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Acaricidal effect of *Cassia fistula* Linn. leaf ethanolic extract against *Rhipicephlaus (Boophilus) annulatus*

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Abstract. The present study evaluates the acaricidal properties of crude ethanolic extract of *Cassia fistula* leaves for controlling *Rhipicephalus (Boophilus) annulatus* based on adult immersion test (AIT). The percentage of adult mortality, inhibition of fecundity and hatching of ova laid were studied at different concentrations of the extract ranging from 50 to 100 mg / ml. The results were compared using one-way ANOVA. The extract produced complete inhibition of hatching of eggs at concentrations above 80 mg / ml of the extract. Mortality of adult engorged female ticks and inhibition of fecundity were concentration dependent. The LC_{50} value of extract against *R. (B.) annulatus* was 97.1 mg / ml.

INTRODUCTION

Ticks account for significant loss to livestock production in terms of the direct and indirect damages that they cause. Some of the pathogens they transmit can be fatal to the host animals. At present, tick and tick borne disease (TTBD) control is mainly effected by the use of acaricides like organophosphates, carbamates, pyrethroids, BHC-cyclodines, amidines, macrocyclic lactones and benzoyl phenyl ureas leading to various problems such as resistance to acaricides, residues, environmental pollution and high cost. These factors reinforce the need for alternate approaches to control tick infestation (Ghosh *et al*., 2007). The ethno-veterinary medical knowledge offers a range of drugs that need to be evaluated for their insecticidal and acaricidal properties. A number of reports are available on the effects of various herbal extracts on different tick species (Abdel - Shafy & Zayed, 2002; Borges *et al*., 2003; Ribeiro *et al*., 2008; Ravindran *et al*., 2011, 2012; Juliet *et al*., 2012).

Cassia fistula Linn. (Golden shower tree) is a flowering plant in the family Fabaceae, native to southern Asia, from southern Pakisthan east through India to Myanmar and south to Sri Lanka. It is a medium sized, fast growing tree that can reach a height of 10 - 20 m. The leaves are deciduous or semi–green, 15 - 60 cm long, pinnate with 3 - 8 pairs of leaflets, each leaflet 7 - 21 cm long and 4 - 9 cm broad (Govindarajan, 2009).

Various medicinal uses of the plant (Rizvi *et al*., 2009) and the phytochemical constituents of the plant have been reviewed (Bahourn *et al*., 2005). Recently, the larvicidal, ovicidal and repellant effects of *C. fistula* against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* were also reported (Govindarajan *et al*., 2008; Govindarajan, 2009). However, there is a lack of literature on the acaricidal effects of this plant.

Rhipicephalus (Boophilus) annulatus is reported to be the most common species in southern India (Jagannath *et al*., 1979; Koshy *et al*., 1982; Rajamohanan, 1982). The

objective of the following study was to evaluate the acaricidal activity of ethanolic extract of the plant *C. fistula* against *R. (B.) annulatus*.

MATERIALS AND METHODS

The leaves of the plant *C. fistula* (popularly known as Kanikonna in local language) were collected from Wayanad district of Kerala in December, 2008. The plant was identified by a plant taxonomist and voucher specimen herbarium was deposited at Department of Botany, University of Calicut, (CALI, Accession number: 6633), Kozhikode, Kerala.

Plant leaves were dried in the shade at room temperature. Dried plant leaves were pulverized using a plant sample grinder. The powdered plant material (100 g) was used for ethanolic extraction in a soxhlet extraction apparatus attached to a rotary vacuum evaporator (Rotavac, Butchi, Switzerland). Solvent was completely removed by drying at room temperature. Required quantity of extract was weighed and dissolved in distilled water for making six different dilutions (50 mg / ml, 60 mg / ml, 70 mg / ml, 80 mg / ml, 90 mg / ml and 100 mg / ml).

Fully engorged adult *R. (B.) annulatus* female ticks were collected from infested animals, washed with water and blotted dry with tissue paper. The average weight of engorged ticks ranged from 0.15 to 0.18 g. These females were used for *R. (Boophilus) annulatus* AIT (Drummond *et al*., 1973).

