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Full Length Research Paper

Evaluation acaricidal efficacy of *Camellia sasanqua* thumb seed oil against the cattle tick *Rhipicephalus (Boophilus) microplus* and the dog tick *Rhipicephalus sanguineus*

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Our study was the first research to investigate the *in vitro* and *in vivo* effect of *Camellia sasanqua* thumb seed oil on the cattle tick *Rhipicephalus (Boophilus) microplus* and the dog tick *Rhipicephalus sanguineus*, which are the most common ticks infested on cattle and dogs in Vietnam. The lethal dose 50 (LD50, the dose that induced the death of 50% experimental ticks within 360 minutes tested time) and the lethal dose 100 (LD100, the dose that induced the death of 100% experimental ticks within 360 minutes tested time) of the crude oil were 4.61 ± 0.4% and 9.18 ± 0.7% with *Rhipicephalus (Boophilus) microplus* and 5.43 ± 0.6% and 9.50 ± 0.3% with *Rhipicephalus sanguineus* in the larval immersion tests. The trial clinical treatment performed on dogs infested with *Rhipicephalus sanguineus* showed that only one treatment including 2 times of 10.0% oil spraying at 24 hours interval was able to eliminate all of the ticks. Our study proved both of *in vitro* and *in vivo* acaricidal effect of *Camellia sasanqua* thumb seed oil and therefore suggested the potential of applying this botanical oil in the tick control.

Key words: *Camellia sasanqua* thumb, acaricide effect, seed oil, *Rhipicephalus (Boophilus) microplus*, *Rhipicephalus sanguineus*, tick, dog, cattle.

INTRODUCTION

The application of chemical acaricides in the tick control has many significant drawbacks such as side-effect, handling hazard, environmental pollution, drug residues and especially the development of resistance. Many researchers have reported the increased resistance in target species to chemical acaricides (Clark et al., 1996; Currie et al., 2004; Fernandes and Freitas, 2007; Halley et al., 1993; Nong X et al., 2012; Nong X et al., 2013a; O´Brien, 1999; Ribeiro et al., 2007; Terada et al., 2010).

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In view of these problems, there has been an increasing interest in searching for alternative sustainable control methods of ticks in recent years (KH Pirali-Kheirabadi and JA Teixeira da Silva, 2011). Phytotherapy is considered a viable alternative to the use of synthetic compounds for the control of tick infestation in livestock (Madzimure et al., 2011; Moyo and Masika, 2009). Plants with acaricidal properties could be used as an alternative which would help to limit the well-documented damage that the synthetic acaricides can cause to the environment. Some studies have used herbal medicine for the control of the ticks (Fernandes and Freitas, 2007; Kaaya GP et al., 1995; Pirali-Kheirabadi KH et al., 2007; Pirali-Kheirabadi KH et al., 2011; Ribeiro et al., 2007). Botanical acarcides have many advan-

tages features: they can be degraded in the environment, do not remain in livestocks, are not as prone to resistance and are relatively safe for humans, animals and the environment (Nong X et al., 2012).

In Vietnam, *Camellia sasanqua* thumb seed oil (CST oil) can be used as an edible oil and is a good raw material for the manufacturing of soap (Quang Tri prefecture Online library of science and technology, 2013). CST is also considered as medicine plants as this seed oil has been traditionally applied for the treatment of constipation, intestinal obstruction, ascarid infection, scabies, louse in human and has also been used as insecticide in agriculture (Đo Tat Loi et al., 1970; Le Tran Đuc, 1977; Information institute- Vietnamese central library, 2013). The commercial CST oil in Vietnamese market is mainly sold as the insecticide and still there is no research about its acaricidal activity. Based on traditional knowledge and the recommendation of herbalists, in the present study, we tried to investigate the *in vitro* and *in vivo* effect of CST oil on the ticks.

