A Preliminary Study: Comparative Toxicity of Extracts from *Tinospora tuberculata* Beumee and *Lumnitzera racemosa* Willd on *Aedes aegypti* Linnaeus Larvae (Diptera: Culicidae)

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Research has been widely made on the potential use of herbal plants as effective agents for mosquito vector control. However, only a few studies have been carried out to investigate the potential insecticidal activity of mangrove plants against these dengue vectors. This study was carried out to examine the comparative toxicity of two mangrove plants, *Tinospora tuberculata* and *Lumnitzera racemosa* against the *Aedes aegypti* larvae. The leaf and stem of *T. tuberculata* and *L. racemosa*, were extracted using 95% methanol and these extracts bioassayed against the 3rd instar larvae of *aegypti* under laboratory conditions. The LC₅₀ values of the crude extract of *T. tuberculata* stem and leaf were 0.5778 mg/ml and 0.7213 mg/ml, respectively. For *L. racemosa*, the LC₅₀ value for stem was 1.2833 mg/ml and 1.1957 mg/ml for leaf. However, no significant differences in toxicity were detected for the stem and leaf of both plants. Both plants, especially *T. tuberculata* might contain insecticidal components toxic to larvae of *Ae aegypti*.

Key words: Toxicity; *Tinospora tuberculata*; *Lumnitzera racemosa*; *Aedes aegypti*; herbal plants; mosquito vector; dengue vectors

Some mosquitoes are known to transmit many serious diseases to humans. Among these mosquitoes, the commonest genus that bites humans are *Anopheles*, *Culex*, *Aedes*, *Mansonia*, *Haemogogus*, *Sebethes* and *Psorophora*. In Malaysia, there are 381 species in 20 genera of mosquitoes, some of which are medically important vectors of arboviruses (Ken *et al.* 2002).

The principal mosquito vector that transmits the arbovirus of dengue is *Aedes aegypti*. The dengue (Flaviviridae) virus is arguably the most important arbovirus affecting humans (Curtis 1986). Dengue fever and dengue hemorrhagic fever are caused by one of four closely related

but antigenically distinct virus serotypes, DEN-1 DEN-2 DEN-3 and DEN-4. Infection by one of these serotypes provides immunity to only that serotype for life, so people living in a dengue-endemic area could have more than one dengue infection during their lifetime (Glubber 1989).

The incidence of dengue has attained levels that are of considerable concern to local authorities. Until today, there are no effective dengue vaccines available. The only way of decreasing the incidence of the disease is the suppression of *Ae. aegypti*. Experience has shown that spraying of chemical insecticides against this mosquito was not effective, since

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it was highly domesticated and many adults rested indoors in hidden places such as closets. One of the possible ways of reducing mosquito densities to a level where dengue epidemics did not occur was to control the immature stage. Therefore, systematic treatment of the breeding places using larvicide could be effective.

In recent years, the use of environmentally friendly and biodegradable agents of plant origin has received renewed interest for disease vector control (Alkofahi *et al.* 1989). They are potentially suitable for use in integrated vector management programmes as the selective pressure imposed by conventional insecticides was enhancing resistance in mosquito populations. Today, over 2000 species of plants that possess some insecticidal activity are known (Jacobson 1989). The demand for new products that are environmentally safe, target-specific, and degradable enhance interest for natural products. Considerable effort has been focused on plant derived material for potentially suitable useful products such as commercial insecticides. The first compound that was used extensively against adult mosquitoes was the flower extract of *Chrysanthemum cinerariaefolium* (Senthil *et al.* 2005). Plant derived chemicals also show some potential in reducing the biological activities of insects rather than synthetic insecticides. For example, the oil extracts from *Cinnamomum pubescens*, *C. micropyllum* and *Litsea elliptica* (Family: Lauraceae) show effective larvicidal activity (Wong 2001). Other plants such as *Bougainvillea glabra* (Family: Nyctaginaceae) also have the potential as pesticides (Thangam & Kathiresan 1990).

