

Evaluations of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non target fish

SUWANNEE PROMSIRI¹, AMARA NAKSATHIT², MALEEYA KRUATRACHUE² and USAVADEE THAVARA³

¹The Biology Program, Faculty of Science, Rajabhat Songkhla University, Songkhla province, ²Department of Biology, Faculty of Science, Mahidol University, Bangkok, and ³National Institute of Health, Department of Medical Science, Ministry of Public Health, Nonthaburi province, Thailand

Abstract A preliminary study was conducted to investigate the effects of the extracts of 112 medicinal plant species, collected from the southern part of Thailand, on *Aedes aegypti*. Studies on larvicidal properties of plant extracts against the fourth instar larvae revealed that extracts of 14 species showed evidence of larvicidal activity. Eight out of the 14 plant species showed 100% mosquito larvae mortality. The LC₅₀ values were less than 100 µg/mL (4.1 µg/mL–89.4 µg/mL). Six plant species were comparatively more effective against the fourth instar larvae at very low concentrations. These extracts demonstrated no or very low toxicity to guppy fish (*Poecilia reticulata*), which was selected to represent most common non-target organism found in habitats of *Ae. aegypti*, at concentrations active to mosquito larvae. Three medicinal plants with promising larvicidal activity, having LC₅₀ and LC₉₀ values being 4.1 and 16.4 µg/mL for *Mammea siamensis*, 20.2 and 34.7 µg/mL for *Anethum graveolens* and 67.4 and 110.3 µg/mL for *Annona muricata*, respectively, were used to study the impact of the extracts on the life cycle of *Ae. aegypti*. These plants affected pupal and adult mortality and also affected the reproductive potential of surviving adults by reducing the number of eggs laid and the percentage of egg hatchability. When each larval stage was treated with successive extracts at the LC₅₀ value, the first instar larvae were found to be very susceptible to *A. muricata* and the second instar larvae were found to be susceptible to *A. graveolens*, while the third and fourth instar larvae were found to be susceptible to *M. siamensis*. These extracts delayed larval development and inhibited adult emergence and had no adverse effects on *P. reticulata* at LC₅₀ and LC₉₀ values, except for the *M. siamensis* extract at its LC₅₀ value.

Key words *Aedes aegypti*, guppy fish, larvicidal activity, medicinal plants, *Poecilia reticulata*

DOI 10.1111/j.1744-7917.2006.00080.x

Introduction

One primary vector of yellow fever, chikungunya fever,

dengue fever, dengue haemorrhagic fever (DHF) and dengue shock syndrome, is *Aedes aegypti* (Grantz, 1993). DHF is a major cause of child morbidity and hospitalization in Thailand as well as in other countries (Yasui, 1993). Control has been mainly affected by use of conventional insecticides but these have caused their own problems, such as adverse effects on the environment and the encouragement of pesticide resistance in some mosquitoes (Su & Mulla, 1998). These problems stimulated a search for safer

Correspondence: Amara Naksathit, Department of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand. Fax: +66 2 2470079; e-mail: grans@mahidol.ac.th

alternative anti-mosquito control.

It has been found that herbal extracts are one safer alternative method of control, especially the extracts of certain medicinal plants. One early report on the use of plant extracts against mosquito larvae was that of Campbell *et al.* (1933) where it was found that plant alkaloids like nicotine, anabasine, methyl anabasine and lumpinin extracted from the Russian weed, *Anabasis aphylla*, killed the larvae of *Culex* sp. Monzon *et al.* (1994) reported that some medicinal plants containing natural toxins were effective against mosquito larvae. Not only can medicinal plant extracts be effective but also they may greatly reduce the risk of adverse ecological effects and they do not induce pesticide resistance in mosquitoes. Since these chemicals are taken from medicinal plants, they are expected to have low human toxicity and a high degree of biodegradation (Choochote *et al.*, 1999).

Recently, many studies conducted in Thailand and around the world have shown that chemicals from medicinal plants have larvicidal, pupicidal and adulticidal effects on mosquitoes (Monzon *et al.*, 1994; Choochote *et al.*, 1999; Palakulk *et al.*, 1999; Rongsriyam & Baskoro, 1998; Shama & Shama, 1995). In the south of Thailand where many tropical rain forests are found, a vast number of medicinal plants have not had their mosquitocidal potential assessed. The identification and eventual use of indigenous medicinal plants in the control of mosquito larvae is beneficial to developing countries such as Thailand and its Southeast Asian neighbors. Thus, the principal objectives of this paper are, to screen the medicinal plants found in southern regions of Thailand for potential larvicidal effects, to test the effects of these extracts on the growth, survival, development and other life cycle aspects of the *Ae. aegypti*, and to study their impacts on fish, the most common group of non-target organisms in mosquito habitats.

Materials and methods

Plant collection

Samples of 112 medicinal plant species in 111 genera belonging to 50 families were collected from the southern part of Thailand from November 2000 to March 2002. Three methods of collection were used. Random sampling was the first method, *i.e.* taking any plant which could be collected in sufficient quantity and quality. The second method was to follow a chemotaxonomic approach, selecting the medicinal plants belonging to specific families which have been reported in scientific journals to have certain larvicidal or insecticidal properties. The third method was based on ethnopharmacological information (Martin,

1995): *i.e.* those plants already used as insecticides by local people. All parts of the plants were collected and kept separately in plastic bags and brought to the laboratory for extraction. Each herbarium specimen was prepared from each plant and was identified by: (i) comparing the morphological characters, habit and habitat with those described in the taxonomic literature; (ii) Dr Chamlong Pengklai and Dr Kongkanda Chayamarit, the Forest Botany Division, Forest Herbarium, National Park, Wildlife and Plant Conservation Department where voucher specimens were deposited.

