Wabo *et al.*, 2011). It is possible that the low doses used in the present study could not support the attainment and maintenance of adequate plasma concentration that could produce therapeutic effect. The saponin content of the stem bark of *S. longipedunculata* may have been responsible for the toxicity observed, thus low safety margin. Plant with higher concentration of saponins than other secondary metabolites tend to be more toxic (Williams, 1978). Therefore, the plant may contain potent toxic secondary metabolites which further explain the selection of low doses for therapeutic evaluation in this study. Recent reports showed that *S. longipedunculata* is toxic to rats, with variable levels of toxicity on vital organs (Ajiboye *et al.*, 2010).

Stem-bark of *S. longipedunculata* showed promising antitrypanosomal effect, particularly its crude methanol extract. It was observed to suppressed the level of parasitaemia in the experimental rats. Furthermore, rats treated with in crude methanol extract lived longer than those in fractions treated groups and PBS group. The antitrypanosomal effect observed in this study may be attributed to the flavonoid present in this extract. Only crude methanol extract was positive for flavonoid while ethyl acetate and aqueous methanol did not contain flavonoid. Incidentally, crude methanol extract exhibited better antitrypanosomal effect compared to other extract treated groups. Flavonoids found in higher plants have been reported to possess antimicrobial and antitrypanosomal activities (Cordell *et al.*, 2001). Nonetheless, the presence of other metabolites may have masked better trypanocidal efficacy observed by antagonism.

It has been established that method of extraction affects composition of plant extract (Handa *et al.*, 2008). Dapar *et al.* (2007) reported higher LD_{50} of 36.74 mg/kg using percolation of root-bark of *S. longipedunculata*, whereas soxhlet extraction was employed in this study. Extraction using heat tends to produce higher yield than cold extraction (Handa *et al.*, 2008). It is possible that the hot extraction used in the present study resulted in greater penetration of the solvent into the plant tissue leading to extraction of more of the toxic metabolites. Thus, the proportion of bioactive secondary metabolites may have been shifted in favour of toxic metabolite leading to higher toxicity of the plant. Perhaps this may have affected its pharmacological effect as well. In contrast, Youan *et al.* (1997) reported no difference in the efficacy of extracts obtained from cold and hot extraction against experimental trypanosome infection in mice. Antitrypanosomal effects of plant extracts are associated with the presence of one or more biologically active ingredients (Mbaya *et al.*, 2007). Different solvents used for extraction of plant materials also influence composition of bioactive metabolites in an extract. Atawodi *et al.* (2011) reported varied efficacy of the extracts of *B. dalzielii*, extracted with different solvents. In the present study, extract of the plant extracted using different solvent systems did not produce any significant pharmacological action. This agrees with the findings of Youan *et al.* (1997) and Wabo *et al.* (2013) who reported that extracts from various plants when tested for pharmacological activity did not show any significant difference in their effects.

Pre-treatment of rats before challenge did not affect the onset of parasitaemia and course of the disease, which signified that the stem-bark of *S. longipedunculata* did not have prophylactic effect against *T. brucei brucei*. Similar incidence was observed by Ogbadoyi *et al.* (2011) who reported that *Acacia nilotica* exhibited curative effect but lacked prophylactic effect against experimental trypanosomosis. In another study however,

Acanthopanax senticosus showed only prophylactic analgesic effect (Takahashi *et al.*, 2014). It follows that medicinal plants can be potential sources of compound lead for curative antitrypanosomal drugs like diminazene and homidium, as well as sources of prophylactic antitrypanosomal drugs like isometamidium.

In conclusion, stem-bark of *S.longipedunculata* has high toxicity (low margin of safety) when used by intraperitoneal route. Although the plant showed some level of curative antitrypanosomal effect, low doses evaluated occasioned by its low LD_{50} values may have hindered excellent curative antitrypanosomal effect that could have been obtained. Finally, stem-bark of *S. longipedunculata* does not have prophylactic effect against *T. brucei brucei* in rat.

References
Adams M, Chammartin M, Hamburger M & Potterat O (2013). Case study of the Swiss flora for prior phytochemical and biological investigations. *Journal of Natural Products*, **76**(2): 209–215.

