International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:9, No:11, 2015

# Screening for Larvicidal Activity of Aqueous and Ethanolic Extracts of Fourteen Selected Plants and Formulation of a Larvicide against *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse) Larvae

Michael Russelle S. Alvarez, Noel S. Quiming, Francisco M. Heralde

***Abstract*—**This study aims to: a) obtain ethanolic (95% EtOH) and aqueous extracts of *Selaginella elmeri, Christella dentata, Elatostema sinnatum, Curculigo capitulata, Euphorbia hirta, Murraya koenigii, Alpinia speciosa, Cymbopogon citratus, Eucalyptus globulus, Jatropha curcas, Psidium guajava, Gliricidia sepium, Ixora coccinea* and *Capsicum frutescens* and screen them for larvicidal activities against Aedes aegypti (Linn.) and Aedes albopictus (Skuse) larvae; b) to fractionate the most active extract and determine the most active fraction; c) to determine the larvicidal properties of the most active extract and fraction against by computing their percentage mortality, LC50, and LC90 after 24 and 48 hours of exposure; and d) to determine the nature of the components of the active extracts and fractions using phytochemical screening. Ethanolic (95% EtOH) and aqueous extracts of the selected plants will be screened for potential larvicidal activity against *Ae. aegypti* and *Ae. albopictus* using standard procedures and 1% malathion and a Piper nigrum based ovicide-larvicide by the Department of Science and Technology as positive controls. The results were analyzed using One-Way ANOVA with Tukey's and Dunnett's test. The most active extract will be subjected to partial fractionation using normal-phase column chromatography, and the fractions subsequently screened to determine the most active fraction. The most active extract and fraction were subjected to dose-response assay and probit analysis to determine the LC50 and LC90 after 24 and 48 hours of exposure. The active extracts and fractions will be screened for phytochemical content. The ethanolic extracts of *C. citratus, E. hirta, I. coccinea, G. sepium, M. koenigii, E globulus, J. curcas* and *C. frutescens* exhibited significant larvicidal activity, with *C. frutescens* being the most active. After fractionation, the ethyl acetate fraction was found to be the most active. Phytochemical screening of the extracts revealed the presence of alkaloids, tannins, indoles and steroids. A formulation using talcum powder–300 mg fraction per 1 g talcum powder–was made and again tested for larvicidal activity. At 2 g/L, the formulation proved effective in killing all of the test larvae after 24 hours.

***Keywords*—**Larvicidal activity screening, partial purification, dose-response assay, *Capsicum frutescens.*

## I. Introduction

DENGUE is one of the most rapidly spreading vector-borne diseases in the world. It affects many countries, especially the ones in the tropical areas like Philippines, Cambodia, Malaysia, India, Indonesia, Thailand, Brazil, Argentina, Eastern Africa, and Nigeria, just to name a few. In the Philippines alone, the reported number of dengue cases as of September 2013, was 117, 658 with more than 20, 000 cases belonging to the 1-10 age group; of these, 433 deaths were reported [1]. The children are the most susceptible especially those belonging to the poorest class wherein inadequate water supply, improper waste management, and other conditions are favorable for the growth and multiplication of dengue's vector, *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse). Methods for exterminating the adult mosquitoes involve fumigation, bug zappers, mechanical pest control, and use of adulticides; all of which have been shown to be either inefficient, toxic or both. A better way of controlling mosquito populations – and in turn, control dengue spread – is by targeting the larvae instead of the adults. Larvicidal agents are commonly bacterial (e.g. Bt larvicides) or synthetic (e.g. methoprene, pyriproxyfen) in nature. Efficient and effective these agents may be, they still have certain issues regarding quality control and acceptance [2]. To circumvent the problems posed by the other methods, the usage of plant-derived products can be done instead.