Plant extraction

The method of plant extraction was modified from those of Satoto (1993) and Choochote *et al.* (1999). Five hundred grams of each plant (oven dried) was ground and filtered using a strainer silver number 60. The powder was macerated with 1.5 L of 80% ethanol solution and left to stand at room temperature for 3 days. The mixture was filtered through a Whatman no.1 filter paper by suction and the filtrate was evaporated under vacuum at 40 °C until completely dried, and kept at a constant 4 °C until needed for tests.

Rearing of Aedes aegypti

Ae. aegypti eggs were obtained from a colony maintained at the Medical Entomology Laboratory of the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.

Filter paper with attached eggs was dipped into a plastic tray containing 500 mL of dechlorinated water for 30–40 min, time enough to allow for eggs to hatch into larvae. They were reared indoors at 26 ± 2 °C, $70\% \pm 10\%$ RH and a 14:10 light: dark photoperiod and they were fed daily with ground mouse feed until such time as they molt to become pupae. They were moved into a mosquito cage where the emergent adults were fed with a 10% sucrose solution. Five days after emergence, female mosquitoes were allowed to blood-feed on white mice for 2–3 hours. A few days after having a blood meal, the gravid mosquitoes laid their eggs.

Larvicidal test

Larvicidal tests were assessed by the standard method of WHO (1981) with some modifications. Medicinal plant extracts, which produced more than 50% larval mortality in preliminary screening, were serially diluted at concentrations of 100, 50, 25, 12.5, 6.3, 3.2, 1.6, 0.8, 0.4, and 0.2 µg/mL. One hundred milliliters of each test solution was

placed in a plastic drinking cup along with (a standard group of) 25 fourth instar larvae. Each experiment was conducted with (alongside) four replicates and a concurrent control group. A control group consisted of 0.03 mL of absolute ethanol and 99.97 mL of distilled water and an untreated one, which contained only 100 mL of distilled water. No food was provided during the treatment. Observations were made 24 and 48 hours after treatment; dead larvae were counted and preserved in a 50% ethanol solution. Subsequently, the lowest concentration of crude extract that had successfully produced more than 50% larval mortality rate was used in a toxicity test on a non-target organism. A probit analysis using a computer program (SPSS/PC+, Finney, 1964) was employed on the results to determine LC₅₀ and LC₉₀ values.

Effects on growth and development of Ae. aegypti

The effects of the three promising medicinal plant extracts, that were found to be effective as larvicides, were studied as follows:

Immature stages mortality and adult emergence

Each cup from a larvicidal test (part 1 above), that was still holding living larvae after a 48-hour period, was placed into a separate mosquito cage. All larvae and emergent adults were fed daily. Larval, pupal and adult mortality as well as adult female and male emergence rates was determined.

Egg and larval number reduction A determination was made of the extract impact on fertility and of fecundity in emerging adults. After blood feeding and mating, females were isolated in another cage and allowed to lay eggs. The eggs were collected, counted, and recorded daily until all females died, the eggs then being allowed to hatch after being dried for a 3-day period. The number of viable larvae was recorded at the fourth instar stage. The larval reduction in the F₁ generation due to exposure to medicinal plant extract was calculated using the following formula (Thangam & Kathiresan, 1992).

$$\text{Larval reduction (\%)} = [(A - B/A)] \times 100\%$$

A = Average number of larvae that hatched per female per cage in the control (The average was calculated in relation to the total number of mosquitoes tested). B = Average number of larvae hatched per female per cage for mosquitoes treated with a particular plant extract.

Growth retardation and prolongation of development Groups of 25 of the first, second, third and fourth instar larvae of *Ae. aegypti* were each exposed to LC₅₀ doses of the three specific kinds of (selected) medicinal plant extract. There was no fixed duration of exposure:

exposure was sustained from the onset of egg hatching until larval death or else of successful adult emergence. The larvae were fed with ground mouse feed for the duration of their exposure. Data on growth and survival percentage was collected and the Harley index (1967) was used for comparing the relevant effects of the various extracts, the Harley formula being:

Mean index = Percentage of individuals pupation + Percentage of individuals reaching adulthood/median day of pupation.

Toxicity to guppy fish

A test to determine the susceptibility of a selected non-target organism to plant extracts followed Mittal *et al.*'s approach (1994). The guppy fish, *Poecilia reticulata*, was selected as the non-target organism. The following stage test was conducted.

Stage one assessment of toxicity, at the lowest concentration of crude extract to produce a more than 50% larval mortality rate in a larvicidal test. This test was conducted on guppy fish. Thirty guppy fish were placed in a rectangular, glass aquarium containing 400 mL of plant extract water solution in three replicates. Each group of 30 fish was exposed to a test solution. A control, consisting of 30 fish in dechlorinated tap water, was studied at the same time. The number of dead fish was recorded first at a 24-hour point and then at a 48-hour point and the percentage mortality calculated. All of these bioassay tests were conducted at a room temperature of approximately 27–28 °C, without aeration or renewal of water.

Stage two assessment of toxicity first at a LC₅₀ value (point) and then at a LC₉₀ value (point), which showed no toxic effects (had suffered no illness) to fish in stage one, was conducted in the same manner.

Results

Larvicidal tests

Preliminary screening 14 species of plant extracts were found to show high toxicity with a more than 50% mortality rate at of the fourth instar larvae stage in a concentration of 100 µg/mL at the 48 h after treatment point (Table 1).

Larvicidal tests Eight out of fourteen plant species, *Anacardium occidentale*, *Mammea siamensis*, *Phyllanthus pulcher*, *Anethum graveolens*, *Kaempferia galanga*, *Cinnamomum porrectum*, *Costus speciosus*, and *Acorus calamus* at a concentration of 100 µg/mL showed 100% larval mortality after an exposure of 48 hours (Table 2).

