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Research Article
Acaricidal Activity of *Cassia fistula* Linn. Ripe Pod Extract Against *Rhipicephalus sanguineus* Semi-Engorged Females

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Abstract

Background and Objective: The present study evaluates the acaricidal activities of *Cassia fistula* ripe pod extract on mortality rate, laying rate and morphological changes of *Rhipicephalus sanguineus* for search a new alternative method of controlling of ticks population. **Materials and Methods:** A total of 90 semi-engorged female ticks were divided into 3 groups (0.25% w/v fipronil as a synthetic acaricide, *C. fistula* ripe pod extract and distilled water) with 3 replications. All ticks in each group was evaluated by immersion test, then the ticks were incubated for 14 days and dead ticks were fixed with Karnovsky's solution for scanning electron microscopy examination. **Results:** *Cassia fistula* extract showed acaricidal activity. The laying rate of female ticks treated with extracts of the *C. fistula* pod extract was 33.33% which was significantly less ($p<0.05$) than the control group (50.00%) and there was no significant difference between mortality of the *C. fistula* pod extract group (83.33%) and the fipronil group (100%). All ticks in this study showed normal external structure by scanning electron microscopy observation. **Conclusion:** Extract from *C. fistula* ripe pod affected mortality and inhibited oviposition of semi-engorged female ticks and could be a promising natural acaricidal product. Further studies are needed to identify the active ingredients present in this plant and explore its possible mechanisms of action.

Key words: *Cassia fistula*, *Rhipicephalus sanguineus*, mortality, laying rate, extraction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ticks are one of the most important ectoparasites in dogs[1,2] and are involved in the transmission of several diseases in both dogs and humans[3]. The dominant tick reported in dogs in Thailand is *Rhipicephalus sanguineus*, known as the brown dog tick. It is the main vector of tick-borne pathogens which cause critical infections that are potentially fatal, e.g., *Ehrlichia canis*, *Babesia canis* and *Hepatozoon canisin* canines[4-6] and *Rickettsia rickettsii*, *Rickettsia conorii* in humans[7-8]. The incidence of tick-borne pathogens has been reported to have increased worldwide in recent years, seriously threatening human and animal health[9]. Therefore, extensive studies have been carried out to search for methods for controlling and prevention ticks, including both synthetic and non-synthetic methods[2]. Synthetic acaricidals has been one of the most used methods to control the ectoparasite but there are several reports that revealed side effects on animals and humans and acaricidal resistance of *R. sanguineus*[10-13]. Due to these problems, new alternative, safer and more environmentally friendly methods of controlling ticks, such as using plant extracts, need to be developed[14-16].

Cassia fistula Linn. (family: Fabaceae) is a medical plant, commonly known as the Golden Shower[17]. It is native to southern Asia and is one of the most widespread plants in Thailand. Various medicinal uses of several parts of plant and their phytochemical activities have been reviewed[18]. It has reported to have various therapeutic potentials such as antitumor[19], anti-inflammatory[20] and antibacterial[21] activity. Sartorelli *et al.*[22] studied the extract from the *C. fistula* fruits, showing significant anti-leishmanial activity against the promastigote form of *Leishmania chagasi*. Moreover, lethal effects of *C. fistula* leaf extract against *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles stephensi* and *Rhipicephalus* (*Boophilus) annulatus* were also reported by Sunil *et al.*[15], Govindarajan[17] and Govindarajan *et al.*[23].

This study aimed to evaluate the *in vitro* acaricidal activity of *C. fistula* ripe pod extract on laying rate, mortality and morphological changes of *R. sanguineus* semi-engorged females compared with fipronil which is a synthetic acaricidal product.

MATERIALS AND METHODS

Tick preparation: A total of 90 *R. sanguineus* semi-engorged females, average weight 30 mg, from one dog were used in this experiment. The ticks were separated into groups of 10, with 3 repetitions for each treatment with (1) The acaricide fipronil (0.25% w/v), (2) *Cassia fistula* ripe pod extract and (3) Control groups. Before treatment, all ticks were morphologically examined under a stereoscope (Nikon, Tokyo, Japan). The experiment was reviewed and approved by the Institutional Animal Care and Use Committee, Mahasarakham University (approval number: 0026/2017).

Plant extract preparation: The ripe pods of *C. fistula* were collected from Maha sarakham province, Thailand in April, 2017. A decoction extract was processed following the method of Sakulpanich and Gritsanapan[24]. About 10 g of the fresh ripe pod was separated from *C. fistula* pods and boiled with 100 mL distilled water for 1 h at 95-98°C. The mixture was filtered through a Whatman size 1 filter paper and the pod was re-extracted several times until the extraction was paled. The decoction extracts were mixed, filtered again and the filtrate was evaporated to dryness on a boiling water bath to yield a dry plant extract. The dry crude extract was dissolved in distilled water at a concentration of 100 mg mL^{-1}.