Several plant species have promising properties that could make them good larvicidal agents against *Ae. aegypti* and *Ae. albopictus*. *Cymbopogon citratus* is traditionally used as an insect repellent [3]. Previous researchers showed the larvicidal activity of its methanol extract against *Anopheles arabiensis* [4] and its essential oil against *Ae. aegypti* [5]. *Capsicum frutescens* is also traditionally used as an insecticide [6]. In fact, previous researches showed the larvicidal activity of its ethanol extract against *Anopheles stephensi* and *Culex quinquefasciatus* [7] and its leaf and fruit methanol extract against *Ae. aegypti* larvae [8]. *Eucalyptus globulus* is also traditionally used as an insecticide and insect/pest repellent, larvicidal activity of its essential oil against *Ae. aegypti* [9]. *Euphorbia hirta*, on the other hand, is not traditionally used as an insecticide, but as a nematicide [10]. Its petroleum ether extract has been shown to have larvicidal activity against *Ae. aegypti* and *C. quinquefasciatus* [11]. *Jatropha curcas* is also known for its toxic activities, as well as its purgative action [12]. A larvicidal screening by [13] showed the activity of its methanol extract against *C. quinquefasciatus.* The steam distillate of *Murraya koenigii* leaves its use as a stomachic, purgative, febrifuge, antihelminthic and anti anemic agent [14]. In fact, its chloroform, methanol, dichloromethane,

M. R. Alvarez, N. S. Quiming, F. M. Heralde are with the Department of Physical Sciences and Mathematics, College of Arts and Sciences, University of the Philippines Manila, Philippines (E-mail: russellealvarez@gmail.com, noel_quiming@yahoo.com, fheralde@yahoo.com).

hexane and chloroform extracts have been shown to have larvicidal activity against *Ae. aegypti* and *A. stephensi* [15]. Its acetone and petroleum ether extract have also been studied for larvicidal activity against *Ae. aegypti* [16]. *Psidium guajava*, on the other hand, is not traditionally used as an insecticide; nevertheless, its acetone extract has been shown to be active against *Ae. aegypti* [17]. *Ixora coccinea* is another plant not traditionally used as an insecticide, but still possessed larvicidal activities against *Ae. aegypti* and *A. stephensi* [18]. *Gliricidia sepium*, although traditionally used as an insecticide [19], still have not been studied for its larvicidal activity against mosquito larvae.

The general goal of the study is to determine the most active plant extract that could serve as a larvicide against *Ae. aegypti* (Linn.) and *Ae. albopictus* (Skuse) larvae and formulate it into a form suitable for application to spawning grounds of the larvae.

## II. Materials and Methods

### A. Reagents

The positive controls used were Malathion (bought as a pesticide concentrate from a hardware store) and the ovicide/larvicide from the Department of Science and Technology (DOST).

The solvents used in the extraction and column chromatography were analytical grade solvents bought from Chemline Inc., (Quezon City, Metro Manila, Philippines) The Dimethyl sulfoxide (DMSO) used in the dissolution of the extracts and the silica plates and silica gel used in the thin layer- and normal phase column chromatography were bought from Merck International (Merck Millipore, Philippines). Talcum powder used in the formulation was bought from a local drugstore.

### B. Plant Samples

Plants were obtained from various locales in the island of Luzon, the Philippines. Powdered forms of *C. dentata, C. capitulata, E. sinnatum* and *S.a elmeri* were obtained from dried leaves from the Laboratory 202 in the College of Arts and Sciences, University of the Philippines Manila; originally obtained from Mt. Isarog, Camarines Sur and was already identified prior to the study. *Euphorbia hirta, M. koenigii, A. speciosa* and *C. citratus* leaves were obtained from the Bureau of Plant Industry, Quirino Ave., Malate, Manila. *Eucalyptus globulus* and *J. curcas* leaves were obtained from Isabela State University – Echague Campus. *Psidium guajava* and *G. sepium* leaves were obtained from backyard plants at Echague, Isabela. *Capsicum frutescens* fruits were bought from local markets at Santiago City, Isabela. *Ixora coocinea* leaves were obtained from ornamental plants in Valenzuela City, Manila. The plants *E. hirta, M. koenigii, A. speciosa, C. citratus, E. globulus, J. curcas, P. guajava, G. sepium, I. coccinea* and *C. frutescens* were identified by the Research Division office at the Bureau of Plant Industry – Quirino office and the Botany Division of the National Museum, add an address. *Ae. aegypti* and *Ae. albopictus* third instar larvae were obtained from the Research Institute of Tropical Medicine, add an address.

