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## SCREENING OF INDIGENOUS MEDICINAL PLANTS FOR THEIR ACARICIDAL ACTIVITY AGAINST CATTLE TICKS UNDER *IN VITRO* CONDITION

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**ABSTRACT:** The aqueous and alcoholic leaf extracts of *Annona squamosa*, *Azadirachta indica* and *Calotropis procera* were tested against the cattle ticks. The fresh and leaf powder extracts were tested individually and also in combinations under *in vitro* conditions. The extracts of *A. indica* showed maximum mortality rate of ticks followed by *A. squamosa* and *C. procera* when tested individually. In combination, the hot water extracts of leaf powder showed 100% mortality whereas ethanol and methanol extracts showed 83% and 80% of mortality respectively. Based on the above experimental results, it is confirmed that the selected plant materials possess more acaricidal activity against cattle ticks. Extracts in combination are more effective than single drug used. Since the herbal drugs are cost effective and easy to process, it can be used as an alternative medicine to control cattle ticks.

**INTRODUCTION:** Ethnoveterinary medicine is frequently used for animal healthcare by different people around the world. Ethnoveterinary medicinal plants used extensively for primary health care treatment to make domestic animals productive and healthy. Ectoparasitic infestation is one of the major veterinary problems affecting livestock.

Among ecto-parasites, ticks have been recognized as the notorious threat due to severe irritation, allergy, toxicosis and cause lowered productivity and mortality. Plants have the ability to synthesize chemical compounds that help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals.

This investigation is to study the *in vitro* acaricidal activity of the *Annona squamosa*, *Azadirachta indica* and *Calotropis procera* against cattle ticks.

### MATERIALS AND METHODS:

**Collection and preparation of plant extract:** The plant materials used for this study are the leaves of *Annona squamosa*, *Azadirachta indica*, and *Calotropis procera*. They are collected, shade dried and mechanically powdered. The leaf powder of selected plants was extracted with solvents like ethanol, methanol and water.

### Preparation of extracts from dried leaf powder:

- 1. Cold extract:** The powder was soaked in known volume of distilled water to make an extract. After 24 hours the extract is filtered through muslin cloth.
- 2. Hot extract:** Pre weighed plant powder material was soaked in known volume of distilled water and kept in hot water bath for 6-7 hours at 70°C and cooled it under room

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temperature. After 18 hours the extract is filtered using muslin cloth and the filtrate is stored for further use (Hot water extract).

#### Preparation of extracts from fresh leaves:

- Cold water extract:** The fresh leaves were collected and washed thoroughly in tap water, crushed using mixer grinder and soaked in distilled water for 24 hours and filtered.
- Hot water extract:** The fresh leaves were collected and washed thoroughly in tap water, crushed using mixer grinder and soaked in known volume of distilled water and kept in hot water bath for 6-7 hours at 70°C and cooled it under room temperature. After 18 hours the extract is filtered using muslin cloth and the filtrate is stored for further use.

**Preparation of combination of leaf extracts:** The fresh and dried leaf materials of all the three plants were extracted individually with different solvents. Different concentrations of extracts (ranging from 12% -24%) are prepared, combined together in 1:1:1 ratio and used to test the mortality rate of ticks under *in vitro* conditions.

**Collection of ticks:** The ticks were collected from the infected cows of organized farm at different ages. The adult ticks were collected from different parts of the body by way of hand manipulation method. The collected ticks were put in vials and wrapped with dry cotton cloth for oxygen supply and used for further testing.

**In vitro study:** *In vitro* effect of acaricidal activity of different extracts of selected plants was tested on ticks collected from naturally infected cows. The collected ticks are randomly divided and each group contains 7 ticks. *In vitro* study was carried out by pour on method<sup>1</sup> and the ticks were transferred into plastic cups containing different concentrations (12%, 14%, 16%, 18%, 20%, 22% and 24%) of leaf extracts. Control was maintained without plant extracts. Mortality rate of the ticks in all concentrations were recorded after 2 hours. LC<sub>50</sub> value was calculated by Kaber's method<sup>2</sup> using the formula;

$$LC_{50} = \frac{\sum \text{Mean death} \times \text{Concentration difference}}{\text{Number of organisms per group}}$$

**RESULTS:** The fresh and dried leaves of *A. squamosa*, *A. indica* and *C. procera* were extracted with different solvents like ethanol, methanol and water in hot (Soxhlet extraction) and cold (Room temperature) conditions. The extracts were tested individually as well as in combination against the ticks collected from infested cows to find out their efficacy in controlling ticks. All the leaf extracts of *A. indica* showed highest mortality rate (86%) at the concentration of 24% followed by *A. squamosa* and *C. procera*. The acaricidal activity of methanol extract of *A. squamosa* occupy second place among the tested plants (Tables 1-3).

The leaf extracts of selected plants were also tested in combination against cattle ticks at different concentrations. Among the extracts tested, aqueous extract revealed 100% mortality of ticks followed by alcohol extracts at the concentration of 24% (Table 4).