Various dilutions (50-100 mg / ml) of ethanolic extract of the plant leaves were tested using AIT. A total of 192 ticks were used for the experiment. Four replicates of six numbers of ticks were used for each dilution of the extract. Groups of six ticks were weighed prior to the experiment and were immersed for 2 minutes in 10 ml of the respective dilution in a 50 ml beaker while gently being agitated. Distilled water was used as negative control. Analytical grade pure compound of deltamethrin (99%) (Accu standard USA) was used at a rate of 30 ppm as a positive control. Ticks were recovered from the solution, blotted dry using tissue paper and placed in separate plastic specimen tubes (25 X 50 mm). The tubes were incubated at 28°C and 80% relative humidity in a BOD incubator.

The specimen tubes were observed and adult tick mortality within 15 days of treatment was determined in comparison to the control. The eggs laid by the ticks were collected, weighed, and maintained under the same environmental conditions as above. Eggs were observed for the next 30 days for visual estimation of hatching rate. Ticks under different treatments were compared with that of the controls.

The percentage inhibition of fecundity was calculated as follows:

Index of egg laying (IE) = weight of eggs laid (mg) / weight of females (mg)

Percentage inhibition of fecundity (IF) = [IE (control group) – IE (treated group)] x 100 / IE (control group) (FAO, 2004).

Data were expressed as the mean ± SEM. Groups were compared using one-way ANOVA for repeated measurements using SPSS software. Duncan's test was used for post-hoc analysis. A value of $P < 0.05$ was considered significant. Dose response data were analyzed by probit method (Finney, 1952). The LC_{50} values of crude extract against *R.* (*B.*) *annulatus* were determined by applying regression equation analysis to the probit transformed data of mortality.

RESULTS

The results of the AIT using the ethanolic extract of *C. fistula* leaves in comparison to deltamethrin are shown in Table 1. The efficacy of the acaricide against *R. (B.) annulatus* females was assessed by measuring the percentage of adult mortality, inhibition of fecundity and hatching rate. Treatment with deltamethrin at 30 ppm caused 16.66% adult tick mortality and an inhibition of fecundity of 57.3 % respectively. No egg hatching was observed at this concentration.

Table 1. Effects of different concentrations of ethanolic extract of *C. fistula* against *R. (B.) annulatus*

Acaricide	Mean ticks weight per replicate ± SEM(g)	Mean % adult mortality within 15 days ± SEM	Mean eggs mass per replicate ± SEM (g)	Index of fecundity ± SEM	Percentage Inhibition of Fecundity (%)	Hatching % (Visual)
CFE 50 mg/ml	0.9752±0.0379^{a}	12.495±4.1650^{ab}	0.4746±0.0171^{c}	0.4870±0.0080^{cd}	2.83	10
CFE 60 mg/ml	1.0024±0.0286^{a}	20.830±7.9780^{abc}	0.4687±0.0130^{c}	0.4678±0.0092^{cd}	6.66	10
CFE 70 mg/ml	0.9664±0.0036^{a}	33.330±6.8055^{bc}	0.4431±0.0244^{bc}	0.4585±0.0248^{cd}	8.52	10
CFE 80 mg/ml	0.9723±0.0408^{a}	33.330±6.8055^{bc}	0.4133±0.0226^{bc}	0.4248±0.0126^{bc}	15.24	0
CFE 90 mg/ml	1.0625±0.0424^{a}	37.499±10.4857^{c}	0.4452±0.0186^{bc}	0.4199±0.0154^{bc}	16.22	0
CFE 100 mg/ml	0.9513±0.0647^{a}	58.330±4.8093^{d}	0.3676±0.0638^{b}	0.3782±0.0449^{b}	24.54	0
Water	0.9315±0.0518^{a}	0±0^{a}	0.4638±0.0086^{c}	0.5012±0.0199^{d}	0	100
Deltamethrin (30 ppm/0.03 mg/ml)	0.9653±0.0361^{a}	16.662 ±6.803^{abc}	0.2081 ±0.0276^{a}	0.2140±0.0236^{a}	57.3	0

n = 4, Values are Mean ± SEM, means bearing different superscripts a, b, c or d (P<0.05), indicate significant difference when compared with the control and recommended concentration of deltamethrin; CFE- *C. fistula* ethanolic extract

