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In vitro assessment of the acaricidal properties of aqueous extract of *Cassia sieberiana* DC (*Caesalpiniaceae*) stem bark on *Boophilus* (Curticei 1891) larvae

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ABSTRACT

This study was conducted to evaluate the 'acaricidal effect' of aqueous extract of *Cassia sieberiana* stem bark on the larvae of *Boophilus* ticks, using the *In vitro* immersion method (IIM). Freshly hatched larvae were divided into 4 replicates of 20 each and exposed to five graded concentrations of the aqueous extract (5, 10, 15, 20 and 25%) for 1 hour and mortality rates recorded post inoculation for each concentration. The mean ± standard deviation (range) larval mortality varied between 4.25±4.1 (2-12) (at 5%) and 11.63±5.3 (6-19) (at 25%) compared with distilled water 1.88±1.3 (0-4) and untreated (normal controls) 0.0±0.0 ($p < 0.05$). Mean mortality rates also ranged between 31.3% (at 5% concentration) to 58.1% (at 25%) compared with 9.4% for distilled water and 0.0% for untreated control ($p < 0.05$).This study has observed a positive correlation between graded concentrations of aqueous extract of *Cassia sieberiana* stem bark and duration of larval immersion *In vitro*.

Keywords: *In vitro* assessment, Acaricidal properties, *Cassia sieberiana, Boophilus* larvae.

INTRODUCTION

Ticks are obligate haematophagous ectoparasites of domestic and wild animals, and man worldwide, transmitting diseases which are responsible for substantial economic losses (Habeeb, 2010), with an estimated global cost of control and productivity losses at 7 billion US dollars annually (Nchu *et al*. 2012). Tick control worldwide is based mainly on the repeated use of synthetic chemical acaricides, which have resulted in problems related to

environmental pollution, milk and meat contamination, and the development of resistance leading to increased cost of control (Dipeolu, *et al*. 1991; Habeeb, 2010; Thembo *et al*. 2010; Pirali-Kheirabadi and Teixeira da Silva, 2011). Synthetic chemicals have also been reported to have genotoxic and cytotoxic effects on human target cells (Pirali-Kheirabadi and Razzaghi-Abyaneh, 2007). In view of these problems, few biocontrol programmes have been developed for ticks (Samish *et al*. 1999). There is a need for alternative tick control measures that are effective, safe and environmentally acceptable, through the use of natural plant products (Webb and David, 2002). Plant products are a rich source of bioactive organic chemicals and offer an advantage over synthetic pesticides as these are less toxic, less prone to development of resistance and easily biodegradable (Deore and Khadabadi, 2009). Over the years, plant extracts and essential oils have been widely used against haematophagous arthropods and have been described to have bioefficacies such as acaricidal, ovicidal, repellant, antifeeding and biocidal activities (Pirali-Kheirabadi *et al*. 2009). The use of botanicals for the control of ticks is compatible with traditional practices in Africa, where most resource-poor-farmers use plant materials to treat endoparasites and ectoparasites of livestock (Nchu *et al.,* 2012). Acaricidal activity of crude extracts from stem and leaves of different plants against cattle ticks has been reported by Kaaya *et al*., (2009); Bagavan *et al.,* (2009); Rosado-Aguilar *et al*., (2010); Habeeb, (2010); Madzimure *et al*., (2011); Ravindran *et al*., (2011). *Cassia sieberiana*, a small shrub Savannah tree with short bole, fissured bark, grey to brown with blackish stripes (Vander-Maesen, 2007), common to northeastern Nigeria, with its roots, pods, leaves and stem bark widely used in traditional medicine to treat many diseases that include AIDS and helminthiasis (Obidah *et al*., 2009), and phytochemical studies have shown that it contains calcium oxalate, flavones, resins and tannins which have antibacterial and anti-inflammatory activities, and reported to possess an immune-boosting effect in experimental albino rats (Buratai *et al.,* 2011). An attempt has been made in this study to evaluate the acaricidal activity of aqueous extract of *Cassia sieberiana* stem bark on the larvae of *Boophilus* species, one of the commonest ticks in Northeastern Nigeria, and a one host tick requiring 3-4 weeks to complete its life cycle resulting into heavy tick burden and acaricide resistance as problems in control efforts (CFSPH, 2009).

MATERIALS AND METHODS

Plant material: *Cassia sieberiana* was obtained from the University of Maiduguri campus and identified by a plant taxonomist from the Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria and a set of voucher herbarium (species No. LCMC 228) was sent to the Department of Chemistry, University of Maiduguri, Nigeria.