### C. Extraction

At least twenty grams of the dried plant material were serially extracted with 95% ethanol and double distilled water in a 1 g plant material: 10 mL solvent ratio. The plant materials were extracted twice with ethanol by soaking at room temperature for 48-72 hours and with water by soaking at 4°C for 24 hours. After filtration, the ethanolic filtrates were concentrated with a rotary evaporator and subsequently evaporated in a water bath at 40°C while the aqueous filtrates were freeze-dried and lyophilized.

### D. Screening for Larvicidal Activity

The larvicidal assay was based on the Guidelines for Laboratory and Field Testing of Mosquito Larvicides [20] with slight modifications. The extracts were tested against *Ae. aegypti* and *Ae. albopictus* larvae separately. Prior to assaying, larvae were allowed to acclimatize in double distilled dechlorinated water (dddH 2O) for 2-3 hours. Glass assay cups containing 60 mL $dddH_2O$ (for aqueuous extracts) or 1% DMSO (for ethanolic extracts) were prepared as negative controls while 60 mL of 0.1 ppm Malathion was used as positive control. Ethanolic extracts were reconstituted in 1% DMSO while aqueous extracts were reconstituted in dddH 2O, making the final concentrations of the extracts 800 ppm. There were 3 replicates for each treatment – positive control, extracts and negative controls. Batches of 15 third instar larvae were put into the assay cups containing 60 mL of 800 ppm reconstituted extracts and left for 48 hours. Throughout the assay, the ambient temperature was kept at 30±2°C. Dead larvae were counted after 24 and 48 hours. Larvae were counted as dead if they cannot be induced to move when the cups were shaken, or prodded with a needle or if they are moribund. After statistical analysis using One Way ANOVA with Tukey's and Dunnett's post-hoc tests, the extract with the significantly highest mortality was subjected to partial purification and dose-response assay.

### E. Partial Purification

The most active extract was subjected to normal phase column chromatography in order to separate it into its components. Silica gel G60 was used as the stationary phase whereas the varying solvent combinations of increasing polarity were used as the mobile phases. The slurry used to pack the column was composed of silica gel and hexane. The packed column used was 3 cm in diameter and 36 cm in height for all runs. The samples were prepared in an evaporating dish by adsorbing 1.55 g of the crude extract to 3.9 grams of silica gel G60 and drying on a water bath at 40°C. Elution was done with solvent systems of gradually increasing polarity using hexane, ethyl acetate, methanol, deionized water and deionized water-acetic acid. The following ratios of 100% hexane 50 hexane: 50 ethyl acetate, 100% ethyl acetate, 80 ethyl acetate: 20 methanol, 60 ethyl acetate: 40 methanol, 40 ethyl acetate: 60 methanol, 20 ethyl acetate: 80 methanol, 100% methanol, 70 methanol:30 deionized water, 60

methanol:40 deionized water, 100% deionized water, 4 deionized water:1 acetic acid, and 2 deionized water: 1 acetic acid were sequentially used in the elution process. Measured volumes (200 mL) of each solvent combination were gradually poured into the column. Fifteen (15) mL eluents were collected and were pooled using a solvent system of 5 chloroform:1 methanol. Pooled fractions were then concentrated using a rotary evaporator at 40℃ and then evaporated at 40℃ to remove the solvent. In order to determine the most active fraction, each fraction was reconstituted in 1% DMSO and subjected to larvicidal assay as described above with 15 larvae per assay cup but using concentrations adjusted to 300 ppm in solution.