Table 1 Fourteen medicinal plants with high toxicity to the fourth instar larvae of *Aedes aegypti* in preliminary screening.

| Family and scientific name | Plant habit | Thai common name | English common name | Part use |
|---|-------------------|---------------------|---------------------|----------------|
| Malvaceae <i>Abutilon indicum</i> | Under shrub | Fan si | Country mallow | Roots |
| Leguminosae- Mimosoideae <i>Samanea saman</i> | Tree | Chamchuri | Rain tree | Stem bark |
| Costaceae <i>Costus speciosus</i> | Herb | Ueang mai na | Spiral flag | Roots |
| Araceae <i>Acorus calamus</i> | Herb | Wan nam | Sweet flag | Roots |
| Myristicaceae <i>Knema globularia</i> | Tree | Lueat raet | — | Seeds |
| Stemonaceae <i>Stemona tuberosa</i> | Herb | Non tai yak | — | Roots |
| Strychnaceae <i>Strychnos nux-vomica</i> | Shrub | Salaeng chai | Snake wood | Seeds |
| Zingiberaceae <i>Kaempferia galanga</i> | Herb | Pro hom | — | Roots |
| Lauraceae <i>Cinnamomum porrectum</i> | Tree | Thep tharo | — | Wood |
| Euphorbiaceae <i>Phyllanthus pulcher</i> | Shrub | Wan thorani san | — | Leaves & twigs |
| Anacardiaceae <i>Anacardium occidentale</i> | Exotic shrub tree | Mamuang Him ma phan | Cashew nut tree | Seed shell |
| Guttiferae <i>Mammea siamensis</i> | Tree | Saraphi | Negkassar | Flower |
| Umbelliferae <i>Anethum graveolens</i> | Exotic herb | Thain khoa Pluea | Dill | Leaves & twigs |
| Annonaceae <i>Annona muricata</i> | Exotic shrub tree | Thurian thet | Durian belanda | Seeds |

These plant extracts showed high larvicidal activity, their LC_{90} after 48 hours < 100 $\mu\text{g}/\text{mL}$, 13.9–56.2 $\mu\text{g}/\text{mL}$ (Table 2). Six remaining medicinal plant species, *Strychnos nux-vomica*, *Knema globularia*, *Stemona tuberosa*, *Samanea saman*, *Annona muricata*, *Abutilon indicum* at a concentration of 100 $\mu\text{g}/\text{mL}$ showed moderate percentage mortality of larvae (93%, 88%, 80%, 78%, 69% and 57%, respectively), their LC_{90} after 48 hours < 200 $\mu\text{g}/\text{mL}$, 82.6–130.3 $\mu\text{g}/\text{mL}$ (Table 2).

Effects on growth and development of *Ae. aegypti*

Immature stages mortality and adult emergence

The effects of the extracts on still living larvae were monitored continuously after an initial 48-hour period of exposure. It was found that slightly more pupae and also more adults died following exposure to solutions containing *A. muricata* and *A. graveolens* extracts than did larvae.

Details of the comparative effects of three medicinal plant extracts on mortality of *Ae. aegypti* are shown in Table 3. It was found that three of the medicinal plant extracts, *M. siamensis*, *A. graveolens* and *A. muricata* caused very low mortality among larvae, actual rates of mortality being 0%–3%, 0%–6%, and 0%–10%, respectively. The mean mortality rates of larvae for all concentrations of these plant extracts were 1%, 2%, and 2%, respectively. Mortality of pupae ranged from 2%–7%, 2%–9%, and 0%–13%, respectively. The mean number of dead pupae listed in the same order, were 3%, 5%, and 7%, respectively. Similarly, mortality at the adult stage ranged from 2%–26%, 4%–35%, and 9%–20%, respectively. The mean numbers of dead adults were 10%, 18% and 13%, respectively. Adult emergence from pupae after permanent exposure to three medicinal plant extracts ranged from 2%–79%, 9%–81%, and 6%–87%, respectively, while the means were 34%, 42%, and 63%, respectively.

Table 2 Toxicity of 14 medicinal plant extracts on *Aedes aegypti* fourth instar larvae.

| Scientific name | 24 h | | 48 h | |
|-------------------------------|---------------------------------------|---------------------------|--------------------------|--------------------------|
| | LC ₅₀ [†] (µg/mL) | LC ₉₀ (µg/mL) | LC ₅₀ (µg/mL) | LC ₉₀ (µg/mL) |
| <i>Abutilon indicum</i> | 94.2 acg (81.3–112.1) | 136.3 k (116.5–170.9) | 89.4 a (78.0–106.3) | 130.3 m (112.9–162.3) |
| <i>Samanea saman</i> | 79.2 bf (68.5–92.8) | 121.11 s (103.8–145.5) | 69.2 bf (59.7–75.5) | 104.5 n (90.0–121.9) |
| <i>Costus speciosus</i> | 98.5 ac (83.1–164.8) | 171.2 m (113.1–387.5) | 33.7 ci (28.5–39.3) | 53.6 os (45.4–64.1) |
| <i>Acorus calamus</i> | 67.2 de (9.1–79.2) | 103.6 ns (90.4–123.6) | 40.15 d (34.6–46.0) | 56.2 os (50.0–68.7) |
| <i>Knema globularia</i> | 72.1 def (3.8–107.1) | 114.4 ls (87.0–183.2) | 53.2 e (39.9–76.4) | 82.6 p (64.5–126.6) |
| <i>Stemona tuberosa</i> | 75.2 bef (5.2–88.8) | 114.9 ls (100.2–140.3) | 65.8 bfg (56.4–78.0) | 105.0 n (91.2–128.4) |
| <i>Strychnos nux-vomica</i> | 90.0 ag (6.5–108.4) | 137.7 k (115.7–171.7) | 62.8 bfg (55.7–74.6) | 92.2 q (82.3–112.7) |
| <i>Kaempferia galanga</i> | 30.7 h (7.5–37.0) | 44.9 o (39.8–55.1) | 29.5 hi (24.9–34.5) | 43.6 rs (36.3–52.3) |
| <i>Cinnamomum porrectum</i> | 43.5 i (9.5–73.5) | 73.6 p (53.0–136.7) | 31.5 hi (26.7–36.3) | 49.8 ors (42.1–58.3) |
| <i>Phyllanthus pulcher</i> | 25.8 h (9.9–66.2) | 50.7 o (21.2–76.1) | 15.9 j (9.6–28.0) | 28.0 t (22.7–68.1) |
| <i>Anacardium occidentale</i> | 9.1 j (6.5–11.4) | 15.3 q (10.8–19.9) | 7.7 k (4.9–9.6) | 13.9 u (8.7–17.3) |
| <i>Mammea siamensis</i> | 5.9 j (4.53–7.6) | 27.8 r (17.7–40.1) | 4.1 k (3.0–5.1) | 16.4 u (11.2–24.5) |
| <i>Anethum graveolens</i> | 27.4 h (17.1–41.9) | 50.0 o (31.6–79.4) | 20.2 l (12.5–40.9) | 34.7 t (24.2–81.9) |
| <i>Annona muricata</i> | 69.25 def (52.1–101.0) | 113.9 lns (86.0–175.1) | 67.4 bf (50.8–98.8) | 110.3 n (85.2–173.3) |

