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Original Research Article

A Study on the Acaricidal Effects of Extracts from *Nicotiana rustica L.* and *Stemona tuberosa* Lour on Dog Ticks

Nguyen Thi Kim Lan[1*], Pham Dieu Thuy[1], Dao Van Cuong[1], Nguyen Thi Thanh Ha[2], Do The Manh[3]
[1]Faculty of Animal Science and Veterinary Medicine, Thai Nguyen University of Agriculture and Forestry, Thai Nguyen city, Thai Nguyen province, Vietnam
[2]Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi, Vietnam
[3]Quang Ninh Sub Department of Animal Health, Tran Hung Dao, Ha Long city, Quang Ninh province, Vietnam

***Corresponding Author:**
Nguyen Thi Kim Lan
Email: nguyenthikimlan@tuaf.edu.vn

Abstract: Our study investigated the effects of extracts from tobacco (*Nicotiana rustica L.)* and stemona (*Stemona tuberosa* Lour.) on parasitic ticks infested on dogs, in order to give a pharmacological explanation for their traditional uses in ectoparasite diseases. The comparison of extracts from different solvents with different extracting times revealed that NaOH 5% with water was the best extracting solvent, while 24 h was the best extracting time for the extraction of these plants' acaricidal effects. After applying these optimal solvent and extracting time to yield those extracts of the highest effects, we examined their effects on ticks at different concentrations, and the results showed that they exerted their best effects when being applied at 20%. These effects were later confirmed by clinical trials, which demonstrated that the extracts also had high treatment effects on those dogs that had been heavily affected with tick diseases. These results partly provide scientific explanations for the therapeutic uses of tobacco and stemona in ectoparasite diseases. In addition, the promissory treatment observed in clinical trials is a step forwards to widen the uses of these medicinal plants in tick diseases, and thus suggesting that follow-up researches are worth to exploit their potentials.
Keywords: *Nicotiana rustica L., Stemona tuberosa* Lour., acaricidal effect, dog tick, ectoparasite

INTRODUCTION

Ticks is a common ecto-parasite infested on Vietnamese dogs [1], and they usually not only significantly decrease the dog health but also have high capacity to carry many dangerous pathogenic microorganisms that affect both of animals and human [2-8]. In these recent years, the resistance of dog ticks to synthetic acaticides has been well recorded in many parts of the world [9-13]. In Vietnam, even that there was no scientific evidences about the resistance of dog ticks to the synthetic drugs, due to lack of researches on this field, many clinical veterinarians have recorded the less effectiveness of usually applied drugs in treatment of dog ticks [14], and incriminated the drug-resistance as the main cause for the significant increment in time and costs of treatment [15]. Failure of many synthetic drugs in the control of diseases has prompted researches to go back to ancient healing methods which use medicinal plants, and many of thousands of plant species growing throughout the world have been demonstrated to exert high pharmacological actions [16]. In the view of this problems in parasitic diseases, the search for alternative sustainable control methods for ticks in recent years has resulted in a number of researches reporting high anti-parasitic effects of plants [9, 14, 17-23]. Researchers also reported many advantages features of botanical acarcides, such as they can be degraded in the environment, do not remain in livestock, are not as prone to resistance and are relatively safe for humans, animals and the environment [24-28]. In addition, in many developing countries like Vietnam, thanks to the availability of diverse plant source, medicine plants are also well-known as the cheap and easy-access treatment therapies [14].

In Vietnamese traditional medicine, tobacco (*Nicotiana rustica L.) and* stemona (*Stemona tuberosa* Lour.) are well recognized as anti-parasitic plants for animals [29]. The insecticide and parasiticide effects of tobacco [27, 30-32] and stemona [29, 30, 33-36] have also been preliminary accessed by several researchers in Vietnam. They are generally considered as natural anti-parasitic therapies which exerts low toxic on host animals [29]. In this study, we focus on investigating their treatment effects with dog ticks, in order to provide evidences to exploit their uses for tick diseases in Vietnam.

MATERIALS AND METHOD

Collect and process of plant materials

Plants were collected in Vietnam. The plants in scientific, English and local names, along with their

collected parts and processing methods are shown in Table 1. Their identity was confirmed by Professor Nguyen Thi Kim Lan, PhD, DVM, Faculty of Animal Science and Veterinary Medicine, Thai Nguyen University of Agriculture and Forestry, Vietnam.

