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Acaricidal Effect of Practical Crude-extracts of Plants against Tropical Cattle Ticks

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ABSTRACT

The acaricidal activity of the plant crude-extracts to tropical cattle ticks were studied in the following plants; *Calotropis gigantica* R.Br., *Calotropis procera* R.Br., *Crytostegia grandiflora* R.Br., *Homalomena aromatica* Linn., *Kalanchoe pinnata* Lamk., *Pachyrrhizus erosus* Linn., *Polyscias balfouriana* Baill, *Rauwolfia serpentina* B.K., *Rhinacanthus nasutus* Linn., *Spilanthes acmella* Murr., *Streblus asper* Lour., and *Thunbergia erecta* T. Anders. The crude-extracts of these plants were extracted with 10% ethanol, water, boiling water and boiled water added with the ethanol to 10%. These crude-extracts were tested for the acaricidal activity to the engorged female tropical cattle ticks (*Boophilus microplus*). Only the crude-extracts of *P. erosus* with boiling water showed delayed acaricidal activity (anti-laying activity). The crude-extracts obtaining by different times of extraction with boiling water and hot water were stored in refrigerator and tested on both larvae and engorged female ticks with different dilution. The boiling-water and the hot-water crude-extracts for 20 and 40 min. showed high delayed acaricidal activity at 1:6 and 1:5 dilution, respectively. Both of them were stored in refrigerator for 7 days after extraction. The water boiling for 5-40 min. crude-extracts were stored in the refrigerator for 20 days, exhibited relatively high larvicidal activity at 1: 220 dilution. While the hot water extract for 5-40 min. showed high larvicidal activity at 1: 220 dilution after storing in the refrigerator for 12 days.

This indicates that acaricidal substances of *P. erosus* (yam bean) seeds can be easily extracted, and the practical application of this crude-extracts to controlling the tropical cattle tick is expectable.

Keywords: practical extraction, yam bean seeds, acaricide, tropical cattle ticks

INTRODUCTION

It is well known that synthetic insecticides are delayed in degradation, remain residue in agricultural environment and induce resistant strains of pests. The tropical cattle ticks (*Boophilus microplus*) can also develop resistant strains against the synthetic acaricides (O'Sullivan and Green, 1971; Roulston, 1971; Drummond, 1977; Howell, 1977). Therefore, the natural plant crude-extracts are required to substitute the synthetic acaricides. Various kinds of practical extraction showed the larvicidal and acaricidal activities of some number of plants which exhibited high or relatively high larvicidal or acaricidal activities in the previous studies (Chungsamarnyart, et al., 1991a, b).

MATERIALS AND METHODS

The ethanol crude-extract of the following plants showed high or relatively high larvicidal or acaricidal activities; *Calotropis gigantica* R.Br., *Calotropis procera* R.Br., *Crytostegia grandiflora* R.Br., *Homalomena aromatica* Linn., *Kalanchoe pinnata* Lamk., *Pachyrrhizus erosus* Linn., *Polyscias balfouriana* Baill,, *Rauwolfia serpentina* B.K., *Rhinacanthus nasutus* Linn., *Spilanthes acmella* Murr., *Streblus asper* Lour., and *Thunbergia erecta* T.Anders (Chungsamarnyart et al., 1991 a; b). The part of these plants were cut into small pieces or ground in mill and extracted with water and 10% ethanol (2 times of the sample weight). Four kinds of extraction were used;

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extracted by 10% ethanol, by water, by boiling with water 15 min. and by the adding ethanol to 10% of boiling with water. The crude-extracts were bioassayed for acaricidal activity on engorged female ticks (Chungsamarnyart, et al., 1990). The crude-extracts which exhibited high acaricidal activity were extracted by different times and tested on both larvae and engorged female ticks with different dilution. The mortality of the adult ticks was observed at 24 h, 48 h and 7 days mark after dipping. The mortality of larvae was only observed at 24 h mark after dipping. The bioassay was replicated 5 times (20 adult ticks/replication and 150-400 larvae/replication). Abbott's formula (Abbott,1925) was used to calculate the corrected mortality of larvae and adult ticks, or corrected non-laying of engorge female ticks.