Considering the large number of plants that are reputed to possess some form of insecticidal activity, only a few local plants have been scientifically evaluated. In this current study, we were interested in examining the potential insecticidal activity of the herbal plant, *Tinospora tuberculata* (Ranunculales:

Menispermaceae) and the mangrove plant, *Lumnitzera racemosa* (Angiospermaceae: Combretaceae) on 3rd instar *Ae. aegypti* larvae. There have been very few studies focused on *T. tuberculata* and *L. racemosa* as insecticides or pesticides (Thangam & Kathiresan 1990; Lee & Hishamudin 1990). We chose *L. racemosa* as this mangrove plant was abundantly found in Terengganu. Furthermore, some studies have reported that the fruit, bark and flower of *L. racemosa* have been used as treatment for asthma, diabetes and snake bite (Premanathan *et al.* 1992). Attempts also have been made to compare the different toxicity effects of crude extract from the leaf and stem of both plants. The outcome of the study could hopefully contribute to the discovery of an alternative bio-insecticide for the control of *Ae. aegypti* larvae.

MATERIALS AND METHODS

Sample Collection and Extraction

T. tuberculata was collected from an area around Wakaf Tembusu in Terengganu, whereas *L. racemosa* was obtained from a mangrove area near the nursery of Universiti Malaysia Terengganu (UMT). The plant material according to the part needed, were crushed into small particles and dried at room temperature (~27°C). Dried plant particles were ground into powder separately. 100g of the powder from each needed part of the plant were mixed with 1000 ml of methanol (95%) and stirred for 3 days in a stirrer machine. Filtration was done on the suspension using Whatman No.1 filter paper and filter funnel into a beaker. The filtered solution was placed into a flask and concentrated by evaporation using a low pressure rotary evaporator at 40°C–45°C. Extractions were done until all the solvent was evaporated leaving the solid crude. The crude extract was stored in a beaker covered with aluminum foil at 20°C for future bioassay. The same process was done for all needed parts of both plants.

Preparation of Stock Solution and Test Concentrations

Stock solutions of extracts from both plants were prepared with concentrations of 50 mg/ml. These were obtained by diluting 5 g of crude extract in 100 ml of solvent (1% of DMSO). The stock solution was then serially diluted, in triplicate, with distilled water to prepare test solutions of 2 mg/ml, 1 mg/ml, 0.500 mg/ml, 0.250 mg/ml and 0.125 mg/ml crude extract. Sterile water was used to dilute the crude extract and preparation of the dilutions of leaf and stem for each plant species were the same using the method as above.

Aedes aegypti Larvae

Eggs of *Ae. aegypti* were obtained from the insectarium of the Unit of Medical Entomology, Institute for Medical Research, Malaysia. Larvae of *Ae. aegypti* were obtained by placing a filter paper with dried mosquito eggs into a tray filled with water. The eggs were left for 1 h to 48 h to hatch. One teaspoon of dried ox liver powder was added into the container as larval food once the eggs had hatched. The larvae were maintained at room temperature ($27^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and at a relative humidity of between 75%–85%. The water in the container that was used to rear the larvae was changed frequently to ensure that it was clean and clear. The container was covered with muslin cloth to ensure that other mosquitoes did not lay eggs into the tray.

Bioassay Procedure

Bioassays were performed on the late 3rd or early 4th instar larvae of *Ae. aegypti*. One hundred ml of test solutions of five different concentrations were prepared. At each concentration, 10 larvae were tested. In control runs, 100 ml of distilled water without the extract was prepared. The treatments were replicated three times and each replicate set

contained one control. Experiments were conducted at room temperature ($\sim 27^{\circ}\text{C}$), photoperiods of 12 h light followed by 12 h dark (12L : 12D) and at a relative humidity of 75%–85%. Mortality counts were made after 24 h of exposure. In the analysis, both dead and moribund larvae were considered as dead, whereas numbers of live larvae were counted separately.