In Vietnam, the most common ticks on dogs is *Rhipicephalus sanguineus* (RS) (Nguyen Van Thanh et al., 2012) and the most common ticks in cattle is *Rhipicephalus (Boophilus) microplus* (RBM) (Phan Dich Lan, 1974; Phan Trong Cung et al., 1977; Trinh Van Thinh et al., 1982). In the north of Vietnam, *Bophillus*.spp occupies 95% of parasitic ticks in cattle (Nguyen Huu Ninh, 1984). Due to the wide distribution in Vietnam, we decided to focus on those ticks in the investigation of acaricide property of CST oil. The present study examined the *in vitro* and *in vivo* acaricidal property of CTS oil against the dog ticks RS and cattle ticks RBM to evaluate the potential of applying this oil as the botanical control agent for the ticks.

MATERIALS AND METHOD

Extraction Methods

The rippen fruits with yellowish-brown skin of *Camellia sasanqua* thumb were collected from The Garden of Medicine plants for veterinary (Faculty of Veterinary medicine, Hanoi university of Agriculture, Hanoi, Vietnam) during the harvesting season (from September to October). The fruits were then dried in sunlight from 4-5 days for the seeds to be automatically ruptured. The dried seeds were de-husked and the husks were carefully separated from the kernels. The kernels were roasted in fried-pan for 30 minutes and finely ground using the kitchen blender before pressed by family oil extruder to obtain the CST oil. This oil was heated to 60-65 °C and filtered through the 3 layers of cheese cloth. This crude oil had the pH value ranged from 4 to 5 and was named acid-oil in the study. NaOH 40% was used to adjust pH of the crude oil to the neutral value (6.8 to 7.2) and this adjusted oil was named neutral-oil in the study. Those extracts were diluted in distilled water and glycerol (1:1) to obtain the concentrations used in experiments.

Tick Collection and Rearing

Adult cattle ticks were collected from naturally infested cattle in the farms located near faculty of Veterinary Medicine, Hanoi University of Agriculture, Trau Quy commune, Gia Lam district, Hanoi, Vietnam. Adult dog ticks were collected from naturally infested dogs in Professional Dog Research Center, faculty of Veterinary Medicine, Hanoi University of Agriculture, Trau Quy commune, Gia Lam district, Hanoi, Vietnam. Animals were suspensed for acaricidal treatment for at least 2 months priory to the tick collection. Female ticks were selected morphologically (full engorged) and maintained in the laboratory at 26 °C and 80% relative humidity in test tubes for laying eggs. After the eggs hatched, tick larvae were fed on healthy dogs and cattle. Fully engored larvae between 14 to 21 days-old were collected and taken to the laboratory within 3-4 hours to perform experiments.

Larval Immersion Test

This test was performed following the mthod of Nong X., et al (2013b) with some modification. A volumne of 2 ml of the respective extract and respective concentration was added to Petri diskes (10 cm diameter, 2 cm height) containing filter paper disks to absorb the liquid. 10 larval ticks were placed on the filter paper, sprayed twice with the extract to be tested and incubated in an incubator at 25-27 °C and 70-80% relative humidity, in which each treatment consisting of 3 replicates. For the control, three petri dishes each containing 2 ml of distilled water and glyceron (1:1) were used. The pH of the control dishes was adjusted by condensed HCl to the values that similar to the respective extract. The viability of ticks was checked regularly by stimulating with a homemade needle and ticks were recorded as dead if no reaction was shown. The maximum time for observation was 360 minutes. If after this time, there were some ticks still alive, the experiments were stopped but the numbers of alive ticks were recorded and the percentages were calculated.

Clinical Treatment of Dog Ticks

Due to the difficulty of gathering cattle in the same places for clinical experiments, the trial treatment was performed only with dogs infested with RS. The animal experiment was performed in Professional Dog Research Center, faculty

Table 1. The LT50 and LT100 of acid-oil and neutral-oil

Concentration	Extract	Time (minute) LT50		LT100	
		Cattle tick RBM	Dog tick RS	Cattle tick RBM	Dog tick RS
10.0 %	Acid-oil	150.3 ± 8.8 [a]	180.7 ± 9.1 [a]	170.3 ± 7.6 [a]	200.7 ± 8.4 [a]
	Neutral-oil	185.3 ± 10.3 [b]	230.3 ± 11.9 [b]	220.7 ± 9.5 [b]	270.7 ± 9.9 [b]
5.0 %	Acid-oil	(48.3 ± 3.2*)	(45.7 ± 3.3*)	(48.3 ± 3.2*)	(45.7 ± 3.3*)
	Neutral-oil	(34.7 ± 4.2*)	(30.3 ± 2.5*)	(34.7 ± 4.2*)	(30.3 ± 2.5*)