RESULTS

The crude-extracts from each plant species showed no significant acaricidal activity at 24 h mark after dipping. The acaricidal activity of crude-extracts at 48 h and 7 days mark after dipping were shown in Table 1. Most of them exhibited no significance acaricidal activity execpt the crude-extracts of *P. erosus* (Table 1). The water boiling crude-extracts of *P. erosus* showed high delayed activity (97% corrected non-laying). The water boiling extract + ethanol to 10% crude-extract of *P. erosus* exhibited relatively high delayed activity (75% corrected non-laying). In addition, the crude-extract by hot water (1: 2 W/V, 20 min.) also exhibited relatively high delayed activity (73% corrected non-laying).

Table 1 The acaricidal activity of crude-extracts of twelve plant species, using various extraction methods, to engorged female tropical cattle ticks.

Name of plants (Scientific and Thai name) and extraction	Part of plants	Corrected mortality 48 h[1]	Corrected non-laying 7 days[2]
1. *Calotropis gigantica* R.Br. (Ruk-dok-muang);	Stem & leaf		
10% Ethanol extract (1:2 W/V; 24 h)		6 bc	26 d
Water extract (1:2 W/V; 24 h)		9 ab	46 c
Water boiling extract (1:2 W/V; 15 min.)		1 dc	4 fg
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		0 e	3 fg
2.*Calotropis procera* R.Br. (Ruk-dok-khao);	Stem & leaf		
10% Ethanol extract (1:2 W/V; 24 h)		3 cde	12 ef
Water extract (1:2 W/V; 24 h)		0 e	4 fg
Water boiling extract (1:2 W/V; 15 min.)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		0 e	0 g
3. *Crytostegia grandiflora* R.Br. (Baan-burii-sii-muang);	Flower		
10% Ethanol extract (1:2 W/V; 24 h)		0 e	0 g
Water extract (1:2 W/V; 24 h)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		0 e	0 g
4. *Homalomena aromatica* Linn. (Toaw-kied)	Rhizome		
10% Ethanol extract (1:2 W/V; 24 h)		0 e	0 g
Water extract (1:2 W/V; 24 h)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		1 ed	2 fg
5. *Kalanchoe pinnata* Lamk. (Kwam-taai-ngaay-pen)	Leaf		
10% Ethanol extract (1:2 W/V; 24 h)		4 cd	4 fg
Water extract (1:2 W/V; 24 h)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.)		0 e	12 ef
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		9 ab	22 de

Table 1 The acaricidal activity of crude-extracts of twelve plant species, using various extraction methods, to engorged female tropical cattle ticks. (con't)

Name of plants (Scientific and Thai name) and extraction	Part of plants	Corrected mortality 48 h[1]	Corrected non-laying 7 days[2]
6. *Pachyrrhizus erosus* Linn. (Man-kaew)	Seed		
10% Ethanol extract (1:2 W/V; 24 h)		2 de	55 c
Water extract (1:2 W/V; 24 h)		2 de	24 d
Water boiling extract (1:2 W/V; 15 min.)		3 cde	97 a
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		11 a	75 b
7. *Polyscias balfouriana* Baill. (Lep-krut)	Leaf		
10% Ethanol extract (1:2 W/V; 24 h)		1 de	5 fg
Water extract (1:2 W/V; 24 h)		1 de	1 g
Water boiling extract (1:2 W/V; 15 min.)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		0 e	0 g
8. *Rauwolfia serpentina* B.K. (Ra-yom)	Root		
10% Ethanol extract (1:2 W/V; 24 h)		0 e	1 g
Water extract (1:2 W/V; 24 h)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		1 de	1 g
9. *Rhinacanthus nasutus* Linn. (Thong-pan-chang)	Stem & leaf		
10% Ethanol extract (1:2 W/V; 24 h)		0 e	0 g
Water extract (1:2 W/V; 24 h)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		0 e	0 g
10. *Spilanthes acmella* Murr. (Phak-krad-hou-waen)	Stem & leaf		
10% Ethanol extract (1:2 W/V; 24 h)		0 e	5 fg
Water extract (1:2 W/V; 24 h)		2 de	10 fg
Water boiling extract (1:2 W/V; 15 min.)		1 de	3 fg
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		1 de	3 fg
11. *Streblus asper* Lour. (Koiy)	Stem & leaf		
10% Ethanol extract (1:2 W/V; 24 h)		0 e	0 g
Water extract (1:2 W/V; 24 h)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		0 e	0 g
12. *Thunbergia erecta* T. Anders. (Chong-naang);	Leaf		
10% Ethanol extract (1:2 W/V; 24 h)		0 e	0 g
Water extract (1:2 W/V; 24 h)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.)		0 e	0 g
Water boiling extract (1:2 W/V; 15 min.) + Ethanol to 10%		0 e	0 g

