***Original Research Article***

# In-vitro study on acaricidal efficacy of methanolic extracts of *Calotropis gigantea* against cattle tick (*Rhipicephalus (B.) microplus*) in Udaipur (Rajasthan)




## Abstract

The present research was designed to evaluate acaricidal efficacy of methanolic extracts of ***Calotropis gigantea*** against cattle tick *Rhipicephalus (Boophilus) microplus* in Udaipur (Rajasthan). Acaricidal efficacy was evaluated Larval packet Test (LPT) and Adult Immersion Test (AIT). Four concentrations of the ***Calotropis gigantea*** extract (12.5%, 25%, 50%, and 100%) and one control group with twice replications for each concentration were used in the bioassay. The Highest efficacy in both in-vitro tests (AIT and LPT) was recorded in 100% methanolic extracts of ***Calotropis gigantea***. The methanolic extracts of ***Calotropis gigantea*** has highest IO% at 100% concentration which was 67.46% in AIT. The 100%concentrations of methanolic extracts showed minimum reproductive index (0.16). In AIT the decrease in reproductive index with increase in the percent inhibition of oviposition, was evident during the study. In LPT, methanolic extracts of ***Calotropis gigantea*** has highest efficacy at 100% concentration which was 64%. Larval mortality increased with increasing concentrations of plant extracts.


**Comment [m1]:** % or mg/ml???

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**Comment [m3]:** of 64%

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Keywords: ***Calotropis gigantea***, ticks, larval packet test (LPT) and adult immersion test (AIT)

## 1. Introduction

India is predominantly an agricultural country where livestock and agriculture are closely

associated with each other. For control of the economically important tick species, organophosphates, pyrethroids and macrocyclic lactones are used at frequent intervals leading to the development of resistance in ticks to most of these chemicals. In Indian sub continent, the incidence of acaricide resistance has been widely reported in one host ticks of the genus Boophilus (Shyma et al. 2013)[6]. In order to tackle the problem of resistance and other environmental issues linked with chemical control, efforts have been made to develop sustainable immunological means for controlling ticks and tick-borne diseases through alternative ecofriendly anti-tick natural products. Amongst the natural products, plant extracts and essential oils have been shown to have significant activity against economically important tick species like *Rhipicephalus (Boophilus) microplus.* (Sunil *et al*., 2013)[8]. The plant extracts can be made from easily available local plants which have history of some pesticidal or medicinal properties and traditionally used by the people. *Calotropis gigantea* (crown flower) is a species of *Calotropis* native to Cambodia, Indonesia, Malaysia, Philippines, Thailand, Sri Lanka, India, China, Pakistan, Nepal and tropical Africa. The latex of *Calotropis gigantea* contains cardiac glycosides, fatty acids and calcium oxalate. *Calotropis* is a poisonous plant and the active principles are uscharin, calotoxin, calactin and calotropin. It possess fungicidal and insecticidal properties (Daniel, 2006)[2].

## 2. Materials and Methods

Plant materials were selected on the basis of available scientific literature and brought into the laboratory. After complete drying, the plant material was powdered in mortar pestle and grinder. The powder of ***Calotropis gigantea*** parts was processed for extract preparation by using maceration methods (Shyma et al., 2014)[5]. 100 gm powder from extracted using 400 ml methanol as solvents. The mixtures were kept for 2 days in tightly sealed vessels at room temperature and stirred several times daily with a sterile glass rod. These mixtures were filtered through muslin cloth. Further extraction of residue was done repeating 3-5 times untila clear colorless supernatant extraction liquid was present indicating that no more extraction from the

plant material was possible. The extracted liquid was subjected to water bath evaporationat 40℃ to remove the solvent. The semi-solid extract was kept under a ceiling fan to dry.

**3. Collection of ticks**

The ticks were collected from animals and the cattle sheds during early morning from Vallabhnagar tehsil of Udaipur district (Rajasthan). The ticks collected were preserved in70% alcohol in clean, well-stopper glass vials and labeled properly. Permanent mounts of ticks were prepared as per standard keys. The damaged and discolored ticks were removed and remaining engorged female ticks were selected for AIT. The engorged female ticks collected from a particular area were labelled and kept individually in labeled glass tubes covered with muslin cloth. Ticks were put in desiccators maintained at room temperature and 85±5% relative humidity (RH) for oviposition. The eggs were collected after 7 days from commencement of incubation. Each tube containing the first week egg production was labeled to ensure the selection of more uniform batch of larvae for each LPT. The eggs laid were allowed to hatch under uniform conditions of incubation and 14- 21days old unfed larvae were utilized for the performance of Larval Packet Test (LPT).

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**3.1. Preparation of working solutions of plant extracts**

The working solutions of plant extracts were prepared as per standard protocol. Dried powder was kept in room temperature for 15-20 minutes. Required quantity of extracts were weighed and dissolved in methanol for making four different dilutions at the rate of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml. In control group only distilled water was used.

**4. Statistical Analysis**

The collected data from the experiment was subjected to statistical analysis using SPSS, version 20.0 for analysis of variance (Snedecor and Cochran, 1980)[7]. The treatment means were compared by Duncan's multiple range test (Duncan, 1995)[3] at 5% level of significance (P<.05).