Immersion test: *Rhipicephalus sanguineus* semi-engorged female ticks were washed with distilled water and then dried on absorbent paper. Afterwards, the ticks were immersed in test solutions in petri dishes for 5 min following the method given by Juasook *et al.*[14]. The three treatment groups consisted of 0.25% w/v fipronil (FP), *C. fistula* ripe pods extract (CE), while ticks in the control group (CON) were immersed in distilled water for the same time period. The ticks were dried on absorbent paper and placed in an incubator (Memmert, Schwabach, Germany) under controlled conditions, 28±1°C, RH >80% and a 12 h photoperiod, for 14 days.

Mortality and laying rate: The acaricidal effect on semi-engorged ticks was investigated and recorded everyday of the experiment for calculation of mortality rate and laying rate. Dead ticks were identified by loss of motility and pedal reflex. Dead ticks were fixed with Karnovsky's solution for scanning electron microscopic examination. The calculations used in this experiment were:

$$\text{Mortality rate} = \frac{\text{Dead ticks}}{\text{Total number of ticks}} \times 100$$

$$\text{Laying rate} = \frac{\text{Laying ticks}}{\text{Total number of ticks}} \times 100$$

Scanning electron microscopy: To observe the surface structure of the tick post-treatment, ten dead ticks from each group were washed several times with 0.2 M cacodylate buffer (pH 7.2) and fixed with Karnovsky's solution at 4°C overnight. After washing for 10 min, 3 times, with the phosphate buffer, samples were post-fixed with 1% OsO_4 in 0.1 M phosphate buffer (pH 7.2) for 1 h, followed by washing again for 10 min, 3 times, in phosphate buffer. Samples were then dehydrated through a graded ethanol series (30, 50, 70, 90, 95 and 100% alcohol, 2 times), dried with a critical point dryer, coated with gold using a JFC-1100E ion sputtering device (JEOL, Tokyo, Japan) and observed with a JEOL JSM-7800F scanning electron microscope (SEM) at a 1.0 kV accelerating voltage.

Statistical analysis: The mortality rate and laying rate were analyzed by one-way ANOVA (SPSS version 22.0). Tick survival was also evaluated by Kaplan-Meier analysis. A $p \leq 0.05$ was considered statistically significant.

RESULTS

Laying rate: The effect of each treatment on the oviposition of *R. sanguineus* semi-engorged females was examined by the laying rate, which was observed during incubation of ticks. This experiment showed that fipronil had the highest acaricidal activity, completely inhibiting oviposition of female ticks. Interestingly, the *C. fistula* ripe pod extract had efficacy in controlling egg laying by ticks, although with lower activity than the chemical product. The laying rates of female ticks treated with extracts of the *C. fistula* ripe pod extract were 33.33%, significantly less ($p<0.05$) than the control group (50.00%) (Fig. 1).

Mortality rate: Ticks received various treatments and were then incubated to study the mortality rate. After treatment, dead ticks were identified by loss of motility and pedal reflex and the number of deadticks in each group was observed and recorded everyday post-treatment (Fig. 2). The tick mortality in all groups started at days 4-6 post-treatment with peak mortality on days 9-10. Remarkably, this study revealed no significant difference of mortality rate in the FP and CE groups (100 and 83.33%, respectively). At the end of the experiment, the lowest mortality was found in the CON group (46.67%).

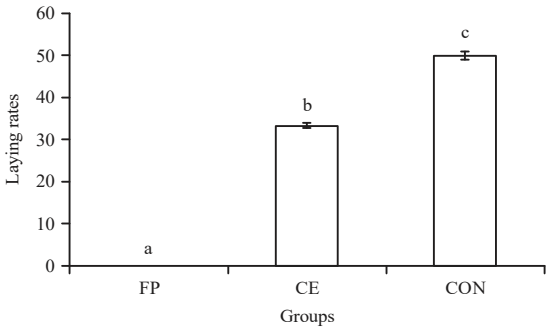

Fig. 1: Laying rates of *R. sanguineus* semi-engorged females
FP: Treated with fipronil, CE: Treated with *C. fistula* pod extract, CON: Control, a-c indicated a significant difference at $p<0.05$