*F. Dose-Response Assay*

Batches of 15 third instar larvae were transferred into glass assay cups containing 60 mL of treatments. For the active extract, the treatments were 200, 300, and 400 ppm extract while for the active fraction; the treatments were 100, 200, 300 and 400 ppm fraction. For each treatment, three replicates were performed. Throughout the assay, the ambient temperature was kept at 30±2℃. Dead larvae were counted after 24 and 48 hours of exposure. The $LC_{50}$ and $LC_{90}$ after 24 and 48 hours of exposure to the most active extract and its most active fraction against *Ae. Aegpyti* and *Ae. Albopictus* were determined using probit analysis.

*G. Phytochemical Screening*

Phytochemical analysis was done on the active extracts that exhibited significantly high mortality. The extract was screened for the presence of flavonoids, steroids, tannins, alkaloid, anthraquinone, anthrones, coumarins and indoles via spray tests.

*H. Formulation of Larvicide*

At least the $LC_{90}$ of the most active fraction was mixed per gram of a vehicle such as talcum powder and allowed to dry for 3 to 4 hours to form a solid intermediate. Then, the solid intermediate was ground to obtain the powder form of the extract. This formulation was tested against *Ae. Aegypti* and *Ae. Albopictus* at 1 g/L and 2 g/L concentrations, using $dddH_2O$ and 2 g/L talcum powder in $dddH_2O$ as negative controls and 0.1 ppm Malathion and 1 g/L OL trap as positive controls. The assay was done as described above, in the screening for larvicidal activity.

*I. Statistical Analysis*

Percent mortality was computed for both crude extracts and fractions from Abbott's formula:

$$Corrected\ \%\ mortality = \frac{Mortality\%treatment - Mortality\%\ in\ control}{100 - Mortality\%\ in\ control} * 100 \quad (1)$$

Percent mortality was computed using the following formula:

$$Mortality\% = \frac{PercentSurvival_{control} - PercentSurvival_{treatment}}{Percent\ Survival_{control}} * 100 \quad (2)$$

Mean, standard deviation, and variance was calculated. One-Way ANOVA was used to determine the significance of the difference between the treatments and control. $LC_{50}$ and $LC_{90}$ values were computed using probit analysis.

To determine which fractions exhibited significant percent mortality compared to the negative control, One-Way ANOVA with Tukey's and Dunett's posthoc tests was used. Those extracts that exhibited significant percent mortality were subjected to ANOVA with Tukey's posthoc test to determine the statistically most active extract. The same tests were used to determine the most active fraction. To compute for the $LC_{50}$ and $LC_{90}$, probit analysis was performed.

## III. Results and Discussion

The ethanolic and aqueous extracts at 800 ppm of the fourteen selected plants were tested for larvicidal activity against *Ae. aegypti* and *Ae. albopictus* after 24 and 48 hours of exposure. The results of the screening can be seen in Table I. Of the fourteen ethanolic plant extracts, only eight plants exhibited significant larvicidal activity against *Ae. aegypti (Linn.)*: *M. koenigii* (53.33% after 24 hours and 71.11% after 48 hours), *E. globulus* (11.11% after 24 hours and 62.22% after 48 hours), *C. frutescens* (100% after 24 hours and 100% after 48 hours), *I. coccinea* (2.22% after 24 hours and 42.22% after 48 hours), *J. curcas* (84.44% after 24 hours and 88.89% after 48 hours) and *E. hirta* (22.22% after 24 hours and 48.89% after 48 hours) all had significantly higher percent mortality results compared to the negative control. On the other hand, against *Ae. albopictus* (Skuse): *C.* (2.22% after 24 hours and 15.6% after 48 hours), *G. sepium* (0% after 24 hours and 13.33% after 48 hours), *M. koenigii* (8.89% after 24 hours and 40% after 48 hours), *E. globulus* (64.44% after 24 hours and 73.33% after 48 hours), *J. curcas* (77.78% after 24 hours and 82.22% after 48 hours) and *C. frutescens* (93.33% after 24 hours and 100% after 48 hours) showed significantly higher percent mortalities compared to the negative control. To choose the most active extract, One-way ANOVA with Tukey's test was performed on the eight extracts that exhibited significant larvicidal activity. (The ANOVA table of this test can be provided upon request to the corresponding author.) Based on this test, *C. frutescens* ethanolic extract exhibited significantly higher larvicidal activity compared to the rest of the extracts ($p$-value<0.05).