1 Mean (%) of corrected mortality of 5 replications (20 ticks/rep.); values followed by different letters are statistically siginficant different. LSD .01 = 3.2131

2 Mean (%) of corrected non-laying of 5 replications (20 ticks/rep.); values followed by letters are statistically significant different. LSD .01 = 10.4022

The seeds of *P. erosus* were extracted by water boiling and hot water for 5, 10, 20 and 40 min. and tested the acaricidal activity to both larvae and engorged female ticks with different dilution crude-extracts. The crude-extracts were stored in refrigerator for some days before testing the activity of each dilution. These crude-extracts showed no acaricidal activity at 24 h after dipping. At 48 h and 7 days after dipping, the acaricidal activity of water boiling and hot water crude-exxtracts are shown in Table 2 and 3, respectively. The fresh crude (0 day stored crude) of 20 min. water boiling extract-crude showed the highest delayed acaricidal activity (99% corrected non-laying of 1:1 dilution). Its 7 days stored crude also exhibited the highest delayed acaricidal activity (95% corrected non-laying) at 1:6 dilution with water (Table 2). The crude-extract by hot water for 40 min. showed the highest delayed acaricidal activity (99% corrected

Table 2 Acaricidal activity of crude-extracts of yam bean seeds to engorged female tropical cattle ticks extracted by various times of water boiling.

Crude-extracts	Conc. crude:water (V/V)	Corrected mortality 48 h[1]	Corrected non-laying 7 days[2]	Crude stored days[3]
Water boiling extract (1:2 W/V) 5 min.	1:1	0 c	80 def	0
	1:2	0 c	83 cde	3
	1:3	0 c	90 abcd	7
	1:4	0 c	65 gh	2
	1:5	0 c	47 ij	5
	1:6	0 c	26 k	5
Water boiling extract (1:2 W/V) 10 min.	1:1	0 c	66 gh	0
	1:2	0 c	80 def	3
	1:3	0 c	87 abcde	7
	1:4	0 c	61 h	2
	1:5	0 c	60 hi	7
	1:6	0 c	37 jk	4
Water boiling extract (1:2 W/V) 20 min.	1:1	0 c	99 a	0
	1:2	0 c	100 a	3
	1:3	0 c	97 ab	7
	1:4	1 c	75 efg	3
	1:5	3 c	59 hi	4
	1:6	0 c	95 abc	7
Water boiling extract (1:2 W/V) 40 min.	1:1	0 c	84 bcde	0
	1:2	0 c	97 ab	3
	1:3	0 c	85 bcde	7
	1:4	0 c	67 fgh	3
	1:5	8 a	95 abc	9
	1:6	3 b	63 gh	4

1 Mean (%) of corrected mortality of 5 replications (20 ticks/rep.); values followed by different letters are statistically significant differents. LSD .05 = 2.8892

2 Mean (%) of corrected non-laying of 5 replications (20 ticks/rep.); values followed by different letters are statistically significant differents. LSD .05 = 13.2710

3 Crude-extracts were stored in refrigerator.

non-laying) at 1:5 dilution (Table 3).

The larvicidal activity of crude-extracts by water boiling and by hot water, are shown in Table 4 and 5, respectively. The water boiling extract for 5-40 minutes crudes and stored in the refrigerator for 20 days showed relatively high larvicidal activity at 1: 220 dilution (82-88% corrected mortality; Table 4). The hot water extract for 5-40 minutes crudes and stored in the refrigerator for 12 days exhibited high larvicidal activity at 1: 220 dilution (86-95% corrected mortality; Table 5). The larvicidal activity of crude-extracts decreased when they were stored over 20 days (Table 4 and 5).