Means with different superscripts within each column are significantly different un-pair t test.. (n*) means that there were only n % of experimental ticks died at the end of experiment (after 360 minutes of observation)

of Veterinary Medicine, Hanoi University of Agriculture and was permitted by the chief-director Nguyen Manh Ha (DPRC No. 09). We used 30 Vietnamese male dogs that naturally infested with RS with the body weight from 15 to 17 kgs and the age from 1.5 to 2 years for the experiment. Because those dogs shared the same living environment for at least 3 months priory to the test, they were considered as the same level of tick infestion. Those dogs were randomly devided into 6 groups and then separated to different clean cages with no ticks in surrounding environment. One group was used as control before the experiment, one group was used as control after the first treament, one group was used as the control after the second treatment and other 3 groups were used as experimental groups to test with CST acid-oil at the concentration of 5.0%; 7.5% and 10.0%. We decided to use these 3 concentrations as the representatives of lethal dose 50 (LD50) (5.0%), lethal dose 100 (LD100) (10.0%) and the value in the middle of LD50 and LD100 (7.5%). The 200 ml acid-oil was mixed well and sprayed to wet the whole body of the dogs. The muzzles were used until the oil was dried. After 24 hours, the dogs were check to test the condition of the ticks and if there were any living ticks, the next spraying was applied. After 7 days from the confirmation that there were no ticks alive, the dogs were checked again and if there is any living ticks, the next treatment was performed.

Statistic Analysis

Data was expressed as mean ± standard deviation. Un-pair t-test was used to test for differences between the lethal time 50 (LT50) and lethal time 100 (LT100) of 2 extracts in Table 1. Kruskal Wallis test followed by *Post – hoc* Scheffe's F test was used to test for the difference between the means of death percentage in Table 2. The LT50, LT100 were calculated through the linear regression obtained between the time and the death percentage of the ticks and the LD50, LD100 were calculated through the linear regression obtained between the doses and the death percentages of the ticks. The difference between the LD50 and LD100 of the cattle tick RBM and dog ticks RS in Table 2 was calculated using the unpair t-test. All of those statistical tests were performed on Excel installed with Statcel software (Yanai Hisae, Laboratory of mathematics, Faculty of Science, Saitama University, 1998). The result was considered significant at probability value less than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

The Acaricide Efficacy of 10.0% and 5.0% of Acid-oil and Neutral-oil

In this first step experiment, we investigated and compared the acaricide effect of acid-oil and neutral-oil at the concentration of 10.0% and 5.0%. The LT50 (the time that induced the death of 50% experimental ticks) and the LT100 (the time that induced the death of 100% experimental ticks) are shown in Table 1.

From this table, we see that both of acid-oil and neutral-oil had acaricide effect on cattle ticks RBM and dog ticks RS and this effect was dose-independent because at 5.0%, these 2 extracts were able to kill only from 30.3 ± 2.5% (neutral-oil with dog ticks RBM) to 48.3 ± 3.2% (acid-oil with cattle ticks RS) of tested ticks, while at 10.0% they were able to kill all of tested ticks with the time ranged from 170.3 ± 7.6 to 270 ± 9.9 minutes. We also observed that acid-oil have superior effect to neutral-oil, because the LT50 and LT100 were significantly shorter with both of cattle tick RBM and dog ticks RS. The difference in efficacy of the 2 oil was not due to the pH difference because the control with the same pH values to the extracts did not exert any acaricidal effect.