Preparation of aqueous stem bark extract: Fresh stem bark from *Cassia sieberiana* were collected, cut into small pieces and dried under shade at room temperature in the Veterinary Parasitology Laboratory of the University of Maiduguri. Dried samples were then ground into fine powder to obtain 1000grams, which was extracted in 700mls distilled water at 60^0C for 8 hours using Soxhlet extractor (Quickfit Corning Ltd., Stafford England). The extract was concentrated on an aluminium tray and weighed 251.1grams to obtain a yield of 74.9% w/w which was kept at room temperature until used.

Tick collection: Twenty fully engorged adult female *Boophilus* ticks were manually removed from naturally infested sheep and goats using hand forceps and put into clean Bijou bottles and taken to the Veterinary Parasitology Laboratory of University of Maiduguri for identification as described by Soulsby, (1982).

Experimental studies:

Step I: Ten fully engorged adult female *Boophilus* species were individually incubated into separate test tubes plugged with cotton wool and kept under ambient temperature (27-29°C) and relative humidity (85-90%) to allow for oviposition. Eggs were collected from each tick into test tubes plugged with cotton under the same laboratory conditions for egg-hatch into larvae.

Step II: Freshly hatched larvae were divided into 4 replicates of 20 each and immersed for a duration of 1 hour into extract concentrations of 5, 10, 15, 20 and 25% with control groups as distilled water and normal (untreated) after which they were removed and put on Whatman filter paper (1mm) and incubated into Petri dishes, kept at room temperature and relative humidity to observe for acaricidal activity.

Statistical analysis: Larval mortality was calculated as mean ± SD with differences between them determined by analysis of variance at $p < 0.05$ regarded as significant (Graph Pad Instat, 2003).. Linear

regression was plotted at 95% confidence interval to determine the relationship between the graded extract concentrations and the duration of larval immersion.

RESULTS

The results of this study is shown in Table 1 as mean ± SD of dead larvae at graded concentrations of aqueous extract of *Cassia sieberiana* stem bark. Values of 4.25±4.1, 5.63±3.5, 5.98±1.3, 10.50±5.2, 11.63±5.3, 1.88±1.3 and 0.0±0.0 were obtained for 5, 10, 15, 20 and 25% extract concentrations, distilled water and normal controls respectively after a 1-hour immersion ($p<0.05$). Mean mortality rates (%) after 1-hour immersion were recorded as 31.3%, 28.1%, 29.4%, 52.5%, 58.1%, 9.4% and 0% for 5, 10, 15, 20 and 25% extract concentrations, distilled water and normal (untreated) controls respectively ($p<0.05$) (Table 2). Figure 1 shows the linear regression on the effect of various concentrations of aqueous extract of *Cassia sieberiana* stem bark on *Boophilus* larvae. There was a positive correlation between the graded concentrations of aqueous extract of *Cassia sieberiana* stem bark and duration of larval immersion *In vitro*.

Table 1: Mean ± SD of Dead Larvae Exposed to Graded Concentrations of Extract for 1hour.

Graded extract Concentration (%)	Mean ± SD (range) dead larvae
5	4.25±4.1 (2 – 12)[a]
10	5.63±3.5 (2 – 12)[b]
15	5.98±1.3 (4 – 18)[c]
20	10.5±5.2 (5 – 18)[d]
25	11.63±5.3 (6 – 19)[e]
Distilled water control	1.88±1.3 (0 – 4)[f]
Normal (untreated) control	0.0±0.0 (-)[g]