DISCUSSION

The 95% ethanol extract of *Calotropis gigantica* R.Br., *Calotropis procera* R.Br., *Crytostegia*

Table 3 Acaricidal activity of crude-extracts of yam bean seeds to engorged female tropical cattle ticks extracted by various times of hot water.

Crude-extracts	Conc. crude:water (V/V)	Corrected mortality 48 h[1]	Corrected non-laying 7 days[2]	Crude stored days[3]
Hot water extract (1:2 W/V) 5 min.	1:1	2 bcd	83 cdefg	0
	1:2	0 d	93 abcd	3
	1:3	0 d	91 abcd	8
	1:4	1 cd	85 bcdef	3
	1:5	2 bcd	79 defg	9
	1:6	1 cd	26 i	4
Hot water extract (1:2 W/V) 10 min.	1:1	0 d	43 h	0
	1:2	0 d	86 abcdef	3
	1:3	0 d	85 bcdef	8
	1:4	1 cd	70 g	3
	1:5	0 d	74 fg	4
	1:6	3 cd	81 defg	7
Hot water extract (1:2 W/V) 20 min.	1:1	1 cd	49 h	0
	1:2	0 d	91 abcd	3
	1:3	0 d	100 a	10
	1:4	1 cd	74 fg	3
	1:5	9 a	86 abcdef	4
	1:6	4 b	75 fg	4
Hot water extract (1:2 W/V) 40 min.	1:1	0 d	49 h	1
	1:2	0 d	96 abc	4
	1:3	2 bcd	99 ab	6
	1:4	0 d	90 abcde	5
	1:5	0 d	99 ab	7
	1:6	2 bcd	76 efg	4

1 Mean (%) of corrected mortality of 5 replications (20 ticks/rep.); values followed by different letters are statistically significant differents. LSD .05 = 2.3591

2 Mean (%) of corrected non-laying of 5 replications (20 ticks/rep.); values followed by different letters are statistically significant differents. LSD .05 = 14.1254

3 Crude-extracts were stored in refrigerator.

Table 4 Larvicidal activity of crude-extracts of yam bean seeds to tropical cattle ticks larvae extracted by water boiling with various times.

Crude-extracts	Conc. crude:water (V/V)	Corrected mortality 24 h[1]	Crude stored days[2]
Water boiling extract (1:2 W/V) 5 min.	1:10	99.41 a	7
	1:30	100.00 a	10
	1:60	92.13 abcd	1
	1:90	71.87 gh	27
	1:130	29.25 k	0
	1:130	75.21 fgh	12
	1:160	82.68 cdefg	6
	1:220	12.39 lm	5
	1:220	88.55 abcde	20
Water boiling extract (1:2 W/V) 10 min.	1:10	100.00 a	7
	1:30	99.50 a	10
	1:60	86.37 bcdef	1
	1:90	80.81 defgh	27
	1:130	58.30 i	0
	1:130	91.10 abcd	12
	1:160	94.59 abc	6
	1:220	0.79 m	5
	1:220	87.81 abcde	20
Water boiling extract (1:2 W/V) 20 min.	1:10	100.00 a	7
	1:30	99.86 a	10
	1:60	90.24 abcd	1
	1:90	92.38 abcd	27
	1:130	38.16 jk	0
	1:130	72.51 gh	12
	1:160	68.66 hi	6
	1:220	13.50 l	5
	1:220	85.24 bcdef	20
Water boiling extract (1:2 W/V) 40 min.	1:10	100.00 a	7
	1:30	99.13 a	10
	1:60	77.35 efgh	1
	1:90	89.67 abcde	27
	1:130	44.02 j	0
	1:130	95.52 ab	12
	1:160	93.38 abc	6
	1:220	8.19 lm	5
	1:220	82.52 cdefg	20

1 Mean (%) of corrected mortality of 3 replications (150-400 larvae/rep.); values followed by different letters are statistically significant differents. LSD .05 = 12.3932

2 Crude-extracts were stored in refrigerator.

Table 5 Larvicidal activity of crude-extracts of yam bean seeds to tropical cattle ticks larvae extracted by hot water with various times.