Table 2. The percentages of death ticks induced by acid-oil 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0% and 10.0%

Concentration (%)	Death percentage	
	Cattle tick RBM	Dog tick RS
10.0	100 ± 0.0 [a]	100 ± 0.0 [a]
9.0	100 ± 0.0 [a]	96. 7 ± 1.3 [a]
8.0	95.3 ± 2.5 [b]	91. 7 ± 2.7 [b]
7.0	78.7 ± 4.8 [c]	66. 7 ± 3.3 [c]
6.0	69.3 ± 6.3 [d]	53.3 ± 5.4 [d]
5.0	49.7 ± 3.9 [e]	43.7 ± 3.8 [e]
4.0	40.0 ± 5.6 [e]	33.3 ± 4.9 [f]
Representive linear regression between concentrations and % of death ticks	y = 10.95 x – 0.507	y = 12.30 x – 16.78
R^2 value	0.941	0.961
LD50	4.61 ± 0.4 [A]	5.43 ± 0.6 [A]
LD100	9.18 ± 0.7 [A]	9.50 ± 0.3 [A]

Means with different superscripts (a, b, c, d, e, f) within each column are significantly different ($p < 0.05$) by Kruskal Wallis and *Post – hoc* Scheffe's F test. The means with the same superscripts (A) within each row have no significant different by un-pair t-test.

The mechanisms by which acid-oil had better effect than neutral-oil can not be explained based on the results of current study. The folow-up reseach is necessary to determine the difference in phytochemical fractionation of these 2 CST oil to understand the mechanisms and also to identify the bioactive components by which they express the acaricidal activity. We suggested that low pH might be a favorable condition to dissolve the active acaricidal ingredients in CST oil. The next-study is on-going to elucidate this difference. However, based on current results, we decided to use acid-oil in further investigation because without pH adnjustment, CST oil had significantly higher efficacy.

The Acaricide Efficacy of Acid-oil at Different Concentrations

Because in Table 1, we saw that acid-oil at 5% was able to kill 45.7 ± 3.3% of dog ticks and 48.3 ± 3.2% of cattle ticks, and 10.0% was able to kill 100% of both ticks, we dicided to used the concentration range: 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, 10.0% to test in order to calculate the LD50 (the dose that induce the death of 50% experimental ticks) and LD100 (the dose that induce the death of 100% experimental ticks). The percentages of death ticks induced by the extract at different concentrations after 360 minutes of observation are shown in Table 2.

From Table 2, we observed that CST acid-oil exerted dose-dependent acaricidal effect on both RBM ans RS ticks, because following the decrement of the concentrations, there was a significant decrement in the percentages of death ticks induced by this oil. At the concentration of 10.0%, this oil was able to terminate all of both CST and RS ticks with the death percentage of 100 ± 1.0%. This reseach is the first study investigated the acaricidal property of CST oil. However, some other researchers have reported the acaricidal property of many essential oil derived from other medicine plants. Ioro et al. (2005) reported the effect of tea tree oil (*Melaleuca alternifolia* oil) against nymph of *Ixodes ricinus.* Abdel-Shafy and Zayded (2002) reported the effect of neem seed oil (*Azadirachta indica* A. Juss) against the adult stage of *Hyalomma anatolicum excavatum* (Ixodoidea: Ixodidae). Prates et al. (1993) reported larvicidal activity of chemical components of the essential oil of *Melunis minutiflora* Beauv. Chagas et al. (2002) reported that at concentration of 10.0%, essential oil of *E. Staigeriana* and *E. Citridora* induced 100% mortality of *Boophilus microplus*. In our study, 10.0% of CST oil also induced 100% mortality of the *Boophilus microplus*.

There was no significant difference in both of LD50 and LD100 of the extract with cattle ticks RBM and dog ticks RS (4.61 ± 0.4% vs. 5.43 ± 0.6% and 9.18 ± 0.7% vs. 9.50 ± 0.3%, respectively). This result suggests that there was no difference in the sensitivity of these 2 ticks to the extract. From Table 2, because the *in vitro* LD50 and LD100 of the dog ticks were 5.43 ± 0.6% and 9.50 ± 0.3%, we decided to use 3 concentrations, including 5.0% (near the LD50), 10.0% (near the LD100) and 7.5% (middle value between LD50 and LD100) to perform the trial clinical treatment on dogs infested with RS ticks in the next step.