Table 1: Plant materials and sample processing

	Medicinal plant	Sample processing
1	**English name:** Tobacco **Local name:** Cay thuoc lao **Scientific name:** *Nicotiana rustica* L. **Collected part:** Leaves	The processing of tobacco leaves was performed followed the method of Tran Cong Khanh and Pham Quang Hai (1992). In brief, leaves of tobacco trees which had been cultivated from December were collected in the period from April to May of the next year. The harvestmen was performed following the experiences of traditional herbalists (Do Tat Loi, 1991), in which only the leaves of the middle ages (neither young nor old) were selected. Collected leaves were washed by running tap water and after being preliminary dried from this water, they were cut into small pieces and the extraction was performed with this fresh tobacco material.
2	**English name:** Stemona **Local name:** Cu bach bo **Scientific name:** *Stemona tuberosa* Lour. **Collected part:** Bulbs	The processing of stemona roots was performed followed the method described in Medical Dictionary of Vietnam IV (Ministry of Health of Vietnam, 2010) and Dictionary of Medicinal plants and Therapies of Vietnam (Do Tat Loi, 1991). In brief, stemona bulbs were harvested in spring or autumn season, initially washed by running tap water and then plunged shortly into hot water. After preliminary dried from water, these bulbs were slide into small pieces and the extraction was performed with this fresh stemona material.

Extraction and acaricidal effect investigation

All the experiments in this study were carried out follow the scheme which is outlined in Figure 1. The extraction was performed followed the methods which are usually used to extract medicinal plants of anti-parasitic properties in Vietnam [14, 37-39], and with some modification. In brief, four extraction methods, including (1) water with previous soak in NaOH 5%, (2) water with previous soak in HCl 5%, (3) water with previous soak in ethanol 40% and (4) water, were performed to extract the two plant materials. For (1), (2), (3) and (4) methods, firstly 100 g material was wetted with 10 ml NaOH 5%, HCl 5%, ethanol 40% or distilled water (DW) in 1 h, then 90 ml of DW was added, mixed and further left in another 11, 23, 36, 47, 59 h, in order to make the extracts of different total diffusion times, including 12, 24, 36, 48 and 60 h. The obtained solutions were filtered through 2 layers of cheese cloths, and then DW was added into these filtrates to adjust the final volume to 100 ml. Concentrated HCl or NaOH 50% was applied to adjust the pH of these extracts to be from 6.9 to 7.1. These extracts were then named bazo-DW, acid-DW, ethanol-DW and DW extracts in our study, based on the solvents that were preliminarily used in (1), (2), (3) and (4) methods. The concentrations of those extracts were considered as 100% (meaning 100 g in 100 ml), and diluted by PSS to the concentrations of 20%, 10%, 5%, 2.5% and 1.25% to test with ticks.

The evaluation of extract effects on dog ticks were performed followed the methods described by Do T.L. and Ngo X.T. [32] and Bui T.T. *et al.*, [37]. In brief, the effects of extracts on parasites were evaluated by the two parameters, including: the number of death parasites due to the use of extract and the time elapsed before the death. In the first set of experiments, we determined the optimal solvents, optimal extracting times and optimal concentrations for each plant materials, based on the comparison of LT50 and LT100 induced by the extracts, in which LC50 was the concentration able to cause the death of 50% experimental ticks and LC100 was the concentration able to cause the death of 100% experimental ticks. In the second set of experiments, applying the most effective extracts identified by the 1st set of experiments,

we performed the clinical trials on the dogs infested by parasitic ticks, and evaluated their treatment efficacy. We used 120 Vietnamese dogs that naturally but heavily infested with ticks, which were collected from six different communes of Quang Ninh province, including Cai Chien, Duong Hoa and Quang Ha communes of Hai Ha district, and Duc Yen, Quang Tan and Dam Ha communes of Dam Ha district. Dogs were recognized as heavily affected with tick diseases only when there was at least 150 ticks per an individual dog. Those dogs were randomly divided into group of 10 individuals, and treatment were performed by spraying extracts to wet their whole bodies once a day, for continuously 3 ds. After this 3 ds, dogs were carefully checked to determine whether their infested ticks were completely eliminated or not, and for cases that there were still living ticks infested on dog bodies, we performed the next treatment by continue to apply the extracts for another 2 ds. Treatment effects of extracts were then evaluated by calculating the percentages of dogs whose the tick diseases had been eliminated due to the extract treatments.