[†]Value ranges in the brackets stand for LC estimated by probit analysis 95% confidence limit. Letters are indices to show similarities of concentrations at LC₅₀ and LC₉₀. The pair or group of plants that have the same indices have similar concentration at $P \geq 0.05$ or different at $P \leq 0.05$.

Number of eggs and larval reduction In comparison to the control, the three medicinal plant extracts reduced the number of eggs laid, the hatching ability and the larvae in the first generation of *Ae. aegypti* after treatment as fourth instar larvae. The results are shown in Table 3. The mean numbers of eggs laid for the three medicinal plant extracts were 46 eggs, 31 eggs, and 27 eggs, respectively. The percentages of hatched eggs for the three medicinal plant extracts ranged from 47%–60%, 40%–62%, and 35%–63%, respectively, while the mean percentages of hatched eggs were 54%, 51%, and 49%, respectively. The percentage reductions in larvae ranged from 20%–53%, 42%–82%, and 60%–84%, respectively, while the mean reductions in larvae were 44%, 66%, and 70%, respectively.

Growth retardation and prolongation of developmental period The comparative effects of the three medicinal plant extracts, at their LC₅₀ concentrations, on the growth, survival and prolongation of the various instar larvae of *Ae. aegypti* are shown in Table 4. It was found that the first instar larvae were more susceptible to *A. muricata* and the second instar larvae more susceptible to *A. graveolens*. The third and fourth instar larvae were more susceptible to *M. siamensis*. Survival percentage of pupae was reduced when treated at the first and second instar stage with *A. muricata* and at the third and fourth instar stage with *A. graveolens*. Percentage adult emergence was lower for first and second instar larvae treated with *A. muricata*, for third instar treated with *A. graveolens* and for fourth instar treated with *M. siamensis*, than that of the

Table 3 Effects of three medicinal plant extracts on immature stages mortality and adult emergence of *Aedes aegypti*.

