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# Practical Extraction of Sugar Apple Seeds against Tropical Cattle Ticks

**Narong Chungsamarnyart [1,2], Suratwadee Jiwajinda [2], Chainarong Rattanakreetakul [1] and Weerapol Jansawan [2]**

## ABSTRACT

The high acaricidal activity of ethanol crude-extract of sugar apple (*Annona squamosa* L.) seeds on tropical cattle ticks (*Boophilus microplus*) has been reported in our previous papers. The acaricidal activity on larvae was found in column chromatography fractions of 100% toluene; 40%, 60%, 80% ethyl acetate in toluene, and 100% ethyl acetate. The high acaricidal activity on adult ticks was found in fractions of 40%, 60% ethyl acetate in toluene, 100% ethyl acetate, and 5% ethanol in ethyl acetate. This showed that the active substance was dissolved in polar solvents. Therefore, it might be extracted with water for practical use of the crude extract.

For the practical extraction of the active compound from sugar apple seeds, different solvents were examined and tested with regard to effect on adult ticks; water extract, boiling water extract, 10% and 20% ethanol extract, and 11.66% white spirit extract. The 10% ethanol extract and boiling water extract showed high acaricidal activity (87% and 88% mortality of the ticks 48 h after dipping, respectively), even with 2 times dilution by water. The mixture of 10% ethanol extract and its rinsed solution (1:4 W/V, 33%) still showed activity even after 50 days kept in refrigeration.

This indicates that the acaricidal substances of sugar apple seeds are dissolved in polar solvents and simplified extraction for practical use in controlling ticks is possible.

**Keywords :** Acaricide, Sugar apple seeds, crude-extract, Tropical cattle ticks.

## INTRODUCTION

Many previous reports have shown that the tropical cattle tick (*Boophilus microplus*) can develop resistant strains against synthetic acaricides (O'Sullivan and Green, 1971; Roulston, 1971; Drummond, 1977; Howell, 1977). Therefore, new original acaricides from plants are in great demand. Plant originated acaricides tend to have a low toxicity to mammals, rapid degradation and a low incidence of developing resistant strains. The present study is an attempt to ascertain the best simple extraction procedure of the active compound from sugar apple seeds in order to use it practically as an acaricide on cattle ticks.

## MATERIALS AND METHODS

The preliminary test of the acaricidal activity of ethanol crude-extract of sugar apple seeds on the larvae and adults of tropical cattle ticks has been previously reported (Chungsamarnyart et al., 1988, 1990 b).

The ethanol crude-extract (500 gm) was partitioned with ethyl acetate and water. The ethyl acetate part (4.6934 gm) was applied to a column of Silica gel (Wakogel C-100 [R], 150-425 micron) packed in toluene, and eluted with 20% gradually increasing amount of ethyl acetate in toluene (F2-F5), 100% ethyl acetate

1 Faculty of Veterinary Medicine, Kasetsart university, Kamphaengsaen campus, Nakhon Pathom, 73140, Thailand.
2 Environmental Science Unit, Central Laboratory and Greenhouse Complex, Kasetsart University Research and Development Institute, Kamphaengsaen campus, Nakhon Pathom, 73140, Thailand.

(F6), and 5% and 10% of ethanol in ethyl acetate (F7 and F8). Each fraction was bioassayed for acaricidal activity on larvae and engorged females of tropical cattle ticks (Chungsamarnyart and Jansawan, 1990). The concentration of each fraction was 1.14 mg/cm$^2$ for the acaricidal activity test on larvae by the leg contact method and 1-2.5 mg/ml dissolved solvent on adult ticks by the dipping method. The dissolved solvent was 9 parts of 1% aqueous solution polyoxyethylene sorbitan mono-oleate (Tween 80 $^R$) and 1 part of 100% ethanol. Control groups of the adult ticks were dipped in dissolved solvent.

For the acaricidal activity test of the crude extracts, the seeds were ground in a mill and extracted with various solvents (2 times weight of the seeds) as follows; water, boiling water 15 min, 10% ethanol extracted boiled seed mill, 10% and 20% ethanol (Twice collection of 24 h extract) and 11.66% white spirit. These crude-extracts were bioassayed for acaricidal activity on adult ticks. The extracts which showed high activity were tested again with 2-3 times dilution by water or 10% ethanol.

The acaricidal activity of the 10% ethanol extract preserved in a refrigerator was observed at 0, 10, 20, 40 and 50 days after extraction to ascertain the lenght of its active life. The extract was a mixture of 10% ethanol extract solution (milled seed weight: volume of 10% ethanol = 1 : 2 and rinsed solution (10% ethanol in the same volume).