The ethanolic extract of *C. fistula* leaves showed moderately higher acaricidal effect against *R. (B.) annulatus*. It exhibited a significant concentration dependent acaricidal activity with maximum effects at 100 mg/ml. Adult mortality was comparable with deltamethrin (0.03 mg/ml), even at the lower dose of 50 mg/ml. At concentrations of 70 mg/ml and above, *C. fistula* leaf extract showed a significantly higher mortality than deltamethrin.

The percentage of inhibition of fecundity ranged from 2.83 to 24.54 which was significantly less than the effect of deltamethin. However, from a concentration of 70 mg / ml of the extract, egg masses laid by the treated ticks differed significantly.

Another significant observation was that, at concentrations ranging from 50-70 mg/ml, only 10% of laid eggs hatched, while at concentrations above 70 mg / ml, no hatching was observed, which was again comparable to the effect of deltamethrin.

Results of probit analysis are shown in Figure 1. The minimum extract required for 50% mortality of ticks was 97.1 mg / ml.

DISCUSSION

In the present study, AIT was performed to evaluate the acaricidal effects of different concentrations of the ethanolic extract of *C. fistula in vitro*. In India, topical application (mainly by sponging, washing, spraying, pour on or dipping) of acaricides is the widely accepted method for controlling of ticks. The contact time adopted in the present study (2 minutes) almost simulates the practical application of acaricide solution on the animal in the field. The topical application of the acaricides should disrupt the outer epicuticular waxy layer of ticks that serves various functions like preventing water loss, providing protection from physicochemical hazards and producing sex pheromone (Cherry, 1969; Hamilton *et al.*, 1989; Yoder *et al.*, 1996). A three fold increase in the amount of surface wax may occur during the engorgement of adults of *Amblyomma americanum* (Yoder *et al.*, 1996) and *R. microplus* (Cherry, 1969). It can be inferred that the adult fully engorged tick is the toughest life stage in the course of tick development and hence the most suited stage for *in vitro* acaricidal testing. Moreover, this test can evaluate the effect of the drug on tick reproduction.

Cassia fistula is widely used for its medicinal properties. The antimicrobial (Perumal *et al.*, 1998; Agarwal *et al.*, 2004; Muthuswamy *et al.*, 2006; Yogesh & Mohan, 2006) and antifungal (Duraipandian & Ignacimuthu, 2007) activities of *C. fistula*

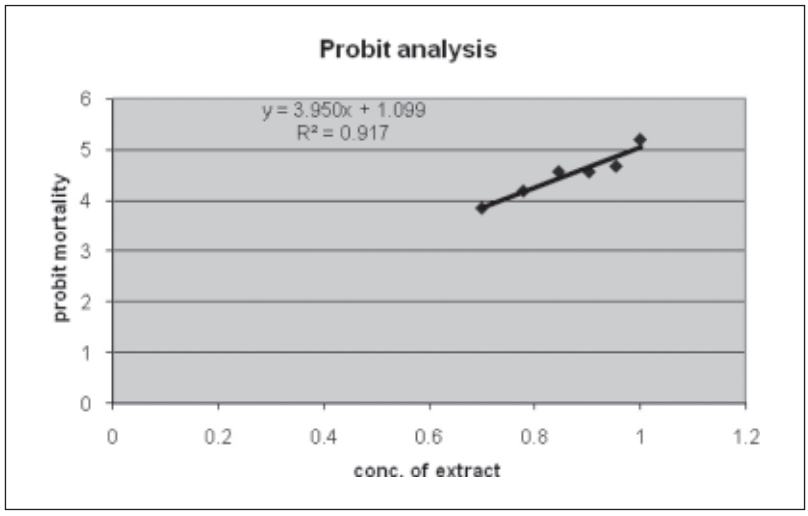

Figure 1. Graph showing log concentrations of ethanolic extract of *C. fistula* on X-axis and probit mortality calculated from Finney's table on Y- axis