Adeyemi OO, Akindele AJ, Yemitan OK, Aigbe FR & Fagbo FI (2010). Anticonvulsant, anxiolytic and sedative activities of the aqueous root extract of *Securidaca longipedunculata (Fresen). Journal of Ethnopharmacology*, **130**(2): 191-195.

Ajiboye TO, Salau AK, Yakubu MT, Oladiji AT, Akanji MA & Okogun JI (2010). Aqueous extract of *Securidaca longepedumculata* root induce redox imbalance in male rat liver and kidney. *Human and Experimental Toxicology*, **29**(8):679-688.

Atawodi SE, Joy JI, Uche SN & Yusufu L (2011). Phytochemical and antitrypanosomal studies of different solvents extracts of *Boswellia dalzielii. International Journal of Biology*, **3**(2), 179-184.

Cock IE (2011).Problems of reproducibility and efficacy of bioassays using crude extracts, with reference to *Aloe vera. Pharmacognosy Communications*, **1**(1): 52-62.

Cordell GA, Quinn-Beattie ML & Farnsworth NR (2001). The potential of alkaloids in drug discovery. *Phytotherapy Research*, **15**(3): 183–205.

Dapar LPM, Aguiyi CJ, Wannang NN, Gyang SS & Tanko MN (2007). The histopathologic effects of *Securidaca longipedunculata* on heart, liver, kidney and lungs of rats. *African Journal of Biotechnology*, **6**(5): 591–595.

Efferth T (2010). Cancer therapy with natural products and medicinal plants. *Planta Medica*, **76**(11): 1035–1036.

Etet PFS & Mahomoodally MF (2012). New insights in staging and chemotherapy of african trypanosomiasis and possible contribution of medicinal plants. *Scientific World Journal*, doi:10.1100/2012/343652.

Handa SS, Khanuja SPS, Longo G & Rakesh DV (2008). *Extraction Techniques for Medicienal and Aromatic Plants*. EXS UNIDO, Trieste. Pp 93-106.

Herbert WJ & Lumsden WHR (1976). *Trypanosoma brucei*: a rapid matching method for estimating the host parasitemia. *Experimental Parasitology*, **40**(3): 427-431.

Juliani HR, Wang MF, Moharram H, Asante-Dartey J, Acquaye D, Koroch AR & Simon JE (2006). Intraspecific variation in quality control parameters, polyphenol profile, and antioxidant activity in wild populations of *Lippia multiflora* from Ghana. *ACS Symposium Series, Oxford Press*, Pp 126 - 142.

Kayser O, Kiderlen AF & Croft SL (2003). Natural products as antiparasitic drugs. *Parasitology Research*, **90**(2): 55-62.

Kristjanson PM, Swallow BM, Rowlands GJ, Kruska RL & de Leeuw PN (1999). Measuring the costs of African animal trypanosomosis: the potential benefits of control and returns to research. *Agricultural Systems*, **59**(1): 79-98.

Lorke D (1983). A new approach to practical acute toxicity testing. *Archive of Toxicology*, **54**(4): 275-287.

Mbaya AW, Nwosu OC & Onyeyili, PA (2007). Toxicity and anti-trypanosomal effects of

Butyrospermum paradoxum (Sapotaceae) stem bark in rats infected with *Trypanosoma brucei and Trypanosoma congolense*. *Journal of Ethnopharmacology*, **111**(3): 526-530.

Mongalo NI, Mcgaw LJ, Finnie JF & Van Staden J (2015). Securidaca longipedunculata Fresen (Polygalaceae): A review of its ethnomedicinal uses, phytochemistry, pharmacological properties and toxicology. *Journal of Ethnopharmacology*, **165**: 215-226.

Newman DJ & Cragg GM (2012). Natural products as sources of new drugs over the30 years from 1981 to 2010. *Journal of Organic Chemistry*, **75**(3): 311–335.

Neuwinger HD (1996). *African Ethnobotany, Poisons and Drugs Chemistry, Pharmacology, Toxicology* London, UK. Chapman and Hall. Pp 743–753.