| Plant | Concentration ($\mu\text{g}/\text{mL}$) | Number of larvae after 48 h | Mortality (%) | | | | | Alive (%) | | | Average number of eggs laid/female | Hatched eggs (%) | Reduction in larvae over control |
|-------------------------------|--|-----------------------------------|---------------|----|----|----|----|-----------|----|----|--|---------------------|--|
| | | | L | P | A | M | F | A | M | F | | | |
| <i>Mammea siamensis</i> | 100.0 | — | — | — | — | — | — | — | — | — | — | — | — |
| | 50.0 | 3 | 1 | 2 | — | — | — | — | — | — | — | — | — |
| | 25.0 | 11 | 1 | 2 | 6 | 5 | 1 | 2 | 1 | 1 | 40 | 53 | 53 |
| | 12.5 | 14 | 3 | 4 | 2 | 2 | 0 | 5 | 3 | 2 | 65 | 55 | 20 |
| | 6.3 | 23 | 2 | 3 | 6 | 5 | 1 | 12 | 8 | 4 | 40 | 58 | 49 |
| | 3.2 | 49 | 1 | 3 | 23 | 11 | 12 | 22 | 5 | 17 | 52 | 48 | 44 |
| | 1.6 | 83 | 3 | 4 | 15 | 14 | 1 | 61 | 18 | 43 | 60 | 47 | 38 |
| | 0.8 | 99 | 1 | 5 | 26 | 20 | 6 | 67 | 28 | 39 | 42 | 52 | 51 |
| | 0.4 | 99 | 2 | 7 | 13 | 8 | 5 | 77 | 45 | 32 | 39 | 56 | 51 |
| | 0.2 | 100 | 0 | 2 | 19 | 17 | 2 | 79 | 33 | 46 | 40 | 60 | 47 |
| | Mean | 48 | 1 | 3 | 10 | 8 | 2 | 34 | 14 | 20 | 46 | 54 | 44 |
| Control | 100 | 0 | 0 | 3 | 3 | 0 | 97 | 57 | 40 | 47 | 94 | — | |
| <i>Anethum graveolens</i> | 100.0 | — | — | — | — | — | — | — | — | — | — | — | — |
| | 50.0 | 9 | 0 | 9 | — | — | — | — | — | — | — | — | — |
| | 25.0 | 24 | 5 | 6 | 4 | 4 | 0 | 9 | 5 | 4 | 59 | 44 | 42 |
| | 12.5 | 46 | 0 | 8 | 11 | 9 | 2 | 27 | 14 | 13 | 33 | 52 | 62 |
| | 6.3 | 86 | 6 | 5 | 27 | 24 | 3 | 48 | 24 | 24 | 18 | 61 | 76 |
| | 3.2 | 100 | 1 | 6 | 35 | 28 | 7 | 58 | 19 | 39 | 28 | 47 | 71 |
| | 1.6 | 100 | 1 | 2 | 31 | 27 | 4 | 66 | 20 | 46 | 16 | 50 | 82 |
| | 0.8 | 100 | 0 | 3 | 30 | 28 | 2 | 67 | 27 | 40 | 33 | 52 | 62 |
| | 0.4 | 100 | 3 | 9 | 27 | 20 | 7 | 61 | 7 | 54 | 21 | 62 | 71 |
| | 0.2 | 100 | 0 | 5 | 14 | 10 | 4 | 81 | 45 | 36 | 40 | 40 | 62 |
| | Mean | 67 | 2 | 5 | 18 | 15 | 3 | 42 | 16 | 26 | 31 | 51 | 66 |
| Control | — | — | — | — | — | — | — | — | — | — | — | — | |
| <i>Annona muricata</i> | 100.0 | 31 | 2 | 3 | 20 | 14 | 6 | 6 | 2 | 4 | 25 | 36 | 80 |
| | 50.0 | 44 | 10 | 12 | 9 | 7 | 2 | 13 | 7 | 6 | 23 | 61 | 69 |
| | 25.0 | 77 | 1 | 6 | 17 | 14 | 3 | 53 | 25 | 28 | 24 | 63 | 67 |
| | 12.5 | 97 | 3 | 12 | 16 | 15 | 1 | 66 | 18 | 48 | 39 | 46 | 60 |
| | 6.3 | 100 | 0 | 13 | 9 | 9 | 0 | 78 | 42 | 36 | 16 | 44 | 84 |
| | 3.2 | 100 | 0 | 6 | 12 | 8 | 4 | 82 | 31 | 51 | 22 | 59 | 71 |
| | 1.6 | 100 | 1 | 5 | 14 | 11 | 3 | 80 | 34 | 46 | 27 | 48 | 71 |
| | 0.8 | 100 | 0 | 4 | 11 | 8 | 3 | 85 | 48 | 37 | 29 | 62 | 60 |
| | 0.4 | 100 | 1 | 5 | 12 | 7 | 5 | 82 | 47 | 35 | 38 | 48 | 60 |
| | 0.2 | 100 | 0 | 0 | 13 | 10 | 3 | 87 | 44 | 43 | 23 | 35 | 82 |
| | Mean | 85 | 2 | 7 | 13 | 10 | 3 | 63 | 30 | 33 | 27 | 49 | 70 |
| Control | — | — | — | — | — | — | — | — | — | — | — | — | |

L = larva, P = pupa, A = adult, M = male, F = female.

control. The larvae took more time to develop to pupae, *i.e.*, 30 days, 33 days, 35 days and 20 days, respectively, in developing to pupation compared to the control, when the first instar were treated with *A. graveolens* extract, the second and the fourth instars with *M. siamensis* extract, and the third instar larvae with *A. muricata* extract, respectively. *Anethum graveolens* produced a marked reduction in Harley's index of 4, 5, 5, and 4 in the first, second, third and

fourth instar larvae, respectively, while *M. siamensis* produced an index of 4 in the fourth instar stage in comparison with the control.

Toxicity to guppy fish

Toxicity at the lowest concentration of crude extracts that produced more than 50% larval

Table 4 Effect of the three medicinal plant extracts at LC₅₀ on survival and prolongation of different larvae instar of *Aedes aegypti*.

| Scientific name | First instar (n) | | | | | | Second instar (n) | | | | | | Third instar (n) | | | | | | Fourth instar (n) | | | | | |
|----------------------|------------------|-----|------|-----|-----|-----|-------------------|-----|------|-----|-----|-----|------------------|-----|------|-----|-----|-----|-------------------|-----|------|-----|-----|-----|
| | L | P/ | A/ | LP | MD | MI | L | P/ | A/ | LP | MD | MI | L | P/ | A/ | LP | MD | MI | L | P/ | A/ | LP | MD | MI |
| | (%) | | (%) | | | | (%) | | (%) | | | | (%) | | (%) | | | | (%) | | (%) | | | |
| <i>M. siamensis</i> | 33 | 20 | 15 | 21 | 14 | 8 | 58 | 46 | 40 | 33 | 19 | 8 | 59 | 45 | 36 | 26 | 15 | 9 | 37 | 13 | 5 | 20 | 14 | 4 |
| | (61) | | (46) | | | | (79) | | (69) | | | | (76) | | (61) | | | | (35) | | (14) | | | |
| <i>A. graveolens</i> | 85 | 36 | 26 | 30 | 19 | 4 | 45 | 19 | 16 | 22 | 15 | 5 | 80 | 23 | 35 | 26 | 15 | 5 | 64 | 20 | 11 | 18 | 11 | 4 |
| | (42) | | (31) | | | | (42) | | (36) | | | | (29) | | (44) | | | | (31) | | (17) | | | |
| <i>A. muricata</i> | 28 | 11 | 8 | 27 | 15 | 5 | 64 | 24 | 18 | 16 | 11 | 6 | 60 | 37 | 33 | 35 | 17 | 7 | 55 | 34 | 23 | 17 | 14 | 7 |
| | (39) | | (29) | | | | (38) | | (28) | | | | (62) | | (55) | | | | (62) | | (42) | | | |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

L = Larva; P = Pupa; A = Adult; LP = Larvae period; MD = Median day; MI = Mean index.

mortality in the larvicide's test Out of 14 plant extracts found to be larvicidal, eight plant extracts were toxic to guppy fish and six extracts were found to be not so toxic. *Abutilon indicum*, *Samanea saman*, *Costus speciosus*, *Acorus calamus*, *Knema globularia*, *Stemona tuberosa*, *Strychnos nux-vomica*, and *Kaempferia galanga* extracts were toxic to guppy fish at concentrations of 100, 100, 50, 50, 50, 100, 100, and 50 µg/mL, respectively. They all produced 100% mortality for guppy fish except for *S. tuberosa* and *K. galanga*, which resulted in 80% mortality. Six plant species, *C. porrectum*, *P. pulcher*, *A. occidentale*,

M. siamensis, *A. graveolens*, and *A. muricata* were not toxic to guppy fish at concentrations of 50.0, 12.5, 6.3, 3.2, 12.5 and 50.0 µg/mL, respectively (Table 5).