TABLE 1: *IN VITRO* EFFECT OF DRIED LEAF POWDER EXTRACTS ON CATTLE TICKS

S. No.	Extracts used	Mortality rate (%) of ticks in different concentration of extracts								LC <sub>50</sub> value of the extracts
		Control (water)	12%	14%	16%	18%	20%	22%	24%	
<b>Hot water extract</b>										
1.	<i>Annona squamosa</i>	0	0	0	14	29	29	43	57	20.5
2.	<i>Azadirachta indica</i>	0	0	14	43	57	57	71	86	17.7
3.	<i>Calotropis procera</i>	0	0	0	0	14	14	29	43	22
<b>Cold water extract</b>										
4.	<i>Annona squamosa</i>	0	0	7	14	14	29	29	43	21.4
5.	<i>Azadirachta indica</i>	0	0	14	29	29	43	57	71	19.4
6.	<i>Calotropis procera</i>	0	0	0	0	14	14	29	29	22.3

**TABLE 2: IN VITRO EFFECT OF FRESH LEAF EXTRACTS ON CATTLE TICKS**

S. No.	Extracts used	Mortality rate (%) of ticks in different concentration								LC <sub>50</sub> value of the extracts
		Control (water)	12%	14%	16%	18%	20%	22%	24%	
<b>Hot water extract</b>										
1.	<i>Annona squamosa</i>	0	0	7	14	29	43	43	57	20
2.	<i>Azadirachta indica</i>	0	0	14	43	57	71	86	86	17.1
3.	<i>Calotropis procera</i>	0	0	7	14	14	29	43	57	20.9
<b>Cold water extract</b>										
4.	<i>Annona squamosa</i>	0	0	14	14	29	29	43	43	20.6
5.	<i>Azadirachta indica</i>	0	0	14	27	43	71	71	86	17.4
6.	<i>Calotropis procera</i>	0	0	7	14	29	43	57	57	19.7

**TABLE 3: IN VITRO EFFECT OF ALCOHOLIC EXTRACT OF DRIED LEAF POWDER ON CATTLE TICKS**

S. No.	Extracts used	Mortality rate (%) of ticks in different concentration								LC <sub>50</sub> value of the extracts
		Control (water)	12%	14%	16%	18%	20%	22%	24%	
<b>Hot water extract</b>										
1.	<i>Annona squamosa</i>	0	0	7	14	29	29	43	43	20.9
2.	<i>Azadirachta indica</i>	0	0	29	43	57	71	78	86	16.9
3.	<i>Calotropis procera</i>	0	0	0	0	14	29	29	43	21.7
<b>Cold water extract</b>										
4.	<i>Annona squamosa</i>	0	0	7	14	29	43	57	57	20
5.	<i>Azadirachta indica</i>	0	0	29	43	57	71	7	86	17.4
6.	<i>Calotropis procera</i>	0	0	0	0	29	29	43	43	21.1

**TABLE 4: COMBINED IN VITRO EFFECT OF LEAF EXTRACTS OF A. SQUAMOSA, A. INDICA AND C. PROCERA ON CATTLE TICKS**

S. No.	Extracts used	Mortality rate (%) of ticks in different concentration							LC <sub>50</sub> value of the extracts
		12%	14%	16%	18%	20%	22%	24%	
1.	Powder+ hot water	0	29	57	57	71	86	100	16.6
2.	Powder+ cold water	0	29	57	57	71	86	100	16.9
3.	Fresh leaves+ hot water	0	14	14	29	29	43	43	19.7
4.	Fresh leaves +cold water	0	14	29	29	43	71	86	18.9
5.	Powder + Ethanol	0	14	43	57	86	71	86	18
6.	Powder+ methanol	0	14	14	29	57	71	86	19.7

**DISCUSSIONS:** In the present study, the leaf extracts of *A. squamosa*, *A. indica* and *C. procera* were tested against cattle ticks as single drug as well as in combinations. Both aqueous and alcohol extracts of *A. indica* showed maximum mortality rate of ticks. The chemical components, Azadirachtin, Nimbin and Nimbinin present in leaves of *A. indica* possess acaricidal activity. This is also confirmed by the earlier studies<sup>3</sup>.

In another report, Neem seed oil and Neem Azal tested against the larvae of *Boophilus decoloratus* ticks showed 100% mortality<sup>4</sup>. Next to *A. indica*, the methanol extract of *A. squamosa* occupy second position in acaricidal activity. This is due to the presence of the compound annonin<sup>5</sup>, squamosin and Acetogenins<sup>6</sup>.

Apart from single herbal drug effect, attempts were also made to find out the combined effect of leaf extracts on cattle ticks. Among the solvents tested, the aqueous extracts showed 100% mortality rate of ticks compared to alcohol extracts. This is due to the synergistic effect of the chemical components present in the plant materials.

In earlier report, the combined effect of leaves of *A. indica* and *Nicotiana tobaccum*, flowers of *C. procera* and seeds of *Trachyspermum ammi* exhibited total larval mortality in *Rhipi cephalus* (*Boophilus*) *microplus*<sup>7</sup>. This may be due to the presence of the secondary metabolites, alkaloids, glycoside and phenolic compounds which initiate the mechanism of action *in vitro* causing mortality of ticks<sup>8</sup>.

Hence, it is concluded that the combination of leaf extracts of these three plants possess more acaricidal properties than single drug used. Thus it can be recommended as an alternative to allopathic medicine.

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