Statistical Analysis

Data obtained from the bioassay toxicity test were analysed by probit analysis (Finney 1971). Probit analysis was used to calculate the confidence intervals for dose-response quantiles (LC_{50}). One-way ANOVA was used to compare the toxicity of leaf and stem crude extract for each part of plants studied. The data was $\log(x+1)$ transformed to ensure normality in calculations of means and ANOVAs. If the control mortality was below 20%, Abbott's formula was used (World Health Organization 1981). However, in tests where the control mortality was above 20%, the experiment was discarded.

RESULTS

The LC_{50} of the leaf extract of *T. tuberculata* was 0.7213 mg/ml against third instar *Ae. aegypti* larvae. In comparison, the LC_{50} of the stem was 0.5778 mg/ml against *Ae. aegypti* larvae. There was no mortality observed in the controls. In this experiment, there was no significant difference detected between the leaf and stem of the herbal plant, *T. tuberculata* ($p = 0.2396$).

The LC_{50} value for stem and leaf of *L. racemosa* was 1.2833 mg/ml (for stem) and 1.1957 mg/ml (for leaf). There was no mortality observed in the controls and no significant difference between the leaf and stem of the mangrove plant, *L. racemosa* was detected ($p = 0.2430$).

Table 1 summarizes the lethal concentration of extract (LC₅₀) for each part of plants against the 3rd instar *Ae. aegypti* larvae. The insecticidal activity of stem and leaf of *T. tuberculata* was higher (0.5778 mg/ml and 0.7213 mg/ml) than the stem and leaf extraction of *L. racemosa* (1.2833 mg/ml and 1.1957 mg/ml). However, the variance between leaf and stem of *T. tuberculata* showed no significant difference ($p = 0.2370$). Similarly, no significant difference between the leaf and stem of *L. racemosa* was detected ($p = 0.243$).

DISCUSSION

Even though there were no significant differences between the leaf and stem for each plant, both plants showed the presence of certain insecticidal chemical compounds. The study suggests that both *T. tuberculata* and *L. racemosa* did have compounds which exhibited insecticidal activity on the target insect. Both *T. tuberculata* and *L. racemosa* plants exhibited different levels of toxic effect on third instar *Ae. aegypti* larvae. *T. tuberculata* exhibited higher toxicity compared to *L. racemosa* against the 3rd instar *Ae. aegypti* larvae. However, no significant differences of LC₅₀ values were detected between leaf and stem of *T. tuberculata*. For *L. racemosa*, both stem and leaf exhibited LC₅₀ values of 1.2833 mg/ml and 1.1957 mg/ml, respectively. Similarly, both LC₅₀ values of leaf and stem of *L. racemosa* showed no significant differences.

The results showed that extracts of *T. tuberculata* stem greatly affected the growth of *Ae. aegypti* larvae compared to extracts from the leaf. Generally, higher concentrations of each part of *T. tuberculata* affected the larvae development and did have a significant impact on the mortality of the larvae. We found most of the larvae exhibited abnormal and erratic movements, dying before reaching the pupal stage when they were exposed to higher concentrations of *T. tuberculata*. The bioactivity of phytochemicals from plants against mosquito larvae could vary significantly depending on the plant species, plant part, age of plant part, solvent used in extraction and mosquito species (Essam *et al.* 2004). Presumably, the toxicity effect exhibited by both plants indicated possible bioactive compounds that were engaged in insecticidal response. Plants from family Menispermaceae had been identified to exhibit pharmaceutical properties and also as insecticides (Essam *et al.* 2004). Previous studies using plants from family Menispermaceae had obtained very good toxicity effects on *Ae. aegypti* larvae. For example, *Stephania japonica*, which was distributed widely in Ulu Anap, Sarawak secondary forest, had been identified to have antimalarial properties (Ibuka *et al.* 1967). Although the toxicity effect possessed by *T. tuberculata* was much lower than other plants from family Menispermaceae, the experiment had proved that the plant exhibited insecticidal activity. *Tinospora tuberculata* contained insecticidal phytochemicals that

Table 1. LC₅₀ values for crude extract of stem and leaf of *Tinospora Tuberculata* and *Lumnitzera Racemosa* against *Aedes Aegypti* larvae after 24 h.