Toxicity of the three medicinal plant extracts to guppy fish at their LC₅₀ and LC₉₀ concentrations

Of the three larvicidal plants *A. graveolens*, *A. muricata*, and *M. siamensis* did not exhibit any noticeable effect on guppy fish after either 24 or 48 hours of exposure at their LC₅₀ and LC₉₀ values. However, *M. siamensis* at LC₅₀ value did affect guppy fish slightly, and produced 17% mortality at the LC₅₀ value and 100% mortality at the LC₉₀ value.

Table 5 Toxicity of extracts of medicinal plants to guppy fish at the lowest concentration that produced more than 50% larval mortality in the larvicidal test.

| Scientific name | Concentration which caused more than 50% larval mortality (µg/mL) | Mortality of selected non-target organism (%) |
|-------------------------------|---|---|
| <i>Abutilon indicum</i> | 100.0 | 100 |
| <i>Samanea saman</i> | 100.0 | 100 |
| <i>Costus speciosus</i> | 50.0 | 100 |
| <i>Acorus calamus</i> | 50.0 | 100 |
| <i>Knema globularia</i> | 50.0 | 100 |
| <i>Stemona tuberosa</i> | 100.0 | 80 |
| <i>Strychnos nux-vomica</i> | 100.0 | 100 |
| <i>Kaempferia galanga</i> | 50.0 | 80 |
| <i>Cinnamomum porrectum</i> | 50.0 | 0 |
| <i>Phyllanthus pulcher</i> | 12.5 | 0 |
| <i>Anacardium occidentale</i> | 6.3 | 0 |
| <i>Mammea siamensis</i> | 3.2 | 10 |
| <i>Anethum graveolens</i> | 12.5 | 0 |
| <i>Annona muricata</i> | 50.0 | 0 |
| Control | 0.0 | 0 |

Discussion

Larvicidal tests

From the 112 species of medicinal plant collected from the southern part of Thailand, 14 species (12.5%) showed toxicity against the third and the fourth instar larvae of *Ae. aegypti* while eight out of these 14 species (7.1%) demonstrated toxicity to the selected non-target organism. Six of the fourteen species (5.4%) showed excellent larvicidal properties against the fourth instar larvae of *Ae. aegypti*. All had LC₅₀ values at 48 hours after treatment under 100 µg/mL (4.1–67.4 µg/mL); therefore, these plants have potential as good mosquitocides.

Effects on growth and development of *Ae. aegypti*

The immature stages mortality and the stage of the emergent adult

Low mortality rates of larvae were observed after permanent exposure to extracts of *M. siamensis*, *A. graveolens*, and *A. muricata*. It was found that some larvae successfully continue to develop up to the pupal stage. Of these, some failed at the pupal stage, but

still some emerged as adults. *Mammea siamensis* demonstrated some inhibition of the growth of larvae more than any correspondent inhibition of pupa and of adults. Extracts of *A. graveolens*, and of *A. muricata* produced larvae mortality rates after 24 hours and 48 hours of relevant exposure, rates that were lower than these produced by *M. siamensis*, and yet these two extracts produced high mortality rates among adults and among pupae. Some of the larvae did not die within the 48 hour period, but instead they died at the pupal or at the adult stage, due to the chronic effects of chemical compounds attributable to the medicinal plant extract (Sukumar *et al.*, 1991). It has already been reported that some plant chemicals produced either larvicidal, pupicidal or adulticidal effects, and that at the various stages of their life cycle, mosquitoes differ markedly in their susceptibility to these phytochemicals (Rongsriyam & Baskoro, 1998).

Number of eggs and larval reduction It was found that the average number of eggs laid by females that emerged from medicinal plant treatment was lower than the number of eggs laid by the females of the control group. Hatchability of these eggs was also low and the size of the first generation was small when mosquitoes were treated at the third and the fourth instar larvae stage, including mosquitoes treated with *M. siamensis* extract. Mahidol *et al.* (2002) reported that plants of the genus *Mammea* were known to be rich sources of various coumarine and xanthenes and coumarines were reported to exhibit diverse biological activities. It seemed possible that *M. coumarins* flowers might inhibit ecdysteroid hormone and juvenile hormone, or that while cytotoxic coumarines and insecticidal compounds stimulated ovaries to produce more eggs, these compounds still had an adverse effect on egg development of *Ae. aegypti* in that they decreased their hatchability. These findings have both biological and physiological significance in that they indicate that some compound had affected reproductive organ development, insinuated itself into the eggs and affected some vital physiological and biochemical processes associated with embryonic development. It would seem that this was the case with all those eggs that failed to hatch (Choochote *et al.*, 1999; Zebitz, 1984). Su and Mulla (1998) report coumarines capable of producing such multiple effects on insects as anti-feeding, growth inhibition, fecundity suppression, sterilization and changes in biological fitness.