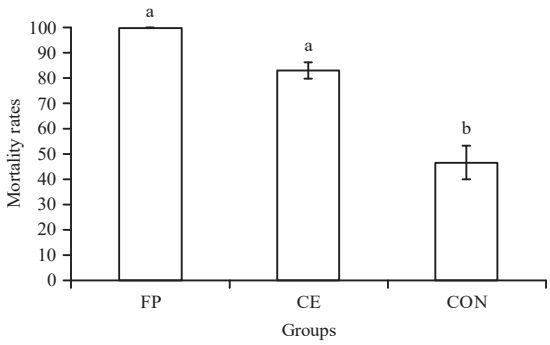

Fig. 2: Mortality rates of *R. sanguineus* semi-engorged females
FP: Treated with fipronil, CE: Treated with *C. fistula* pod extract, CON: Control, a-b indicate a significant difference at $p<0.05$

Moreover, Kaplan-Meier analysis of survival of *R. sanguineus* semi-engorged females (Fig. 3) showed median survival time in the FP group and the CE group (9 and 10 days, respectively). Median survival time in the control group was longer than the both of the other groups which can not be evaluated from the short duration of the experiment.

Scanning electron microscopy: In the present study, dead female ticks in all experiments were fixed and processed for analysis of acaricidal activity on the morphological changes of *R. sanguineus* semi-engorged females. All ticks in this study showed normal external structure, except that ticks exposed to fipronil as a chemical acaricide showed a thin and smooth surface which ticks in the natural treated group and control group had a thick and wrinkled surface (Fig. 4).

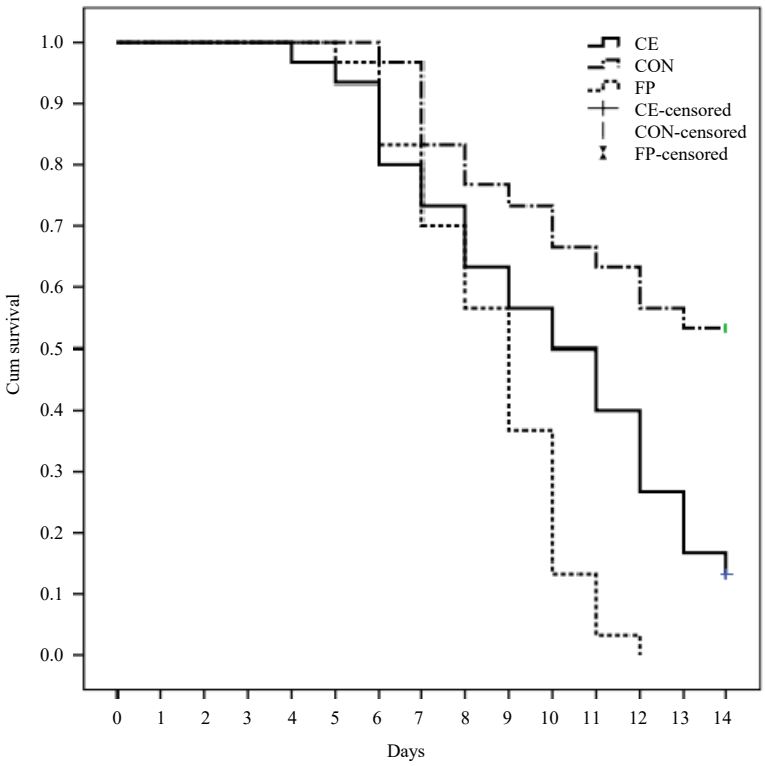

Fig. 3: Kaplan-Meier analysis of surviving *R. sanguineus* semi-engorged females in each treatment group during the experiment
FP: Treated with fipronil, CE: Treated with *C. fistula* pod extract, CON: Control, There were significances in overall survival between all treated groups and control group ($p<0.05$), Hash marks indicate censored events

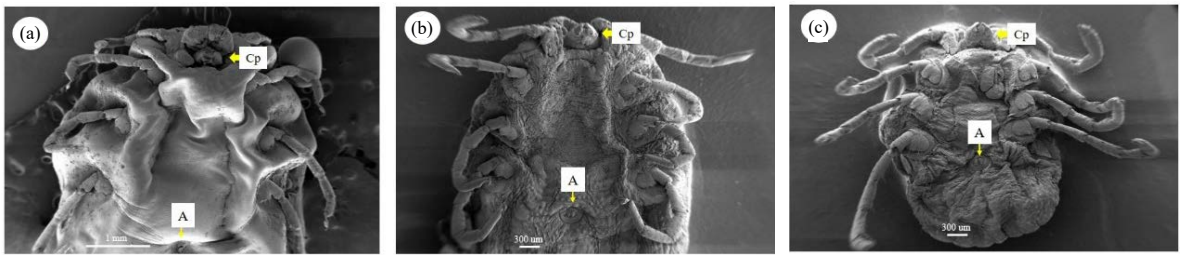

Fig. 4(a-c): Scanning electron micrographs of *R. sanguineus* semi-engorged females ventral view, (a) Treated with fipronil, (b) Treated with *C. fistula* pod extract and (c) Control
Cp: Capitulum, A: Anus