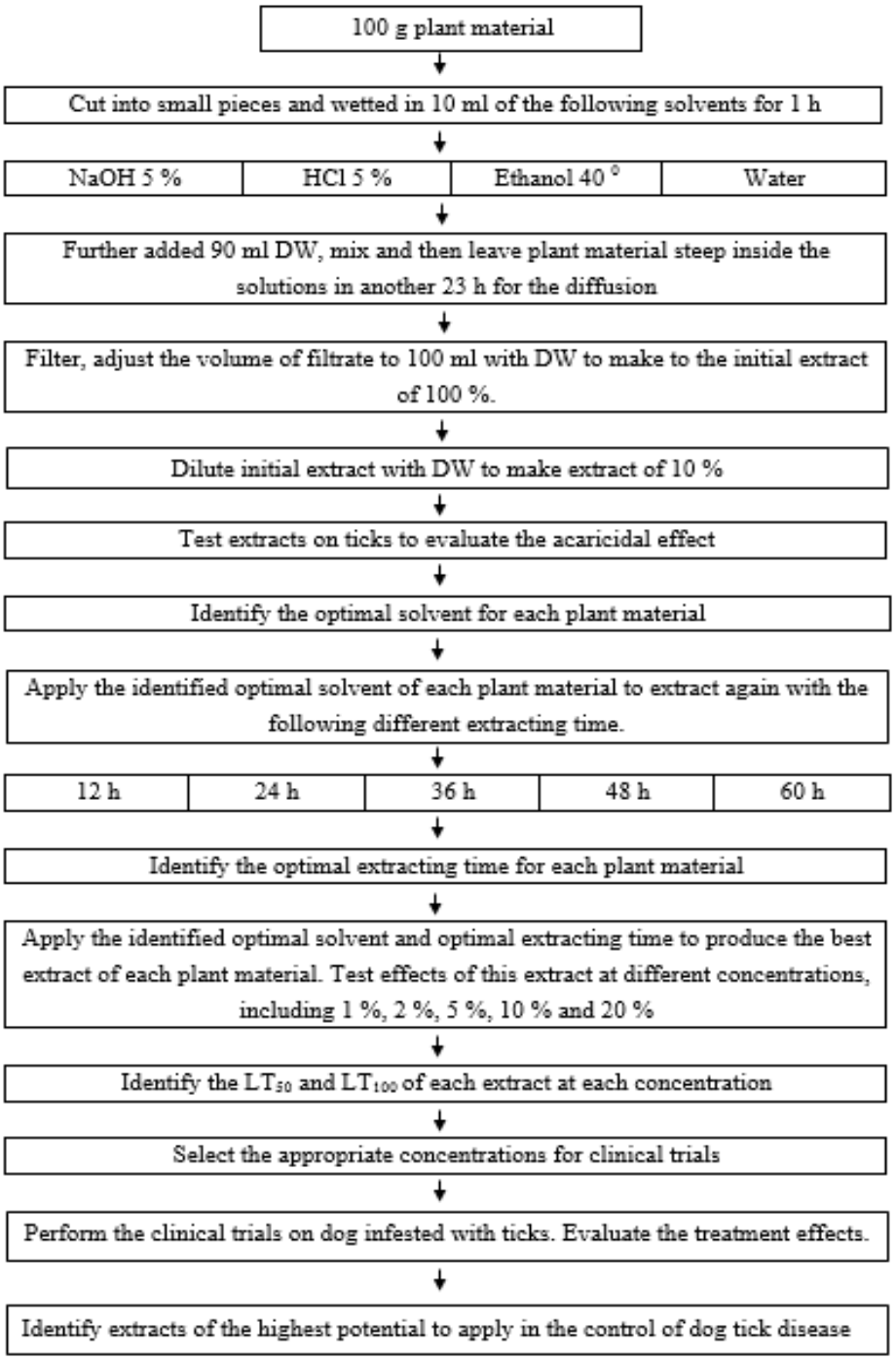

Fig-1: Methodological Scheme. Note: LT_{50} (Lethal time of 50%) and LT_{100} (Lethal time of 50%) mean the necessary treatment time to induce the death of 50% and 100% of experimental ticks

RESULTS

The identification of the optimal extracting solvents

In order to identify which solvents yield the best antiparasitic effects for each plant material, we compared the effects of extracts derived from different solvents at the same concentration of 10%, and the results are shown in Table 2.