Growth retardation and prolongation of developmental period There was a delay in the development of larvae to the pupal stage when the first, second, third or fourth instar larvae were exposed to all three medicinal plant extracts. This was especially noted in *A. graveolens*, followed by *A. muricata*. This may be due to

the presence of high juvenile hormone levels in the larvae or else due to chemical compounds in the medicinal plant, preventing normal pupation and preventing adult emergence from occurring. Zebitz (1984) reports that azadirachtin may act as an anti-ecdysteroid or else otherwise affected the neuroendocrine control of ecdysteroids such that growth is inhibited and that the developmental period is prolonged. Mohtar *et al.* (1999) report the effect of a methanol-aqueous extract of *Nerium indicum* leaf at 100 mg/L on different larval instars of *Ae. aegypti* and show an elongation of the preimago period for all their larvae treated when compared to the control larvae. Many studies have drawn attention to the effects of plant extracts on adult eclosion (Yodbutra *et al.*, 1985; Schwartz *et al.*, 1998). The benefit of elongation is that mosquito larvae numbers are reduced due to the longer period needed for a new generation to complete the mosquito life cycle (Havertz & Curtin, 1967).

This study found that six medicinal plant extracts, *C. porrectum*, *P. pulcher*, *A. occidentale*, *M. siamensis*, *A. graveolens*, and *A. muricata* evinced high larvicidal activity against the fourth instar larvae of *Ae. aegypti*. Furthermore, *M. siamensis*, *A. graveolens*, and *A. muricata* were all found to have a chronic effect on the fourth instar larvae of *Ae. aegypti* after permanent exposure, leading to a reduction in the number of resultant eggs and subsequent larvae of the mosquitoes.

Mode of action of three medicinal plant extracts, *M. siamensis*, *A. graveolens* and *A. muricata* as it impacted on the life cycle of *Ae. aegypti* was studied.

Mammea siamensis crude extract was the most toxic of the three, with a 48-hour LC₅₀ value of 4.1 µg/mL. Other workers have reported similar results. Issakul *et al.* (2004), who investigated insecticidal substances extracted from *M. siamensis*, report insecticidal affects on the eggs of the house fly, *Musca domestica*. Avirutnant and Pongpan (1983) reported that alcohol and water extracts of *M. siamensis* flowers shown inhibitory effects on microorganisms.

The second potential anti-*Ae. aegypti* plant extract was the dill plant, *A. graveolens*, having a 48-hour LC₅₀ value of 20.2 µg/mL. Its leaves are used for their antimicrobial and nematocidal properties, as well as for their properties as insect repellent and insecticide. It has been found to contain insecticidal components and also is synergistic with carbamate and organophosphorous insecticides for some insect species. Carvone and myristicin in the aerial parts of dill, including seeds (“dill green”) act as insecticides and synergists (Lichtenstein *et al.*, 1974). Supavarn *et al.* (1974) report a methanol extract of whole plants of *A. graveolens*, demonstrating low toxicity to the fourth instar larvae of *Ae. aegypti* but high inhibition of pupal

development.

Annona muricata seed was found to be an active larvicide with a 48-hour LC₅₀ value of 67.4 µg/mL. Jacobson (1958) reports similar results for the seed extracts of another *Annona* species, namely *A. cherimola*, *A. glabra* and *A. squamosa*, which had lethal effects on larvae of *Aedes* sp. Its potential as a larvicidal plant was further supported by the results of a recent study by Satoto (1993), who found that *A. squamosa* seed was one of the most effective larvicides against both *Culex tritaeniorhynchus* and *Ae. aegypti*. Grainge and Ahmed (1988) report that the seed alkaloids from *A. muricata* were antifeedants to *Ae. aegypti*, known for about 10 years; they are characteristic of the Annonaceae (*Annona*, *Asimina*, *Goniothalamus*, *Rollinia*, *Uvaria*), where they are mostly concentrated in the seeds. The potential applications of these molecules are due to their marked cytotoxic and antitumor (asimicin, bullatacine), antibacterial (cherimolin) and insecticidal (asimicin) properties.

Toxicity to guppy fish

They were non-toxic to the tested non-target organism, except *M. siamensis* at larval LC₉₀ value for *Ae. aegypti* was slightly toxic to fish. *Mammea siamensis* has been reported to contain proanthocyanidin polymers as the active compounds producing piscicidal effects (Balza *et al.*, 1989). However, dried flowers from these plants have been traditionally used in herbal medicine (Soralum *et al.*, 2001) and they have not been found to be toxic to human beings. The other two plant extracts had no toxic effect on fish.

Investigators in this study were not able to investigate *C. porrectum*, *P. pulcher* and *A. occidentale* although these plants are worthy of further study. Furthermore, *A. occidentale* appears to have strong potential as a larvicide. However, the extraction process, from cashew nuts, is complex as it contains a toxic astringent as well as complex corrosive oily substances.

Acknowledgments

This work was funded by the Thailand Research Fund Organization (PDF/59/2540), Mahidol and Rajabhat Songkhla Universities, Thailand.

References

Avirutnant, W. and Pongpan, A. (1983) The antimicrobial activity of some Thai flowers and plants. *Journal of Pharmaceuti-*