extract are well known. Analgesic, antipyretic, anti-inflammatory, hypoglycemic, antitumor, antioxidant and hepatoprotective activities of *C. fistula* were also reported. These activities were mainly attributed to the presence of alkaloids, triterpene derivatives, anthraquinone derivatives and polyphenolics comprising of flavanoids, catechines and proanthocyanidins (Morimoto *et al*., 1988; Kashiwada *et al*., 1990). Raja *et al*. (2000) reported that the leaf extract of *C. fistula* significantly reduced the egg laying and fecundity of *Callosobruchus maculatus* Fab.

It may be noted that the ethanolic extract of *C. fistula* at the dose rate of 70 mg/ml was better adulticide compared to deltamethrin. Even though, the percentage inhibition of fecundity is less than that of deltamethrin, *C. fistula* extract at higher concentrations completely blocked eclosion. The leaf extract of *C. fistula* was also reported to possess remarkable larvicidal, ovicidal and repellent activity against *Cx. quinquefasciatus* and *Anopheles stephensi* (Govindarajan *et al*., 2008; Govindarajan, 2009).

The presence of (-) epiafzelechin, (-) epiafzelechin-3-O-glucoside, (-) epicatechin, procyanidin B2, biflavanoids, triflavanoids, rhein, rhein glucoside, sennoside A, sennoside B, chrysophanol and physcion (Kaji *et al*., 1968; Mahesh *et al*., 1984) were qualitatively detected in the leaves of *C. fistula*. Sakulpanich & Gritsanapan (2009) identified rhein as the major anthraqunone component in *C. fistula* leaf extract just as the pod extract. Rhein isolated from the ethyl acetate extract of *C. fistula* flowers exhibited antifeedant and larvicidal activities against lepidopteron pests *viz*; *Spodotera litura* and *Helicoverpa armigera* (Duraipandian *et al*., 2011). Rhein, is also a known inhibiter for proliferation of various human cancer cells by inducing apotopsis through caspase dependent pathway (Chang *et al*., 2012). Hence the acaricidal activity observed in the present study could be attributed to the presence of the anthraqunone compound rhein.

The LC_{50} values of methanolic leaf extract of *C. fistula* against *Cx. quinquefasciatus* and *An. stephensi* were 17.97 and 20.57 respectively (Govindarajan *et al*., 2008). In the present study, fifty percent mortality of *R. (B.) annulatus* female ticks (LC_{50}) was achieved at 97.1 mg / ml of *C. fistula* crude extract.

The complete inhibition of egg hatch observed at the highest concentration of *C. fistula* might be due to inhibition of prostaglandins or depletion of waxy water proofing of eggs. Prostaglandins play an

important role in the regulation of reproductive activities *viz.*, peristaltic activity of oviduct and controlling the secretion from tubular accessory sexual gland in ticks. The presence of pro-anthocyanidins and flavanoids were reported from young and old *C. fistula* leaves (Luximon-Ramma *et al.*, 2002). Flavanoids inhibit eicosanoid biosynthesis (Damas *et al.*, 1985) and also inhibit both cytosolic and membrane tyrosine kinase enzyme involved in a variety of biological functions (Formica & Regelson, 1995). This suggests that the extract produced a concentration dependent inhibition of fecundity by inhibiting the PGE_2 mediated pathway. The anti-inflammatory (Yadava & Verma, 2003) and antitumor effects produced by *C. fistula* extract were also attributed to the presence of flavanoids through the same pathway. Further studies are needed to explore the possible mechanism for the ovicidal activity of *C. fistula* extract.

For the first time the acaricidal effects of the ethanolic extract of leaves of *C. fistula* are reported. The extract produced a concentration dependant mortality of adult ticks. Complete blocking of hatching of laid eggs was observed at concentration above 80 mg / ml. Further studies are needed to identify the active ingredient and its possible mode of action.

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