



Research Article

Acaricidal Efficacy of *Cassia sieberiana* DC (*Caesalpinaceae*) Stem Bark Aqueous Extract on *Hyalomma* KOCH, 1844 (Acari: Ixodidae) Reproductive Stages

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ARTICLE INFO:

ABSTRACT

Keywords:

Acaricidal Efficacy,
Cassia sieberiana,
Hyalomma,
Maiduguri,
Nigeria

One of the most significant challenges facing tick control through chemical acaricides is resistance and environmental pollution, encouraging the need for natural plant products that are less toxic and environment friendly. This study was conducted to determine the acaricidal efficacy of *Cassia sieberiana* stem bark aqueous extract on *Hyalomma* species using immersion testing for egg-hatch inhibition and adult mortality. Both tests used ascending extract concentrations of 5%, 10%, 15% and 20%, at durations of 1, 2, 3, 4, 5, 6, 7 and 8 hours. Egg-hatch inhibition peaked at 5% concentration at 8hrs, 10% and 15% at 7 and 8hrs and 20% at 5, 6, 7 and 8hrs while adult mortality was highest at 20% concentration. There was a positive correlation between graded extract concentration, immersion period, egg-hatch inhibition and adult mortality. The LC₅₀ values for adult mortality and egg-hatch inhibition were 13.1 mg/mL and 13.7 mg/mL and coefficient of determination were $y = 3.7x + 1.5$ and $y = 3.062 + 8.12$ respectively. Both LC₅₀ values were almost similar indicating a strong association between extract concentration and acaricidal activity *in vitro*.

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INTRODUCTION

Ticks are arthropods of the Class *Arachnida* and are one of the most important ectoparasites affecting livestock with both veterinary and medical importance (Anderson, 2002; James-Rugu and Jadayi, 2004). *Hyalomma* species reported as vectors of *Theileria annulata* which is the causative agent of theileriosis in livestock and Crimean Congo haemorrhagic fever virus to man (Thembo *et al.*, 2010).

The use of synthetic acaricides to control ticks of veterinary and medical significance is becoming increasingly problematic, due largely to their high cost, drug resistance, product residues (milk contamination), withdrawal of active ingredients, undesirable environmental persistence or pollution and unacceptable risks to non-target organisms (Sanis *et al.*, 2012; George *et al.*, 2014). In view of the evolution of resistance to synthetic acaricides among others, quest has driven research to identifying alternative approaches to control ticks in recent years (Kiss *et al.*, 2012; Bissinger *et al.*, 2014), especially by using natural plant products that are relatively environment friendly (Borges *et al.*, 2003; Ghosh *et al.*, 2007; Bagavan *et al.*, 2009; Pirali-Kheirabadi *et al.*, 2009; Pirali-Kheirabadi and Teixeira da Silva, 2010; Biu *et al.*, 2013). Ethnoveterinary medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing countries including Nigeria (WHO, 2002). Some studies on the use of plant extracts against ticks have been conducted in Pretoria, South Africa (Thembo *et al.*, 2010); Nigeria (Biu *et al.*, 2013; 2014); Iran (Pirali-Kheirabadi and Teixeira da Silva, 2011); Brazil (Giglioti *et al.*, 2011); Burkina Faso (Kabore *et al.*, 2012); Egypt (Abdel-Shafy and Zayed, 2002) and India (Sanis *et al.*, 2012).