Ogbadoyi EO, Garba MH, Kabiru AY, Mann A & Okogun JT (2011). Therapeutic evaluation of *Acacia nilotica* (Linn) stem bark extract in experimental African trypanosomiasis. *International Journal of Applied Research in Natural Products*, **4**(2): 11-18.

Okomolo FCM, Mbafor JT, Ngo Bum E, Kouemou N, Kandeda AK, Talla E, Dimo T & Rakotonirira SV (2011). Evaluation of the sedative and anticonvulsant properties of three Cameroonian plants. *African Journal of Traditional and Complementary and Alternative Medicine*, **8**(5): 181-190.

Perry BD, Randolph TF, McDermott JJ, Sones KR & Thornton PK (2002). *Investing in Animal Health Research to Alleviate Poverty.* International Livestock Research Institute, Nairobi, Kenya. Pp 148.

Simukoko H, Marcotty T, Phiri I, Geysen D, Vercruysse J & Van de Bossche P (2007). The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomiasis on the plateau of eastern Zambia. *Veterinary Parasitology*, **147**(3-4): 231-238.

Stevenson PC, Dayarathna TK, Belmain SR & Veitch NC (2009). Bisdesmosidic saponins from *Securidaca longipedunculata* Roots: Evaluation of deterrency and toxicity to coleopteran storage pests. *Journal of Agricultural Food Chemistry*, **57**(19): 8860-8867.

Takahashi Y, Tanaka M, Murai R, Kuribayashi K, Kobayashi D, Yanagihara N & Watanabe N (2014). Prophylactic and therapeutic effects of *Acanthopanax senticosus* Harms extract on murine collagen-induced arthritis. *Phytotherapy Research*, **28**(10): 1513-1519.

Tauheed AM, Shittu SH, Suleiman MM, Habibu B, Kawu MU, Kobo PI & Yusuf PO (2016a). *In vivo* ameliorative effects of methanol leaf extract of *Lawsonia inermis* Linn on experimental *Trypanosoma congolense* infection in Wistar rats. *International Journal of Veterinary Science and Medicine*, **4**(2): 33-40

Tauheed AM, Suleiman MM, Mamman M & Lawal IA (2016b). *Ex vivo* trypanostatic effect of stem-bark extracts of *Securidaca logipedunculata* (Fress. Holl) against *Trypanosma brucei brucei*. *African Journal of Biotechnology*, **15**(51): 2789-2794.

Trease GE & Evans WC (1983). Drugs of Biological Origin. In: *Pharmacognosy*. Twelfth edition. Balliere Tindall, UK. Pp 309-540.

Van Wyk BE, Van Oudtshoorn B & Gericke N (2009). *Medicinal Plants of South Africa*, Second Ed. Briza Publications, Pretoria, South Africa. Pp 268.

Wabo PJ, Ngankam,NJD, Bilong BCF & Mpoame MA (2011). Comparative study of the ovicidal and larvicidal activities of aqueous and ethanolic extracts of pawpaw seed *Carica papaya* (Caricaceae) on *Heligmosomoides bakeri*. *Asian Pacific Journal of Tropical Biomedicine*, **4**(6): 447-450.

Wabo PJ, Payne VK, Mbogning TG, Komtangi MC, Yondo J, Ngangout AM, Mpoame M & Bilong BCF (2013).*In vitro* anthelminthic efficacy of *Dichrocephala integrifolia* (Asteraceae) extracts on the gastro-intestinal nematode parasite of mice: *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae). *Asian Pacific Journal of Tropical Biomedicine*, **3**(2):100-104.

Williams CM (1978). Toxicity of saponins in Alfombrilla (*Drymaria arenarinoides*). *Journal of Range Management*, **31**(3): 182-184.

Youan BBC, Coulibaly S, Miezan TB, Doua F & Bamba M (1997). In vivo evaluation of sixteen plant extracts on mice inoculated with *Trypanosoma brucei gambiense*. *Bulletin of the World Health Organization*, **75**(4): 343-348.