DISCUSSION

Considering that *R. sanguineus* tick has become an important ectoparasite, it is desirable to investigate new methods of control aiming to find alternatives which are less aggressive to the environment and non-target organisms. This study investigated the acaricidal activity of a natural plant, *C. fistula* ripe pod extract, on laying, mortality and morphology of *R. sanguineus* semi-engorged females compared with fipronil.

Fipronil, is popular for use in tick management in dogs and is a widely accepted acaricide belonging to the phenylpyrazole family[25]. It interferes with the central nervous system of insects by acting mainly in the GABA system[10,26,27]. In this study, ticks treated with fipronil had absolutely mortality and oviposition was entirely inhibited for 14 days after treatment, in agreement with a previous report by Juasook *et al.*[14] and De Oliveira *et al.*[28]. The Scanning Electron microscopy showed a thin and smooth surface of a semi-engorged tick. The mechanism of action of fipronil is not restricted to the tick's nervous system but also inhibits the feeding process by damaging the salivary gland[12]. Therefore, the actions of fipronil on tick's surface are needed to further studies. This result supported previous studies showing that

fipronil also has an impact on the reproduction of the tick[28,29]. Nevertheless, the toxicity of fipronil can contaminate the environment and damage non-target organisms, including animals and humans[10,30] and also has been classified as a possible human carcinogen[31,32]. Therefore, many plant extracts were tested for their acaricidal effects.

Cassia fistula Linn. is a flowering plant which is widespread in Thailand and is known as Khun in Thai language. The whole plant possesses medicinal properties[33] and also antiparasitic, larvicidal and ovicidal activities[17,22]. In Thai traditional medicine, the ripe pods have been used as laxative drug[34]. The ripe pod contains several anthraquinones such as rhein, aloin, emodin, sennosides and aloe-emodin, both in aglycone and glycosides forms[35]. The present study collected *C. fistula* ripe pods and decoction extracted them to investigate their acaricidal activity.

Even though, the acaricidal activity against female brown dog ticks by inhibiting laying rate is less than for the synthetic acaricide, *C. fistula* ripe pods extracts at 100 mg mL^{-1} mortality was not significantly different from the fipronil group. From scanning electron microscopy, ticks in both the *C. fistula* ripe pods extracts and control group demonstrated similar morphology. To evaluated the mechanism of this plant extract for female tick controlling, ultrastructure microscopy need to further experiment.

Previous research, the ripe pod extract of *C. fistula* was also identified rhein as the major anthraquinone component as the leaf extract[34]. Rhein, an anthraquinone derivative is known to exhibit a variety of biological activities such as antimicrobial, antifungal, purgative, anti-inflammatory, analgesic, anti-tumor and hypoglycaemic activities[34,35]. Duraipandiyan *et al.*[36] demonstrated that rhein isolated from the ethyl acetate extract of *C. fistula* flowers exhibited antifeedant and larvicidal activities against cotton bollworm and leaf worm. In addition, the acaricidal effect of *C. fistula* leaf extract against *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles stephensi* and *Rhipicephalus* (*Boophilus*) *annulatus* were also reported by Sunil *et al.*[15], Govindarajan[17] and Govindarajan *et al.*[23]. The acaricidal activity shown in this experiment could be attributed to the presence of the anthraquinone compound rhein.

CONCLUSION

The results of this study demonstrate that *C. fistula* ripe pod extracts affected the mortality and inhibited oviposition of *R. sanguineus* semi-engorged females, indicating that these plant extracts can be active against brown dog tick and could be a promising natural acaricidal product. However, the acaricidal property of the *C. fistula* has not been previously reported. Further studies are needed to identify the active ingredients present in the plant ripe pods and explore its possible mechanism for the inhibiting laying rate and the mortality of ticks.

SIGNIFICANCE STATEMENT

This study discovered that the *C. fistula* ripe pod extracts affected the mortality and inhibited oviposition of *R. sanguineus* semi-engorged females. Thus, this plant extract, which is a natural compound, could be a promising natural acaricidal product to control *R. sanguineus* in dogs. Therefore, the active ingredients present in this plant and its possible mode of action for the control of tick population should be defined in further studies.

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