The plant *Cassia sieberiana* DC (*Caesalpinaceae*) is distributed in Nigeria, Senegal, DR Congo, Uganda and Gambia, with its leaves, roots and fruits widely used in ethnoveterinary medicine (Atinhedou *et al.*, 2002; Khan, 2009; Obidah *et al.*, 2009). The major phytochemical constituents of methanolic stem bark extract of *Cassia sieberiana* are tannins, flavonoids, alkaloids, saponins, carbohydrates and reducing sugars, and that the extract possesses anti-oxidant activity, (Tamboura *et al.*, 2005; Donkor *et al.*, 2013; Ihedioha *et al.*, 2013). The anti-nociceptive activity of the ethyl acetate extract of the root bark has been shown to be attributable to interactions with opioidergic, muscarinic cholinergic and adenosinergic systems (Donkor *et al.*, 2013). It has been reported that the aqueous extract of *Cassia sieberiana* stem bark has acaricidal activity against larvae of *Hyalomma* ticks, and there was a positive correlation between extract concentrations and the larval mortality rate (Biu *et al.*, 2014). Acaricidal properties of plant extracts have been attributed to their bioactive components such as flavonoids, anthraquinones and tannins (Fernandes and Freitas, 2007; Ribeiro *et al.*, 2007; Juliet *et al.*, 2012; Biu *et al.*, 2014) by providing inhibitory effects on vitellogenin during oogenesis (Jonsson, 2004), acceleration of the hatching rate and mortality of *Hyalomma* newly hatched larvae (Abdel-Shafy and Zayed, 2002) and these compounds have been reported as phyto-components of *Cassia sieberiana* (Vander Maesen *et al.*, 2008). This study evaluates the acaricidal activity of *Cassia sieberiana* stem bark aqueous extract using *Hyalomma* species.

MATERIALS AND METHODS

Plant Collection and Extraction

The fresh stem bark of *Cassia sieberiana* plant was collected within the University of Maiduguri campus, identified by a Botanist

with a voucher Number LCMC 228. It was cut into smaller pieces to enhance drying under shade at room temperature ($\pm 27^{\circ}\text{C}$). The dried plant material was ground into fine powder to obtain 1000g, which was extracted in 4000mL distilled water at 60°C for 8 hours in a Soxhlet extractor. The extract was concentrated on an aluminium tray using hot air oven (40°C) to obtain a weight of 251.1 grams equal to 25.1% w/w yield.

Collection and Identification of Ticks

Fully engorged adult *Hyalomma* female ticks were collected from a flock of naturally infested sheep and goats at the Maiduguri livestock market. The ticks were detached from the animals using thumb forceps and transferred into clean bottles and taken to the Veterinary Parasitology Laboratory of the University of Maiduguri for identification based on the keys provided by Walker *et al.* (2013 and further experimental studies.

Egg-hatch Inhibition Assay

Forty-eight (48) fully engorged female *Hyalomma* ticks were incubated into 24 test tubes, two ticks per tubes and plugged with cotton wool, under ambient conditions of temperature ($\pm 27^{\circ}\text{C}$) and relative humidity ($\pm 85\%$), to allow for oviposition. Oviposited eggs were collected using fine hair brush, and spread over a clean ruled Petri dish to obtain a total count of 800 eggs using a digital counter with the aid of a hand lens. The eggs (160) were divided into 8 replicates of 20 eggs each and immersed into extract concentrations of 5, 10, 15, 20% and test tube control (untreated) respectively for a period of 1, 2, 3, 4, 5, 6, 7 and 8 hours respectively. The immersed eggs were then recovered from the various tests, dried on a Whatman filter paper No. 1 and then placed into plastic specimen tubes (25 x 50mm), incubated at ambient room temperature ($27\text{-}29^{\circ}\text{C}$) and relative humidity (85-90%) for a period of 30 days for visual estimation of hatching. The

eggs under various treatments were compared with that of the normal controls. Egg-hatch was measured by subtracting the number of counted larvae from the number of eggs incubated for each replicate (Sharma *et al.*, 2017)

Adult Mortality Testing

One hundred and sixty (160) adult *Hyalomma* ticks, divided into 4 groups (A, B, C and D) of 8 replicates each of 5 ticks were immersed in extract concentrations of 5%, 10%, 15% and 20% for a the period of 1, 2, 3, 4, 5, 6, 7 and 8 hours respectively. Group E also replicated 8 times of 5 ticks each served as normal control (kept in an empty Petri dish) and exposed for the same immersion hours. Extract-tested ticks were then recovered from the various concentrations, dried on a Whatman filter paper No. 1 and then placed under a stereoscope for visual evaluation of adult mortality indicated by absence of motility, pedal reflex and darkened cuticle (Pirali-Kheirabadi and Teixeira da Silva, 2011).