The rest of the ethanolic plant extracts showed no larvicidal or otherwise statistically insignificant activity at 800 ppm. This is probably because the plant in question had larvicidal activity only observed when other solvent systems are used, *e.g. P. guajava acetone extract had larvicidal activity against Ae. aegypti* [21]. A possible explanation as to why these extracts showed minimal larvicidal activity was that the larvicidal components of these plants (if there are any at all) are poorly soluble in the solvents used; hence, the aqueous extracts exhibit low larvicidal activity. For example, capsaicin, a known larvicidal component of *C. frutescens*, has very low water solubility (around 0.0013 g/100 mL); thus, its aqueous extract is expected to have low activity. Another example is the larvicidal-active essential oil components of plants, like

that of *C. citratus* and *E. globulus*. Essential oils are immiscible in water; thus, the aqueous extracts of these plants are expected to have low activity. Additionally, the two-mosquito species also exhibited different susceptibilities to the plant extracts, but generally, *Ae. albopictus* showed higher resistance to the plant extracts compared to *Ae.aegypti*.

Capsicum frutescens ethanolic extract was subjected to dose-response assay to determine the LC50 and LC90 after 24 and 48 hours against *Ae. aegypti* and *Ae. albopictus*. For *Ae. aegypti*, the mean LC50 and LC90 after 24 hours were 231.59 ppm and 430.95 ppm, respectively while after 48 hours, they were 141.92 ppm and 346.74 ppm, respectively. For *Ae. albopictus*, the mean LC50 and LC90 after 24 hours were 300.20 ppm and 408.92 ppm, respectively while after 48 hours, they were 270.61 ppm and 474.60 ppm, respectively. Its most active fraction was also subjected to dose-response assay to determine the LC50 and LC90 after 24 and 48 hours of exposure against *Ae. aegypti* and *Ae. albopictus*. For *Ae. aegypti*, the LC50 and LC90 after 24 hours were 97.22 ppm and 181.13 ppm, respectively while after 48 hours, they were 66.02 ppm and 132.80 ppm, respectively. For *Ae. albopictus*, the LC50 and LC90 after 24 hours were 41.74 ppm and 164.53 ppm, respectively while after 48 hours, they were 24.35 ppm and 83.78 ppm, respectively. Table II summarizes the results of the probit analysis, including the mean LC50 and LC90 values for 24 and 48 hours, fiducial limits, $\chi 2$ and r2 values. The results of the dose-response assay indicate that the activity of *C. frutescens* ethanolic extract is dose-responsive; at increasing concentrations of the extract, the percent mortality also increases. The steep slopes of the probit regression models for *Ae. aegypti* after 24 and 48 hours, and for *Ae. albopictus* after 24 and 48 hours imply that even at low increases in extract concentration, a high larvae percent mortality can be observed. A $\chi 2$ value of less than 5.991 – the critical value for df equals 2 – in all the regression models indicate high goodness-of-fit, while a coefficient of determination (r2) value close to 1 imply that the results of the assays are reliable. Probit analysis of the most active fraction also indicates that the active fraction is dose-responsive. Again, the steep slopes indicate that even at low concentrations of the active fraction, a high larvae percent mortality can be observed. Since the $\chi 2$ value is also less than 5.991 and the r2 value is still close to 1, high goodness-of-fit and linearity of the regression models are observed. Since the LC50 and LC90 values for the active fraction are lower than those of the crude extract, it can be concluded that the active fraction is more potent than the crude extract. No overlaps in the fiducial limits for the LC50 and LC90 values except for the LC50 after 24 hours vs. *Ae. aegypti* imply that these values are significantly different. In comparison to the positive controls used, the activity of the active fraction at 300 ppm was comparable to the positive controls used.