Table 2: The acaricidal effect of 10% extracts of tobacco and stemona extracted for 24 h with different solvents on dog ticks

No	Plant	Extracting solvent	Treatment time that induce the death of 50 % experimental ticks (LT_{50}) (min)	Treatment time that induce the death of 100 % experimental ticks (LT_{100}) (min)
1	Tobacco	NaOH 5%	45	80
		HCl 5%	100	265
		Ethanol 40 ^{0}	60	105
		DW	2 *	
2	Stemona	NaOH 5%	120	205
		HCl 5%	125	255
		Ethanol 40 ^{0}	210	350
		DW	1 *	
Control		There was no death ticks observed		

Note: * indicates that the treatment was not able to induce the death up to 50% or 100% of the tested ticks, and the accompanying numbers are the numbers of death ticks counted at the end of observation time (360 min).

From Table 2, we observed that NaOH 5% was the best extracting solvents for anti-parasitic effects, because regardless of the plant materials, this medium always yielded the shortest LT50 and LT100 values. For tobacco, the LT50 and LT100 of extract from NaOH 5% was 45 min and 80 min, respectively, and were the shortest among all of investigated extracts. Similarly, the LT50 and LT100 of stemona extract from NaOH 5% was 120 min and 205 min, respectively, and were shorter than all of those of from other solvents, including HCl 5%, ethanol 40% and DW.

The identification of the optimal extracting times

In order to identify which extracting times yield the best antiparasitic effects for each plant material, we compared the effects of NaOH 5% extracts derived from different extracting times at the same concentration of 10%, and the results are shown in Table 3.

Table 3: The acaricidal effect of 10% extracts of tobacco and stemona extracted with NaOH 5% for different extracting time on dog ticks

No	Plant	Extraction time (h)	Treatment time that induce the death of 50 % experimental ticks (LT_{50}) (min)	Treatment time that induce the death of 100 % experimental ticks (LT_{100}) (min)
1	Tobacco	12	50	80
		24	45	75
		36	48	85
		48	50	90
		60	65	125
2	Stemona	12	140	260
		24	115	205
		36	135	245
		48	145	260
		60	170	305
Control		There was no death ticks observed		

From Table 3, we observed that 24 h was the best extracting time for anti-parasitic effects, because regardless of the plant materials, this extracting time always yielded the shortest LT50 and LT100 values. For tobacco, the LT50 and LT100 of the extract of 10% of NaOH 5% of 24 h extracting time was 45 min and 75 min, respectively, and were the shortest among all of investigated extracting times. Similarly, the LT50 and LT100 of stemona extract of 10% of NaOH 5% of 24 h extracting time was 115 min and 205 min, respectively, and were shorter than all of those of other extracting times, including 12, 36, 48 and 60 h.

The identification of the optimal extract concentrations

In order to identify which extract concentrations yield the best antiparasitic effects for each plant material, we compared the effects of different conentrations of NaOH 5% extracts with 24 h extracting time derived from the two plant materials, and the results are shown in Table 4.

Table 4: The acaricidal effect of different concentrations of extracts of tobacco and stemona extracted with NaOH 5% in 24 h on dog ticks

No	Plant	%	Treatment time that induce the death of 50 % experimental ticks (LT_{50}) (min)	Treatment time that induce the death of 100 % experimental ticks (LT_{100}) (min)
1	Tobacco	1	#	
		2	70	6 *
		5	50	8 *
		10	40	75
		20	35	55
2	Stemona	1	#	
		2	#	
		5	3 *	
		10	140	220
		20	65	140
	Control	There was no death ticks observed		

Note: * indicates that the treatment was not able to induce the death up to 50% or 100% of the tested ticks, and the accompanying number mean the number of death ticks counted at the end of observation time (360 min). # indicates that there was no ticks died by the treatment until the end of observation time (360 min).

From Table 4, we observed that the extracts of tobacco and stemona possessed the antiparasitic effect and these effects were dose-dependent, because following the decrement of concentrations, there were the increment in LT50 and LT100 values induced by extracts to dog ticks. We also observed that at the concentration of 20%, the two extracts were able to kill all tested parasites with the shortest LT50 and LT100. These values were respectively 35 and 55 min for tobacco, and were respectively 65 and 140 min for stemona extracts.