The mortality of the ticks was observed 24 h, 48 h and 5 days after dipping. The bioassay was replicated 5 times (20 ticks/replication). Abbott's formula (Abbott, 1925) was used to calculate the corrected mortality of larvae and adult ticks.

## RESULTS

The corrected mortality of larvae at the 24 h mark after contact is shown in Table 1. The mean (%) of the corrected larvae mortality was high after contact with the crude-extract and fractions of 100% toluene (F1), 40% ethyl acetate in toluene (F3), 60% ethyl acetate in toluene (F4), 80% ethyl acetate in toluene (F5), and 100% ethyl acetate (F6). The 5% ethanol in ethyl acetate (F7) fraction also showed a relatively high larvae mortality. It indicated that the larvicidal substances were in both polar and non-polar eluted solvents.

The high insecticidal activity (2.5 and 1 mg/ml conc.) was found in fractions of 40%, 60% ethyl acetate in toluene (F3, F4), 100% ethyl acetate (F6), and 5% ethanol in ethyl acetate (F7) (Table 2).

As the best extraction for practical use of sugar apple seeds, the 10% ethanol and water boiling extracts showed high acaricidal activity (Table 3). In particular, the 3 times dilution (33% conc.) of 10% ethanol extract still exhibited high effectiveness (92% mortality of ticks at the 48 h mark after dipping). The mixture of 10% ethanol extract and its rinsed solution (1:4 W/V, 33%) also still showed activity even after 50 days preservation in refrigeration. The mortality of ticks was 50±17.6% and 93±4.5% at the 48 h and 5 days mark after dipping, respectively. The 10% etha-

**Table 1 The *in vitro* acaricidal activity of ethanol crude-extract and fractions of *Annona squamosa* seeds on tick larvae***

| Solvents | Corrected mortality (Mean, %)** of tick larvae after contacted 24 h |
|---|---|
| Ethanol crude-extract | 92.95 ab |
| 100% Toluene (F1) | 81.16 abc |
| 20% Ethyl acetate in toluene (F2) | 55.90 d |
| 40% Ethyl acetate in toluene (F3) | 84.84 ab |
| 60% Ethyl acetate in toluene (F4) | 99.54 a |
| 80% Ethyl acetate in toluene (F5) | 99.32 a |
| 100% Ethyl acetate (F6) | 86.67 ab |
| 5%ethanol in ethyl acetate (F7) | 76.14 bcd |
| 10%ethanol in ethyl acetate (F8) | 62.82 cd |

* 1.14 mg/sq.cm. concentration.

** Mean (%) of corrected mortality of 4 replicates (200-400 larvae/rep.). LSD .05 = 19.8253

**Table 2** The *in vitro* acaricidal activity of fractions of ethyl acetate part of *Annona squamosa* seeds on engorged female ticks.

| Solvents | Conc. (mg/ml) | Corrected mortality (Mean, %)* 24 h** | 48 h*** | 5 d**** |
|---|---|---|---|---|
| 100% Toluene (F1) | 2.5 | 0 f | 0 e | 0 e |
| 20% Ethyl acetate/toluene (F2) | 2.5 | 4 ef | 16 ed | 39 d |
| 40% Ethyl acetate/toluene (F3) | 2.5 | 68 ab | 80 b | 92 a |
| 60% Ethyl acetate/toluene (F4) | 2.5 | 59 acb | 96 ab | 100 a |
| 80% Ethyl acetate/toluene (F5) | 2.5 | 32 cdef | 46 c | 74 b |
| 100% Ethyl acetate/toluene (F6) | 2.5 | 74 a | 96 ab | 100 a |
| 5% Ethyl acetate/toluene (F7) | 2.5 | 60 abc | 100 a | 100 a |
| 10% Ethyl acetate/toluene (F8) | 2.5 | 0 f | 0 e | 0 e |
| 20% Ethyl acetate/toluene (F2) | 1.0 | 0 f | 0 e | 14 e |
| 40% Ethyl acetate/toluene (F3) | 1.0 | 34 cde | 86 ab | 100 a |
| 60% Ethyl acetate/toluene (F4) | 1.0 | 26 edf | 88 ab | 98 a |
| 80% Ethyl acetate/toluene (F5) | 1.0 | 4 ef | 32 cd | 58 c |
| 100% Ethyl acetate/toluene (F6) | 1.0 | 38 bcd | 92 ab | 100 a |
| 5% Ethanol/ethyl acetate (F7) | 1.0 | 54 abcd | 92 ab | 100 a |

* Mean (%) of corrected mortality of 5 replicates (10 ticks/rep.)
** LSD .05 = 29.21,
*** LSD .05 = 16.70,
**** LSD .05 = 15.25

**Table 3** The *in vitro* acaricidal activity of various solvents extracts of *Annona squamosa* seeds on engorged female ticks.