The presence of capsaicinoid compounds, especially capsaicin – a known pest toxicant that causes metabolic disruption, membrane damage and nervous system dysfunction [25].

A formulation of 300 mg active fraction per 1 gram talcum powder was made, and 1 g/L and 2 g/L formulations were tested against both mosquito species. The results show (Fig. 1) that both negative controls had nil percent mortality while both positive controls killed all larvae within 24 hours. Against *Ae. aegypti* the 1 g/L formulation killed 93.33% and 100% of the larvae after 24 and 48 hours of exposure, respectively, and 62.22% and 93.33% of the *Ae. albopictus* larvae after 24 and 48 hours of exposure, respectively. On the other hand, the 2 g/L formulation killed all test larvae within 24 hours of exposure.

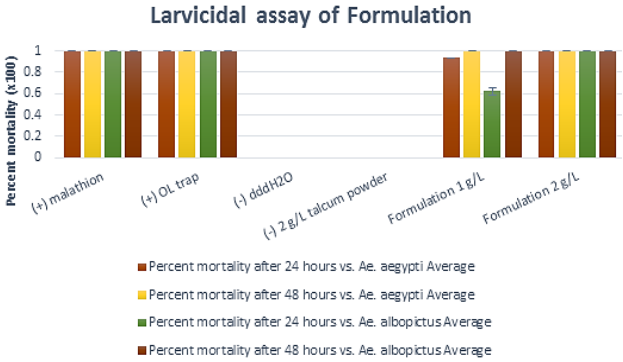


Fig. 1 Mapping nonlinear data to a higher dimensional feature space

Phytochemical screening for the presence of flavonoids, steroids, tannins, alkaloids, anthraquinones, anthrones, coumarins and indoles in the eight active extracts and one active fraction was performed, the results of which is presented in Table III. Of these phytochemicals, alkaloids, tannins, indoles and steroids were the most commonly observed in these active extracts and fraction. Based on the results of the phytochemical screening most if not all of the active plant extracts and fraction-contained tannins, alkaloids, indoles, and steroids. Alkaloids [22], tannins [23] and indoles [24] have been shown to possess toxic activities against insects; hence, their presence in the extracts can be the cause of their larvicidal activity. Phytochemical screening of the active fraction also showed tannins, alkaloids, coumarins, indoles, and steroids.

## IV. Conclusions

Out of the 28 extracts screened, only the ethanolic extracts of *C. citratus, E. hirta, I. coccinea, G. sepium, M. koenigii, E. globulus, J. curcas and C. frutescens* exhibited significant larvicidal activity, with the ethanolic extract of *C. frutescens* exhibiting the highest larvicidal activity of 100% mortality against *Ae. aegypti* after 24 and 48 hours, and 93.33% and 100% mortalities after 24 and 48 hours, respectively, against *Ae. albopictus* at 800 ppm.

Phytochemical screening of these active extracts revealed the presence of flavonoids in the ethanolic extracts of *M. koenigii* and *E. globulus*, tannins in all the significantly active extracts except *E. hirta*, alkaloids in all the significantly active extracts except *E. hirta* and *J. curcas*, anthrones in the ethanolic extracts of *C. citratus*, and *M. koenigii*, coumarins in

the ethanolic extracts of *E. globulus, J. curcas* and *C. frutescens*, and indoles and steroids in all the significantly active extracts.