The clinical treatment of plant extracts on dog infested with ticks

In order to evaluate the treatment effects of plant extracts on dogs infested with tick disease, we performed the clinical trials. We selected to test with the plant extracts of the highest effects, by applying the optimal extracting solvent: NaOH 5%, the optimal extracting time: 24 h and the most effecttive concentration: 20%, which had been identified in part 3.1, 3.2 and 3.3. The results of these clinical trials are shown in Table 5.

Table 5: Results on clinical treatment of extracts of tobacco and stemona at 20% on dogs infested with ticks.

Tobacco extract				
District	Commune	Number of experimental dogs	Number of dogs free from tick disease after treatment	Treatment efficacy (%)
Hai Ha	Cai Chien	10	10	100.0
	Duong Hoa	10	8	80.0
	Quang Ha	10	9	90.0
Dam Ha	Duc Yen	10	10	100
	Quang Tan	10	9	90.0
	Dam Ha	10	9	90.0
Total		**60**	**55**	**91.7**
Stemona extract				
District	Commune	Number of experimental dogs	Number of dogs free from tick disease after treatment	Treatment efficacy (%)
Hai Ha	Cai Chien	10	7	70.0
	Duong Hoa	10	9	90.0
	Quang Ha	10	8	80.0
Dam Ha	Duc Yen	10	8	90.0
	Quang Tan	10	8	80.0
	Dam Ha	10	9	90.0
Total		**60**	**49**	**81.7**

From Table 5, we observed that tobacco and stemona extracts showed promissory treatment effects with dog tick diseases, shown by the high percentage of dogs that became free from ticks after extract treatments (91.7% and 81.7% for tobacco and stemona extracts, respectively).

DISCUSSION

This study demonstrated the acaricidal effects of extracts from tobacco and stemona on dog ticks, and thus providing evidences to explain their traditional therapeutic uses in parasitic diseases, which have been recorded in Vietnamese ethnic medicine [29]. In Vietnam, due to the decrement in the effects of synthetic drugs and their significant side-effects, there has been a significant trend in investigating plants with anti-parasitic properties. In the search of botanical anti-parasitic therapies, researchers in Vietnam have recognized a number of folk medicine plants as the cheap and effective alternations for synthetic drugs in the treatment of parasitic diseases, such as camellia sasanqua seed oil [14], pomegranate bark [15], garlic, lemon grass, fortune bogorchid, cinnamon [40], *Achyranthes bidentata blume, Cassia alata L, Embelia ribes burn, Ipomoea hederacea jacq, Leucaena glauca benth, Solanumtorvum swartz* [41]. Our current study therefore further confirms and adds evidences to the promissory potentials of anti-parasitic therapies derived from plants. In addition, even there has been some studies researching anti-parasitic effects of tobacco and stemona plants, this study represented the first attempt to perform the clinical trials of their extracts on the dogs that heavily affected with ticks, and was able to demonstrate their high treatment efficacy. These results are certainly one step forwards to widen their therapeutic applications, but further investigations are needed if we want to exploit their clinical uses in a larger scale. In our study, alkaline (NaOH 5%) was the best extracting solvent, and 24 h was be the best extracting time for yielding the high acaricidal effects from tobacco and stemona plants. Our results were in accordance to one previous study, which had also investigated the acaricidal effects of other three anti-ectoparasitic plants, including *Annona squamosa* beans, *Pachyrizus erosus* beans and *Derris elliptica* radix, and also reported that alkaline solvent and 24 h extraction were the optimal solvent and the optimal time for extracting the plant acaricidal properties [37]. However, this previous study and our current study have not yet isolated and identified active components in the plants that responsible for these optimal values, and therefore it remains to be further verified in future researches. 20% was the most effective concentration among all of investigated doses, for both of tobacco and stemona, because it brought the highest effects but showed no side effects in clinical trials. Based on these results, we suggested that 20% dose should be used in next follow-up researches, of which we aim to test their clinical treatment in a larger scale.

CONCLUSIONS

Both tobacco and stemona have potentials to apply as botanical anti-ectoparasitic therapies to control the tick diseases on dogs. The extracts applying the alkaline solvent (NaOH 5%), 24 h extracting time and at concentration of 20% showed the best effects, and therefore should be further investigated to evaluate the treatment potentials.

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