## 5. Results and Discussion

### 5.1. Efficacy of methanolic extracts of *Calotropis gigantea* in Larval Packet Test (LPT)

The concentrations of methanolic extracts of seeds of *Calotropis gigantea* of varied from 12.5 to 100 mg/ml. The peak mortality (57%) was recorded at a concentration of 100 mg/ml. A total of four treatment groups and one control group were used in experiment. In control group no mortality of ticks was observed. A significant larval mortality produced by application of extracts of 50 mg/ml, 25 mg/ml and 12.5 mg/ml were 47.33%, 32.33% and 14.33% respectively. With the increase in concentration level the percent mortality rate also increased, as shown in (Table. 1)

**Table 1: Efficacy of different concentrations of methanolic extracts of *Calotropis gigantean* against *Rhipicephalus (Boophilus) microplus* larvae by LPT**

| Concentration of extract (mg/ml) | Live larvae | SE | Dead larvae | SE | % of Larval mortality | SE |
|---|---|---|---|---|---|---|
| Control | 100.000$^{e}$ | 0.000 | 0.000$^{a}$ | 0.000 | 0.000$^{a}$ | 0.000 |
| 100 | 43.000$^{a}$ | 0.577 | 57.000$^{e}$ | 0.577 | 57.000$^{e}$ | 0.577 |
| 50 | 52.670$^{b}$ | 1.453 | 47.330$^{d}$ | 1.453 | 47.330$^{d}$ | 1.453 |
| 25 | 67.670$^{c}$ | 0.882 | 32.330$^{c}$ | 0.882 | 32.330$^{c}$ | 0.882 |
| 12.5 | 85.670$^{d}$ | 2.028 | 14.330$^{b}$ | 2.028 | 14.330$^{b}$ | 2.028 |

Means bearing different superscript in the same column differ significantly P<0.05

### 5.2 Efficacy of *Calotropis gigantea* in methanolic extracts against *Rhipicephalus (Boophilus) microplus* in Adult Immersion Test (IO %)

AIT was used in present study to determine the acaricidal activity against *Rhipicephalus (Boophilus) microplus* with various concentration of *Calotropis gigantean* of methanolic extract; FAO, (2004)[4].The different concentration of methanolic extracts various from 12.5 to 100 mg/ml. In AIT the dependent decrease in reproductive index and increase in inhibition of oviposition was observed from concentration 12.5 to 100 mg/ml. A significant percentage inhibition of oviposition (IO %) at 100, 50, 25 and 12.5 mg/ml the extracts were 64.57, 60.79, 53.76 and 45.56 % respectively. No mortality of ticks was observed at any concentration, as shown in (Table.2).

**Table 2: Acaricidal efficacy of different concentrations Methanolic extracts of *Calotropis gigantean* against *Rhipicephalus (Boophilus) microplus* by AIT**

| Conc. of extract (mg/ml) | Live ticks weight (gm) (Mean) | (SE) | Weight of eggs (gm) (Mean) | (SE) | Repro-duction Index (RI) (Mean) | (SE) | %IO (Mean) | (SE) |
|---|---|---|---|---|---|---|---|---|
| Control | 0.725 | 0.001 | $0.365^{e}$ | 0.002 | $0.503^{e}$ | 0.002 | $0.000^{a}$ | 0.000 |
| 100 | 0.735 | 0.002 | $0.131^{a}$ | 0.002 | $0.178^{a}$ | 0.002 | $64.571^{e}$ | 0.245 |
| 50 | 0.718 | 0.002 | $0.142^{b}$ | 0.002 | $0.197^{b}$ | 0.003 | $60.797^{d}$ | 0.341 |
| 25 | 0.721 | 0.013 | $0.168^{c}$ | 0.002 | $0.233^{c}$ | 0.002 | $53.769^{c}$ | 0.639 |
| 12.5 | 0.706 | 0.002 | $0.194^{d}$ | 0.001 | $0.274^{d}$ | 0.001 | $45.562^{b}$ | 0.042 |

Means bearing different superscript in the same column differ significantly P<0.05

Shyma *et al.*, (2014)[5] have reported 63.2% larval motility were quite similar to our results. Zaman *et al.*, (2012)[9] evaluated acaricide effect of *Calotropis procera* flowers using larval packet test and ear bag method. The extract exhibited lethal effects on total larval mortality at 50 mg/ml. which are similar with the present findings. Acaricide effect of flowers of combined aqueous herbal extracts of *Azadirachta indica* leaves and *Calotropis procera* flowers were evaluated using adult immersion test, larval packet test and ear bag method. The extract exhibited lethal effects on egg laying (index of egg laying, 0.371404 ± 0.00435), hatching (22.35%). Al-Rajhy *et al.*, (2003)[1] tested extract from *Calotropis procera* and reported better efficacy than Azadirachtin on the basis of LC 50 values against adult stages of the camel tick, *Hyalomma dromedarii*. the probit regression analysis of the extracts showed that with the increase in the dose of extracts, a significant increase in the mortality rate of treated ticks with significant inhibition inreproduction which are similar to our findings.

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**Comment [m8]:** Please include the recent article in your discussion In vitro Acaricidal Activity and Phytochemical Screening of Calotropis gigantea Flowers against Rhipicephalus microplus
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