- cal Sciences*, 10, 81–86.
- Balza, F., Abramowski, Z., Neil, G.H., Towers, G.H. and Wiriyachitra, P. (1989) Identification of proanthocyanidin polymers as the piscicidal constituents of *Mammea siamensis*, *Polygonum stagninum* and *Diospyros diepenhorstii*. *Phytochemistry*, 28, 1827–1830.
- Campbell, F.L., Sullivan, W.W. and Smith, L.N. (1933) The relative toxicity of nicotine, nabasine, methylalanine and lupinine for *Culex* mosquito larvae. *Journal of Economic Entomology*, 26, 505–509.
- Choochote, W., Kanjanapothi, D., Panthong, A., Taesotikul, T., Jitpakdi, A., Chaithong, U., Pitasawat, B. (1999) Larvicidal, adulticidal and repellent effects of *Kaempferia galanga*. *South-east Asian Journal of Tropical Medicine and Public Health*, 30, 470–476.
- Finney, D.J. (1964) *Statistical Method in Biological Assay*. 2nd edn., Hafner Publishing Co., New York. pp. 668.
- Grainge, M. and Ahamed, S. (1988) *Handbook of Plants with Pest Control Properties*. John Wiley & Sons, Toronto. pp. 470.
- Grantz, G.N. (1993) What must we do to effectively control *Aedes aegypti*. *Journal of Tropical Medicine*, 35, 243–251.
- Harley, S.L.K. (1967) A note on the influence of a range of plant chemicals on the growth and survival of *Aedes aegypti* L. larvae. *Canadian Journal of Zoology*, 45, 1297–1300.
- Havertz, D.S. and Curtin, T.J. (1967) Reproductive behavior of *Aedes aegypti* sub-lethally exposed to DDT. *Journal of Medical Entomology*, 4, 143–145.
- Issakul, K., Kongtrakoon, W., Dheeranupatana, S., Jangsutthivorawat S. and Jatisatienr, A. (2004) *Insecticidal effectiveness of compounds from Mammea siamensis* Kost. against *Musca domestica* Linn. *ISHS Acta Horticulturae*, 629, XXVI International Horticultural Congress: *The Future for Medicinal and Aromatic Plants*.
- Jacobson, M. (1958) *Insecticides from Plants. A Review of the Literature, 1954–1971*. Agriculture Handbook, U.S.D.A., Washington, D.C., pp. 1941–1952.
- Lichtenstein, E.P., Liang, T.T., Schulz, K.R., Schnoes, H.K. and Carter, G.T. (1974) Insecticidal and synergistic components isolated from dill plants. *Journal of Agricultural and Food Chemistry*, 22, 658–664.
- Mahidol, C., Kawetripob, W., Prawat, H. and Ruchirawat, S. (2002) *Mammea* coumarins from the flowers of *Mammea siamensis*. *Journal of Natural Products*, 65, 757–760.
- Martin, G.J. (1995) *Ethnobotany: a Methods Manual*. Chapman & Hall, London. pp 268.
- Mittal, P.K., Adak, T. and Sharma, V.P. (1994) Comparative toxicity of certain mosquitocidal compounds to larvivoracious fish, *Poecilia reticulata*. *Indian Journal of Malariology*, 31, 43–47.
- Mohtar, M., Yarmo, M.A. and Kadri, A. (1999) The effects of *Nerium indicum* leaf extract on *Aedes aegypti* larvae. *Journal of Tropical Forest Products*, 5, 87–92.

- Monzon, R.B., Alvior, J.P., Luczon, L.L., Morales, A.S. and Mutuc, F.E. (1994) Larvicidal potential of five Philippine plants against *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say). *Southeast Asian Journal of Tropical Medicine and Public Health*, 25, 755–759.
- Palakul, K., Sucharit, S., Komalamisra, N. and Deesin, V. (1999) Larvicidal activity of Thai Ka-lum-pak sa-lad dai *Euphobia antiqurum* Linn. against *Aedes*, *Culex*, *Anopheles* and *Mansonia* larvae in laboratory. Research Abstract, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok. pp. 384.
- Rongsriyam, Y. and Baskoro, T. (1998) Medicinal plants for replacement of insecticides used in vector control. Research Abstract, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok. pp. 683.
- Satoto, T.B.T. (1993) A laboratory study of the biological effects of some medicinal plants on *Culex tritaeniorhynchus* sp. MS thesis in Tropical Medicine, Faculty of Graduate Studies, Mahidol University, Bangkok. pp. 119.
- Schwartz, A.M., Paskewitz, S.M., Orth, A.P., Tesch, M.J., Toong, Y.C. and Goodman, W.G. (1998) The lethal effects of *Cyperus iria* on *Aedes aegypti*. *Journal of American Mosquito Control Association*, 14, 78–82.
- Shama, S. and Shama, K.V.P. (1995) Field studies on the mosquito repellent action of neem oil. *Southeast Asian Journal of Tropical Medicine and Public Health*, 26, 180–182.
- Soralum, P., Choasakool, W. and Bhathantoorak, S. (2001) *Medicinal Plant Encyclopedia. V. I. Gardenherb of Sirerukkachart*. Faculty of Pharmacy, Mahidol University, Bangkok. pp. 253.
- Su, T. and Mulla, M.S. (1998) Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of American Mosquito Control Association*, 14, 204–209.
- Supavarn, P., Knapp, F.W. and Sigafus, R. (1974) Biologically active plant extracts for control of mosquito larvae. *Mosquito News*, 34, 398–402.
- Sukumar, K., Perich, M.J. and Boobar, L.R. (1991) Botanical derivative in mosquito control. A review. *Journal of American Mosquito Control Association*, 7, 210–216.
- Thangam, T.S. and Kathiresan, K. (1992) Smoke repellency and killing effect of mangrove plants against the mosquito *Aedes aegypti* (Linnaeus). *Tropical Biomedicine*, 10, 125–128.
- World Health Organization (1981) *Instructions for determining the susceptibility or resistance of mosquito larvae to insect development inhibitor*. WHO/Vector Biology and Control, 812–881.
- Yasui, K. (1993) Strategies of dengue vaccine development by WHO. Using new biotechnology. *Journal of Tropical Medicine and Hygiene*, 35, 233–241.
- Yodbutra, S., Ketavan, C., Upatham, E.S. and Areekul, S. (1985) Effects of a juvenile hormone analogue on the morphology and biology of *Aedes scutellaris malayensis* Colless (Diptera: Culicidae). *Southeast Asian Journal of Tropical Medicine and Public Health*, 16, 41–48.
- Zebitz, C.P.W. (1984) Effects of some crude and azadirachtin-enriched neem (*Azadirachta indica*) seed kernel extracts on larvae of *Aedes aegypti*. *Australian Journal of Entomology*, 39, 208–211.

Accepted February 21, 2006