RESULTS

Figures 1 and 2 shows the adult mortality and egg-hatch inhibition patterns of graded concentrations of *Cassia sieberiana* stem bark aqueous extract against *Hyalomma* ticks based on immersion time in hours. Adult mortality was highest at 20% concentration, while egg-hatch inhibition peaked at 5% concentration at 8hrs, 10 and 15% at 7 and 8hrs and 20% at 5, 6, 7 and 8hrs. There was a positive correlation between graded extract concentration, immersion period, egg-hatch inhibition and adult mortality. Figure 3 indicates the linear regression line for adult mortality based on graded extract concentrations and number of ticks dead and Figure 4 shows the linear regression line for egg hatch inhibition based on graded extract concentrations and number of eggs inhibited. The LC_{50} values for adult mortality and egg-

hatch inhibition were 13.1 mg/ml and 13.7 mg/ml and coefficient of determination were $y = 3.7x + 1.5$ and $y = 3.062 + 8.12$ respectively. Both LC_{50} values were almost

similar indicating a strong association between extract concentration and acaricidal activity *in vitro*.

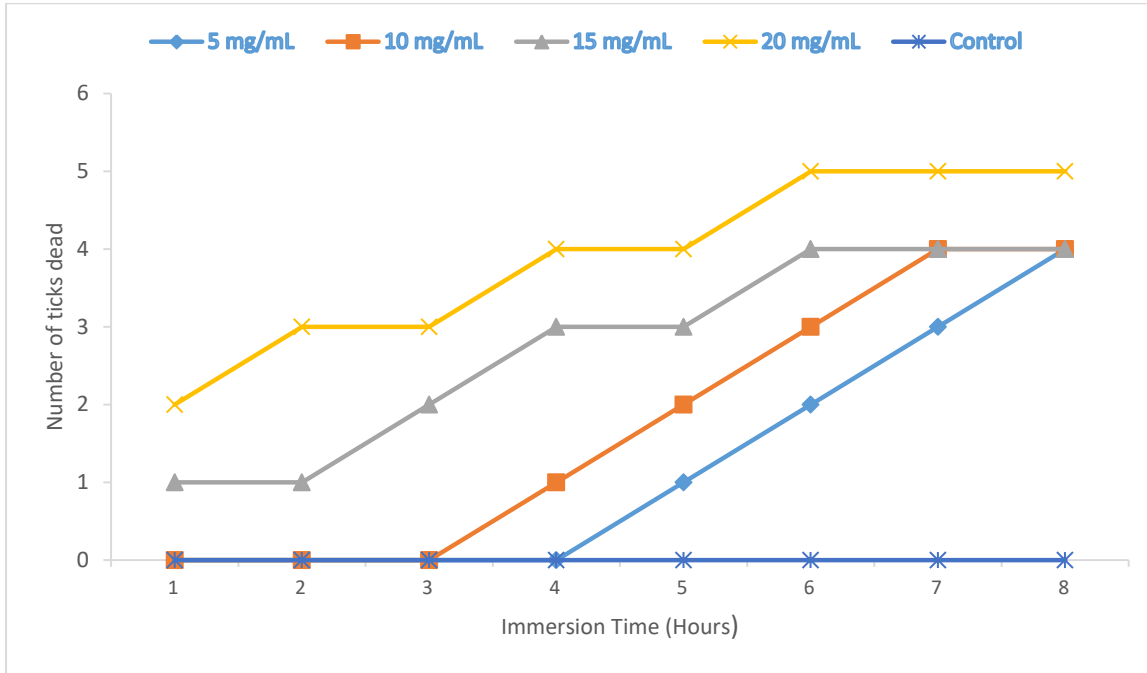


Figure 1: Adult mortality pattern based on graded extract concentrations and immersion time (hours)