TABLE I
SUMMARY OF THE LARVICIDAL SCREENING OF ETHANOLIC AND AQUEOUS EXTRACTS AGAINST AE. AEGYPTI AND AE. ALBOPICTUS AFTER 24 AND 48 HOURS OF EXPOSURE

| Plant species | Exposure (hours) | Against *Ae. aegypti* | | | | Against *Ae. albopictus* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ethanolic extracts | | Aqueous Extracts | | Ethanolic extracts | | Aqueous extracts | |
| | | Average Percent Mortality | *p-value** | Average Percent Mortality | *p-value** | Average Percent Mortality | *p-value** | Average Percent Mortality | *p-value** |
| (-) control | 24 | 0% | --- | 0% | --- | 0% | --- | 0% | --- |
| | 48 | 0% | --- | 0% | --- | 0% | --- | 0% | --- |
| *Selaginella elmeri* | 24 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 |
| | 48 | 4.44% | 0.599 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 |
| *Christella dentate* | 24 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 |
| | 48 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 |
| *Elatostema sinnatum* | 24 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 |
| | 48 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 |
| *Curculigo capitulata* | 24 | 2.22% | 0.671 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 |
| | 48 | 2.22% | 0.852 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 |
| *Cymbopogon citratus* | 24 | 0% | 0.968 | 2.22% | 0.671 | 2.22% | 0.705 | 2.22% | 0.705 |
| | 48 | 4.44% | 0.599 | 2.22% | 0.852 | 15.56% | 0.000 | 2.22% | 0.627 |
| *Psidium guajava* | 24 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 |
| | 48 | 4.44% | 0.599 | 0% | 0.968 | 4.44% | 0.130 | 0% | 0.968 |
| *Gliricidia sepium* | 24 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 | 0% | 0.968 |
| | 48 | 6.67% | 0.301 | 0% | 0.968 | 13.33% | 0.000 | 0% | 0.968 |
| *Murraya koenigii* | 24 | 53.33% | 0.000 | 2.22% | 0.671 | 8.89%% | 0.002 | 0% | 0.968 |
| | 48 | 71.11% | 0.000 | 2.22% | 0.852 | 40% | 0.000 | 0% | 0.968 |
| *Jatropha curcas* | 24 | 84.44% | 0.000 | 0% | 0.968 | 77.78% | 0.000 | 0% | 0.968 |
| | 48 | 88.89% | 0.000 | 0% | 0.968 | 82.22% | 0.000 | 0% | 0.968 |
| *Eucalyptus globulus* | 24 | 11.11% | 0.000 | 0% | 0.968 | 64.44% | 0.000 | 0% | 0.968 |
| | 48 | 62.22% | 0.000 | 0% | 0.968 | 73.33% | 0.000 | 0% | 0.968 |
| *Capsicum frutescens* | 24 | 100% | 0.000 | 0% | 0.968 | 93.33% | 0.000 | 0% | 0.968 |
| | 48 | 100% | 0.000 | 0% | 0.968 | 100% | 0.000 | 0% | 0.968 |
| *Ixora coccinea* | 24 | 2.22% | 0.671 | 0% | 0.968 | 4.44% | 0.235 | 0% | 0.968 |
| | 48 | 42.22% | 0.000 | 0% | 0.968 | 4.44% | 0.130 | 0% | 0.968 |
| *Euphorbia hirta* | 24 | 22.22% | 0.000 | 2.22% | 0.671 | 0% | 0.968 | 0% | 0.968 |
| | 48 | 48.89% | 0.000 | 2.22% | 0.852 | 0% | 0.968 | 0% | 0.968 |
| *Alpinia speciosa* | 24 | 0% | 0.599 | 2.22% | 0.671 | 0% | 0.968 | 0% | 0.968 |
| | 48 | 4.44% | 0.968 | 2.22% | 0.852 | 0% | 0.968 | 0% | 0.968 |

**p-values* were taken from Dunnet's post-hoc test, comparing the extracts against the negative controls at $\alpha$=0.05. *p-value*<0.05 implies statistically significant average percent mortality compared to the negative control.