The Results of Trial Clinical Treatment on Dogs Infested with Dog Ticks RS

During the treatment, there were no abnormal signs of all tested dogs. The dogs remained healthy with normal behaviours. The treated skins were not rashed. After the oil was dried (from 30 minutes to 1 hour) and the muzzles were taken out, the dogs had no tendency to lick the remained

Table 3. The results of clinical treatment of CST acid-oil on dogs infested with RS ticks

Time		Number of ticks/ dog Control before the test	Control after the 1st treatment	Control after the 2nd treatment	5.0 %	7.5 %	10.0 %
Before experiment		36.2 ± 3.4	NT	NT	NT	NT	NT
Treatment 1	1st apply	NT	NT	NT	+	+	+
	2nd apply	NT	NT	NT	+	+	-
	3 rd apply	NT	NT	NT	-	-	No apply
7 days after 1st treatment		NT	38.4 ± 4.3	NT	+	+	-
Treatment 2	1st apply	NT	NT	NT	-	-	No apply
7 days after 2nd treatment		NT	NT	40.5 ± 2.8	-	-	-
14 days after 2nd treatment		NT	NT	NT	-	-	-

NT: no test. (+) means there were some alive ticks on tested dogs. (-) means there were no alive ticks on tested dogs. No apply means the treatment was not performed on the respective groups.

remained oil on their hairs or skins. The results of the trial clinical treatment on dog ticks are shown in Table 3.

From the results shown in Table 3, we saw that 10.0% acid-oil expressed the best efficacy, because only 1 treatment with 2 times of spraying application at 24 hours interval was able to terminate all of the parasitic ticks. The tick-free condition was remain until 3 weeks after the treatment. On the other hand, lower concentrations (5.0% and 7.5%) were necessary to treated twice to terminate the ticks, in which the first treatment included 3 times of spraying at the interval of 24 hours and the second treatment was performed after 7 days from the first treatment, in which 1 time of spraying was applied. The tick-free condition was remain until 2 weeks after the second treatment. The 3 control groups had no difference in the number of infested ticks per dog during the experimental period (36.2 ± 3.4; 38.4 ± 4.3; 40.5 ± 2.8 ticks/dog for control before the test group, control after the 1st treatment group and control after the 2nd treatment group, respectively), proved that the decrement in the number of ticks in treated groups was due to the CST application. Because all of 3 tested doses did not induce any toxic signs on the dogs, we concluded that the 10.0% was the best applicable dose because only one treatment was necessary, so it would help to save the oil and the working time for the dog owners or the workers in the dog center, where the treatment is necessary to apply on a large dog number. This concentration was then applied to control the ticks during the serious infestion occured in the Professional Dog Research Center from May, 2012 to August, 2012.

The resistance related in *Rhipicephalus sanguineus* to some synthetic acaticides has been recorded in many parts of the world (Fernandes, 2000; Fernandes, 2001; Fernandes and Freita, 2007; Miller et al., 2001). In Vietnam, even that there was no report about the synthetic drug resistance of *Rhipicephalus sanguineus*, but following the observation of the workers and veterinarians in Professional Dog Research Center, the usually applied synthetic drugs became less effective and lead to the repeatation of treatment and therefore significantly increased the cost. CST oil can serve as the cheap and effective alternative. This oil is available on the market at the price of 10 dollar for one litre, the amount that after diluted to 10.0% is enough to performed spraying application for 50 times. In addition, with the abundant of this tree in Vietnam and the simple in extract process, it is possible to self-produce this oil in farm or in animal center for the control of the ticks.

CONCLUSION

Our study was the first reseach which investigated the *in vitro* and *in vivo* effect of the CST oil on animal parasitic ticks. The findings showed that CST oil has acaricidal properties and could be useful in controlling of cattle tick *Rhipicephalus (Boophilus) microplus* and the brown dog tick *Rhipicephalus sanguineus*, which are the most popular ticks parasitized on dogs and cattles in Vietnam.

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