Plant species	Part of plant	Regression equation	Chi-square value	LC ₅₀ (mg/ml)
<i>Tinospora tuberculata</i>	Leaves	$y = -0.3623x + 5.6422$	0.8957	0.5778
	Stem	$y = -0.4287x + 5.9133$	0.3968	0.7213
<i>Lumnitzera racemosa</i>	Leaves	$y = -1.1381x + 4.6715$	1.3587	1.2833
	Stem	$y = -1.4967x + 4.6275$	1.1192	1.1957

were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects (Ibuka *et al.* 1967).

Pyrethrum, derris, quassia, nicotine, hellebore, anabesine, azadirachtin, *d*-limonene camphor and turpentine were some of the important phytochemical insecticides which were widely used in many countries before the introduction of synthetic organic insecticides (Thangam & Kathiresan 1990; Bandara 2000). *T. tuberculata* also contained alkaloid as its secondary metabolite. Secondary metabolites or phytochemicals could be extracted from either the whole plant or from specific parts of the plant that were known to contain a concentration of the desired active chemical. Alkaloids are known as an important secondary metabolites which impart resistance against many insect pests. The resistance to feeding insects by a number of well known alkaloids, the plant sources from which they had come and the insect species deterred, have been uncovered (Essam *et al.* 2004; Ibuka *et al.* 1967; Bandara 2000).

Lumnitzera racemosa was considerably less explored compared with other plants, especially herbal plants which were well known for their medicinal properties. That was possibly because mangrove plants were presumed to have no value for medicinal usage and thus very few studies had been conducted to investigate the potential of mangrove plants as an alternative product in medicinal usage. Due to that reason, we would like to explore and understand whether mangrove plants can possibly be used as an alternative to conventional synthetic insecticides, besides the herbal plants. In this study, we have found that the leaf of the *L. racemosa* was more effective than the stem. *L. racemosa* did have a toxic effect on *Ae. aegypti* larvae, with leaf extract more toxic compared with the stem extract. However, the active chemical compounds of *L. racemosa* were unknown and for the future research, we

plan to investigate the chemical properties of *L. racemosa* and their role as potential insecticides for controlling the population of *Ae. aegypti*.

Different mosquito species display different susceptibilities to similar phytochemicals. Thus, this toxicity study might have been much more promising if it had been carried out against different species of mosquitoes. The results of this study however, could be useful in the research for newer, more selective and biodegradable larvicidal compounds. The extracts of these plants can also be combined with synthetic insecticides to obtain more suitable results. For example, leaf and flower extracts of *Bougainvillea glabra*, exhibit higher insecticidal activity when combined with DDT, Benzene hexachloride and malathion (Thangam & Kathiresan 1990). It would be interesting to investigate whether the insecticidal and insect repellent compounds in both mangrove and herbal plants contain tannins and alkaloids or other classes of compounds. Further investigations needed are to isolate these compounds using combinations of liquid chromatography, nuclear magnetic resonance spectroscopy and high-pressure liquid chromatography. These techniques have been used by natural product chemists to isolate and determine the structures of interesting molecules.

CONCLUSION

The results of this study shows promising insecticidal activity from both herbal (*T. tuberculata*) and mangrove (*L. racemosa*) plants towards the 3rd instar *Ae. aegypti* larvae. *T. tuberculata* exhibited higher larvicidal toxicity compared to *L. racemosa*. Both plants, *T. tuberculata* and *L. racemosa* had the potential to produce alternative insecticides effectively against the *Ae. aegypti* larvae. Further research would have to be done to determine the active compounds from both plants that could be used as larvicides.

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