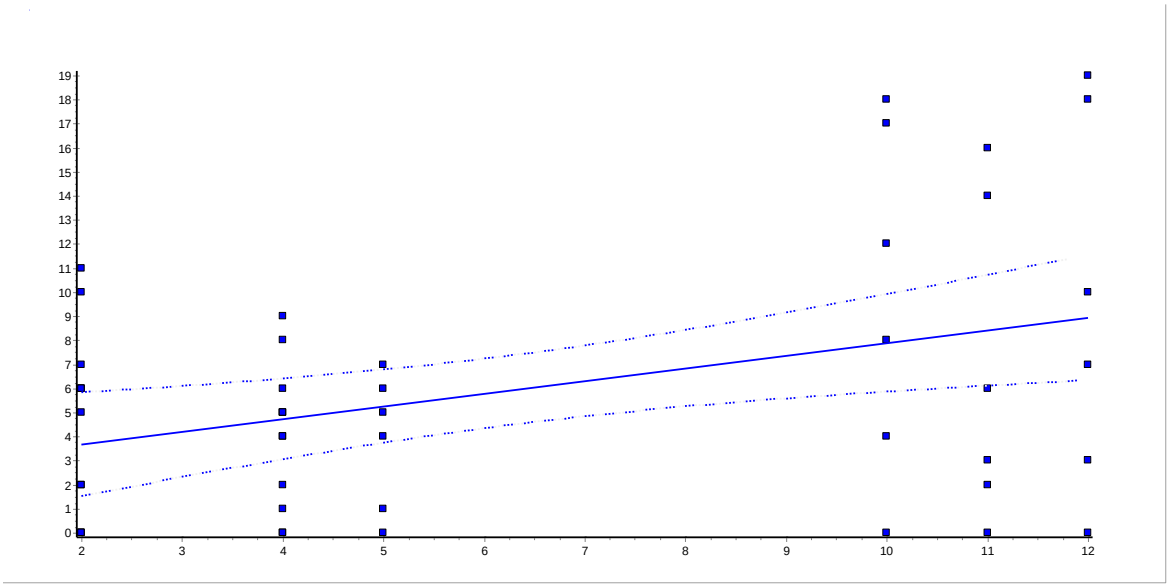
Mean values bearing different superscripts are statistically significant

Table 2: Mortality rates (%) of Larvae Exposed to Graded Concentrations of Extract after One Hour Interval.

Graded concentrations of extract (%)	Mean±SD (range) mortality rates (%) of *Boophilus* larvae
5	31.3±20.5 (10-60)[a]
10	28.1±17.5(10-60)[b]
15	29.4±6.2(20-40)[c]
20	52.5±25.9(25-90)[d]
25	58.1±26.5(30-95)[e]
Distilled water	9.4±6.2(0-20)[f]
Normal (untreated)	0±00(00)[g]

Mean values bearing different superscripts are statistically significant

Figure 1: Linear regression on the effect of various Concentrations of Aqueous extract of *Cassia sieberiana* Stem Bark on *Boophilus* Larvae.



_____ Linear regression

…….. 95% Confidence Interval

Correlation Coefficient ®= 0.3829 Slope= -3.661

DISCUSSION

This study on the acaricidal efficacy of aqueous extract of *Cassia sieberiana* stem bark on *Boophilus* larvae indicated a positive correlation between graded concentrations of the extract, the immersion test-time interval and larval mortality. The extract concentration of 25% produced a mortality rate of up to 95%. This is almost similar to Borges *et al.*, (2011) who reported *Boophilus* larval mortality rate of 100% for chlorofermic extracts and 98% for hexamic extracts of *Azadirachta indica*, Chagas *et al.*, (2002), who indicated that essential oils of *Eucalyptus citrodoro* and *Eucalyptus staigeriana* (Myrtaceae) killed 100% of the *Boophilus* tick larvae at 10% concentration, while *Eucalyptus globoles* had the same efficacy at 20% concentration; and Ribeiro *et al.*, (2007) who reported that the crude extract of *Hypericum polyanthemum* produced a 100%, 96.7%, 84.7% and 52.7% *Boophilus* larval mortality rates at concentrations of 50, 25, 12.5, and 6.25 mg/ml respectively after 48 hours exposure. Khudratulla and Jagannath, (1998) and Tho, (2003) has reported that many factors that include type of solvent, extraction time, extract concentration, plant age, species of ticks and its developmental stage and exposure time affect the acaricidal activity of plant extracts. *Cassia sieberiana* extract molecules have been reported to possess flavonoids, alkaloids, tannins, saponins and terpenes (Mavar-manga *et al.*, 2006; Buratai *et al.*, 2011). Plant derived compounds such as saponins were reported to have acaricidal properties against *Boophilus* ticks (Martin *et al.*, 2001; Oberdorster *et al.*, 2001; Sparg *et al.*, 2004; Pereira and Famoda, 2006; Ribeiro *et al.*, 2008; Deore and Khadabade, 2009; Magadum *et al.*, 2009; Fernandez-Salas *et al.*, 2011). In conclusion, this study has ascertained the acaricidal efficacy of *Cassia sieberiana* aqueous stem bark *In vitro* on *Boophilus* larvae, and it should be recognized as a future alternative acaricide for the control of *Boophilus* ticks.

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