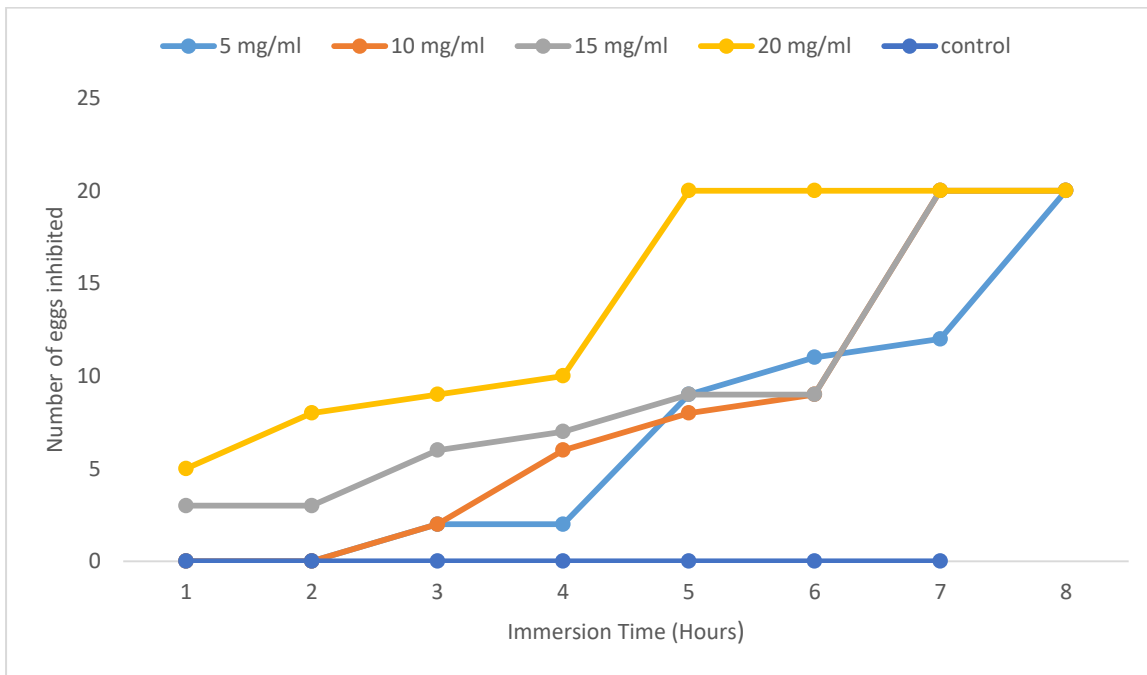


Figure 2: Egg hatch inhibition pattern based on graded extract concentrations and immersion time (hours)