Crude-extracts	Conc. crude:water (V/V)	Corrected mortality 24 h[1]	Crude stored days[2]
Hot water extract (1:2 W/V) 5 min.	1:10	99.60 ab	7
	1:30	97.64 abc	10
	1:60	90.59 abcdefg	6
	1:90	50.01 j	33
	1:130	78.23 hi	2
	1:160	93.26 abcdef	13
	1:220	86.10 defgh	12
	1:220	37.93 k	27
Hot water extract (1:2 W/V) 10 min.	1:10	99.90 ab	7
	1:30	99.45 ab	10
	1:60	78.20 hi	6
	1:90	81.17 ghi	33
	1:130	84.72 efgh	2
	1:160	74.29 i	13
	1:220	88.82 cdefg	12
	1:220	51.15 j	27
Hot water extract (1:2 W/V) 20 min.	1:10	100.00 a	7
	1:30	87.53 abc	10
	1:60	89.55 bcdefg	6
	1:90	96.72 abc	33
	1:130	100.00 a	2
	1:160	93.85 abcdef	13
	1:220	95.15 abcd	12
	1:220	52.01 j	27
Hot water extract (1:2 W/V) 40 min.	1:10	100.00 a	7
	1:30	98.23 abc	10
	1:60	83.67 fghi	6
	1:90	92.33 abcdef	33
	1:130	94.61 abcde	2
	1:160	80.47 ghi	13
	1:220	94.96 abcde	12
	1:220	41.94 jk	27

1 Mean (%) of corrected mortality of 3 replications (150-400 larvae/rep.); values followed by different letters are statistically significant differents. LSD .05 = 10.4282

2 Crude-extracts were stored in refrigerator.

grandiflora R.Br., *Homalomena aromatica* Linn., *Kalanchoe pinnata* Lamk.,*Polyscias balfouriana* Baill,, *Rauwolfia serpentina* B.K., *Rhinacanthus nasutus* Linn., *Spilanthes acmella* Murr., *Streblus asper* Lour. and *Thunbergia erecta* T. Anders. have shown relatively high or high larvicidal, acaricidal and delayed acaricidal activities (Chungsamarnyart, et al., 1991 a, b) but their practical crude-extracts exhibited non acaricidal activity (Table 1). These indicated that the active acaricidal substances of these plants were not much extracted by high polar solvent (water) even by the 10% ethanol solvent. They should be extract by less polarity solvents (eg. 95% ethanol) but this is not practical for the farmers.

The water boiling crude-extract of *Pachyrrhizus erosus* Linn. seeds showed delayed acaricidal activity (anti-laying activity; Table 1). The acaricidal and larvicidal activities of the crude-extracts from different boiling times and diluted with water, were tested and the activities showed in Table 2, and 4. The acaricidal and larvicidal activities were still high in 1:6 and 1: 220 water diluted crude-extracts, respectively. While the hot-water crude-extracts also exhibited high acaricidal and larvicidal activities in 1:5 and 1: 220 water dilutions, respectively (Table 3 and 5). It demonstrated that these practical extraction of *P. erosus* seeds are possible to use for controlling the cattle ticks. Especially, once or twice a week of practical application of 1:220 water diluted crude-extract can control the tick larvae developing to adults. However, the crude-extracts should be stored in refrigerator for 7-12 days since these duration of stored crude-extracts exhibited high acaricidal and larvicidal activities (Table 2-5). The same concentration (1:220 V/V) of the 5 days stored crude-extract and 20 days stored crude-extract of water boiling extraction exhibited much different larvicidal activity (Table 4). The hot-water crude-extract at 1:220 dilution storing 12 and 27 days also showed much significant different activity (Table 5). The water boiling crude-extract and stored for 20 days showed relatively less larvicidal activity than the hot-water crude-extract and stored for 12 days. These indicated that the storing time affected larvicidal activity of crude-extracts. This might be the result of multiplication of some bacteria which can develop in cool temperature. The bacteria fermented and produced the substances in stored crude-extracts, since the endosperm of the seeds are starch. The fermented production substances might improve the larvicidal activities. However, those substances were not identifiable in this experiment. The active acaricidal and larvicidal substances of *P. erosus* seeds extracted by these simplified procedure were also not known. The active substances might be the same substances as showing the insecticidal activity against many insects which have been reported (Grainge and Ahmed, 1988)

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