| Extracted solvents | Conc. (V/V) | Corrected mortality (Mean, %)* 24 h** | 48 h*** | 5 D**** |
|---|---|---|---|---|
| 20% ethanol (1:2, W/V) 24 h | 100% | 5 f | 27 def | 60 bc |
| 10% ethanol (1:2, W/V) 24 h | 100% | 68 abc | 83 ab | 100 a |
| | 50% | 66 abc | 87 ab | 94 a |
| | 33% | 73 ab | 92 ab | 98 a |
| 10% ethanol (1:2, W/V) 48 h | 100% | 28 edf | 52 cd | 74 ab |
| 10% ethanol (1:2, W/V) 24 h | 100% | 3 f | 7 f | 7 e |
| 11.66% White spirit (1:2, W/V) 24 h | 100% | 37 cde | 84 bc | 96 a |
| 48 h | 100% | 25 def | 62 bc | 92 a |
| Water (1:2, W/V) | 100% | 12 ef | 38 cde | 41 cd |
| Water boiling (1:2, W/V) 15 min | 100% | 74 ab | 84 ab | 98 a |
| | 50% | 64 abc | 88 ab | 96 a |
| | 33% | 24 def | 48 cd | 58 bc |
| | 25% | 24 def | 24 def | 24 de |
| Water boiled + ethanol to 10% ethanol | 100% | 60 bc | 98 a | 100 a |
| | 50% | 50 bcd | 64 bc | 80 ab |
| | 33% | 12 ef | 14 ef | 32 d |
| 10% Ethanol of boiled mill, 24 h | 100% | 60 bc | 88 ab | 96 a |

* Mean (%) of corrected mortality of 5 replicated (20 ticks/rep.)
** LSD .05 = 28.40,
*** LSD .05 = 27.15,
**** LSD .05 = 23.29

nol extract of boiled seed mill also showed high acaricidal activity (Table 3).

## DISCUSSION

There are many previous reports on the insecticidal activity of the sugar apple, *Annona squamosa*, on other insects, but there has not yet been a report on its toxicity on the tropical cattle tick. The seed oil of *A. squamosa* has high insecticidal activity against beetles, pumpkin and cabbage aphids, houseflies (Naidu et al., 1953; Grainge and Ahmed, 1988); eggplant lace bugs (Reddy, 1958); human head-lice (Puapatanakul, 1980); leafhoppes (Mariappan and Saxena, 1983); yellow fever mosquitoes, silkworms, red cotton stainers, potato aphids chrysanthemum aphids, black bean aphids, diamondback moths, cotton leafworms, and drugstore beetles (Grainge and Ahmed, 1988). However, the seed oil has far inferior toxicity against the adults of houseflies, mosquitoes, flour beetles, and the larvae of wooling bear, and case-bearing clothes compared with pyrethrins and DDT (Cheema et al., 1958; and Mukerjea and Govind, 1958). The toxicity of the seed oil and its petroleum ether extracts on fruit flies is also poor, but aqueous fractions of water distillation of the seeds and fruit skin has moderate toxicity (Areekul et al., 1987). Most previous works have been carried out by using the non polar-solvent extracts of the seed. In the present work, the polar-solvent extracts (ethanol and water extracts) of the seed were tested. It has been shown that the polar-solvent extract of *A. squamosa* seeds are more intense in insecticidal activity. The extract showed very high toxicity on ticks within 24 h after dipping and showed no significant difference in corrected mortality between 24 h and 48 h after dipping (Chungsamarnyart et al., 1990).

The active insecticidal substance was found in moderate polar solvents (Table 2) and polar solvents (Table 3). The 10% ethanol and boiling water extracts showed high toxicity, especially in the 3 times dilution(33% conc.) of 10% ethanol extracts. The boiling water extract exhibited weaker toxicity than 10% ethanol extract (Table 3). The 10% ethanol extract of the residue also showed high acaricidal activity (Table 3). Therefore, farmers can extract the active compound from milled seeds by themselves with 2 times the weight of 10% ethanol for 24 h and then rinse its residue with the same volume of 10% ethanol for 2 times. The collection of 10% eth[illegible]xtract solution and its rinsed solution can be and directly sprayed on the ticks on the cattle. The 10% ethanol extract can be kept longer than boiling water extract, since it will not ferment rapidly. However, if farmers cannot get the ethanol, they can extract the substance by boiling water and use it within 24 h.

The effective acaricidal substances of *A. squamosa* has not yet been identified. However, the cytotoxic substance, squamocin, has been found in the petroleum ether extract of seeds (Fujimoto *et al.*, 1988). The ethanol extract of bark has also been found acetogenin which has a toxic effect on brine shrimp (Li et al., 1990). The further purification of the active acaricidal substance and determination of the chemical structure are currently under study.

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