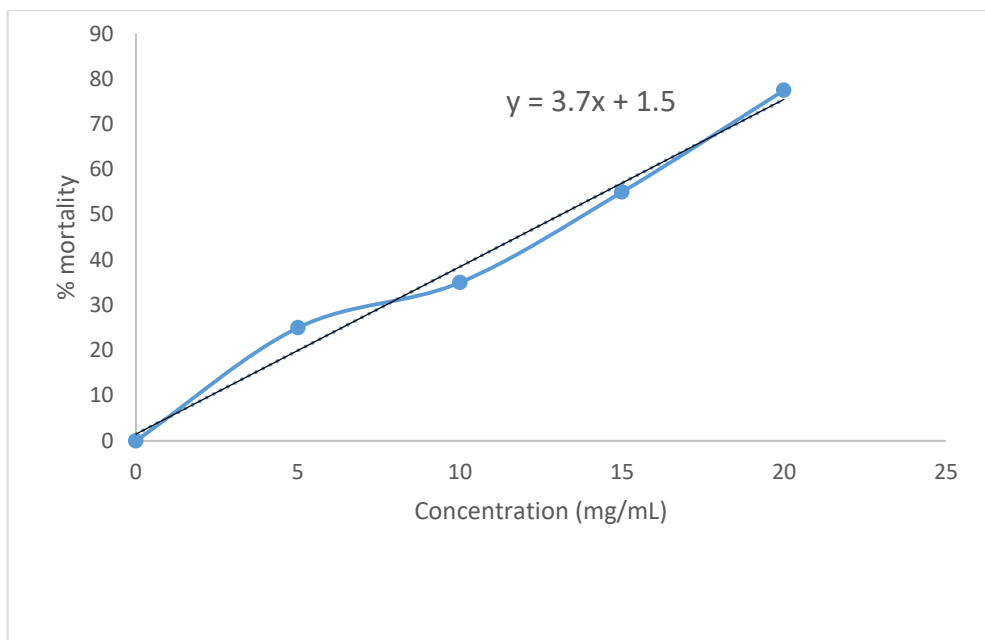


Figure 3: Linear regression line for adult mortality based on graded extract concentrations and number of ticks' dead

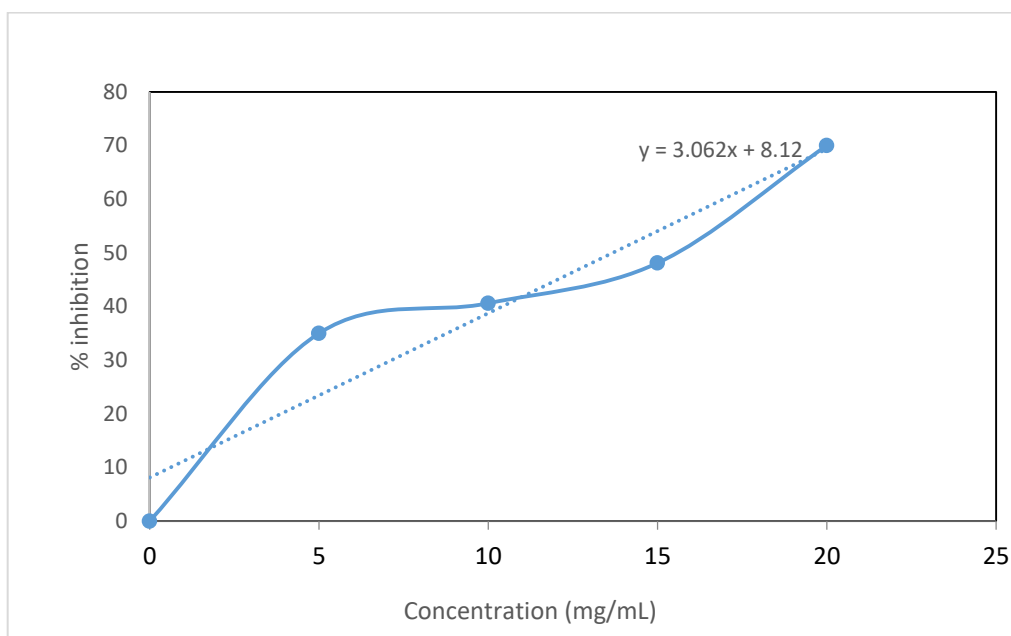


Figure 4: Linear regression line for egg hatch inhibition based on graded extract concentrations and number of eggs inhibited

DISCUSSIONS

The results of this study have shown that the aqueous extract of *Cassia sieberiana* stem bark has egg inhibition and adult mortality effects on *Hyalomma* ticks. There was a positive correlation between graded extract concentration, immersion time, egg-inhibition, and tick mortality. This finding agrees with the reports of Biu *et al.* (2013) who established the acaricidal efficacy of *Cassia sieberiana* stem bark aqueous extract on *Hyalomma* larvae, though the larval stages were typically more susceptible to the treatment of the extract than the adult ticks. Although, the protocol of this study was not designed to address the mode of action of aqueous extract of *Cassia sieberiana* stem bark, Juliet *et al.* (2012) and Biu *et al.* (2013) have attributed its acaricidal activities to its phytochemical composition. Aliyu *et al.* (2013) also reported that extracts of *Cassia sieberiana* had bioactive components such as flavonoids, saponins, glycosides, anthraquinones and tannins. The results of this study showed highest efficacy of the extract at 20% concentration for egg-hatch inhibition and adult mortality. This is in consonance with the findings by Chagas *et al.* (2002) who used essential oils of *Eucalyptus globules*. The results also revealed that the egg-hatch inhibition and adult mortality patterns are directly proportional to the immersion period and concentration of the aqueous extract of *Cassia sieberiana* stem bark. This result concurs with the findings of Piralikheirabadi and Teixeira da Silva (2011) who obtained an increasing mortality rate of *Rhipicephalus (Boophilus) annulatus* exposed to different concentrations of *Zataria multiflora* and *Artemisia annua* essential oils. Costa and Rabitto, (2008) also reported that the hydro-alcoholic extracts of leaves of *Eucalyptus* species had efficacy of 96% against engorged female ticks at a concentration of 10% with increasing post inoculation time.

It is believed that the aqueous extract contains chemicals that can penetrate rapidly into the tick cuticle to reach the body cavity and destroy the epithelial gut cells and finally leading to death of the ticks. This observation is similar to those of Aliyu *et al.* (2013) who used methanol and benzene extracts of *Cassia sieberiana* on malaria parasites and obtained 92% elimination at 10mg/mL after 72 hours, and Ribeiro *et al.* (2007) who used crude methanolic extract of *Hypericum polyanthemum* on *Boophilus microphus*. Also, similar findings were obtained by Chungsamarnyart and Jansawan (2001) who dipped matured *Tamarindus indicus* in water and 10% ethanol for 24hrs, 48hrs and 7 days, and the resultant extracts tested against engorged female cattle tick, *Boophilus microphus*. They reported that the mean correlated mortality of ticks were 56 – 70%, 70 – 89% and 77 – 99% after dipping for 24hrs, 48hrs and 7 days respectively. Another report was made by Piralikheirabadi and Teixeira da Silva (2011), who used the essential oils of *Artemisia annua* and *Zataria multiflora* which ranged from 26.6% (using 10 μ L/cm³) to 100% (using 40 μ L/cm³) and for *Artemisia annua* essential oil it was 33.2% to 100% (using 20 μ L/cm³ and 80 μ L/cm³ respectively). Researchers have indicated that *Cassia sieberiana* is very toxic (Tamboura *et al.*, 2005; Obidah *et al.*, 2009) and this might have justified its acaricidal activities on the *Hyalomma* ticks in this study.

CONCLUSION

In this study, the aqueous extract of *Cassia sieberiana* stem bark was evaluated for possible acaricidal activity on *Hyalomma* ticks using egg inhibition and adult mortality assays. The results reveal a positive correlation between graded extract concentration and immersion time with egg-inhibition, and tick mortality, with the highest efficacy observed at 20% concentration for egg-hatch inhibition and adult mortality. Lastly, the observed acaricidal activity was

attributed to phytochemicals present in the plant extract.

ACKNOWLEDGEMENT

The authors wish to acknowledge immensely the contribution of members of the laboratory unit, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Borno State, Nigeria.

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AUTHORS CONTRIBUTION

Abdullahi A. Biu, Maimunatu A. Abdulkadir, Umar A. Maina, ThankGod E. Onyiche and Saidu I. Ngulde, conceived, designed and coordinated the study, while Raliat Aliyu and Aliyu M. DanZaria conducted the experiments assisted by the appropriate laboratory staff.

CONFLICT OF INTEREST

There is no conflict of interest as regards to this manuscript

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