TABLE II
RESULTS OF THE PROBIT ANALYSIS OF ETHANOLIC CAPSICUM FRUTESCENS EXTRACT AND ETHYL ACETATE FRACTION AGAINST AE. AEGYPTI AND AE. ALBOPICTUS

| Treatment | Mosquito Species | Exposure | $LC_{50}$[1] (LL, UL)[3] | $LC_{90}$[1] (LL, UL)[3] | Slope ± SE | $X^2$* | $r^2$ |
|---|---|---|---|---|---|---|---|
| *Capsicum frutescens* ethanolic extract | *Ae. aegypti* | 24 | 231.5881 (192.9089, 259.7932) | 430.9479 (366.6488, 603.8086) | 4.7518±0.9815 | 0.0205 | 0.999 |
| | | 48 | 141.9191 (52.6713, 188,5988) | 346.7425 (292.7324, 514.5759) | 3.3034±0.9869 | 0.0007 | 0.993 |
| | *Ae. albopictus* | 24 | 300.2024 (280.5081, 320.3958) | 408.921 (375.4258, 467.281) | 9.5481 ± 1.3413 | 2.3576 | 0.974 |
| | | 48 | 270.6146 (252.1678, 288.2887) | 374.6019 (346.4148, 418.9785) | 9.0753 ± 1.1284 | 1.9610 | 0.974 |
| *Capsicum frutescens* ethanolic extract –ethyl acetate fraction | *Ae. aegypti* | 24 | 97.2225 (76.341, 113.3585) | 181.1339 (155.7739, 228.5227) | 4.7426 ± 0.8384 | 0.7031 | 0.943 |
| | | 48 | 66.0239 (29.0088, 86.3972) | 132.8026 (108.3948, 178.3831) | 4.2227 ± 1.2109 | 0.0848 | 0.961 |
| | *Ae. albopictus* | 24 | 41.7358[4] | 164.5353[4] | 2.1512 ± 0.7327 | 3.6250 | 0.895 |
| | | 48 | 24.3486[4] | 83.7848[4] | 2.3880 ± | 0.3828 | 0.907 |

[1] mg extract/L
[3]LL=lower fiducial limit; UL = upper fiducial limit
[4]The LL and UL cannot be computed because their g (index of significance of potency) > 0.04.**df*=2

TABLE III
PHYTOCHEMICAL SCREENING SUMMARY LEGEND: (-): NO REACTION, (+): WEAK INTENSITY REACTION, (++): MEDIUM INTENSITY REACTION; (+++): STRONG INTENSITY REACTION

| Plant sample | Flavonoid | Tannin | Alkaloid | Anthraquinone | Anthrone | Coumarin | Indole | Steroid |
|---|---|---|---|---|---|---|---|---|
| *E. hirta ethanolic* | - | - | - | - | - | - | + | + |
| *C. citratus ethanolic* | - | +++ | ++ | - | + | - | + | + |
| *I. coccinea ethanolic* | - | +++ | ++ | - | - | - | ++ | ++ |
| *G. sepium ethanolic* | - | +++ | ++ | - | - | - | ++ | ++ |
| *M. koenigii ethanolic* | + | +++ | ++ | - | + | - | ++ | ++ |
| *E. globulus ethanolic* | + | + | ++ | - | - | + | ++ | + |
| *J. curcas ethanolic* | - | ++ | - | - | - | + | ++ | ++ |
| *C. frutescens ethanolic* | + | +++ | + | - | - | + | ++ | ++ |
| *C. frutescens ethanolic – ethyl acetate fraction* | - | +++ | + | - | - | + | ++ | ++ |

After dose response assay of the *C. frutescens* ethanolic extract, the $LC_{50}$ and $LC_{90}$ values were determined. The $LC_{50}$ and $LC_{90}$ values were 231.59 and 430.95 ppm, respectively at 24 hours and 141.92 and 346.74 ppm, respectively at 48 hours for *Ae. aegypti*. The $LC_{50}$ and $LC_{90}$ were 300.20 and 408.92 ppm, respectively at 24 hours and 270.61 and 374.60 ppm, respectively at 48 hours for *Ae. albopictus*.

Partial purification successfully obtained the most active fraction of *C. frutescens.* After dose-response assay, the $LC_{50}$ and $LC_{90}$ values of this active fraction were determined. The $LC_{50}$ and $LC_{90}$ values were 97.22 and 181.13 ppm, respectively at 24 hours and 66.02 and 132.80 ppm, respectively at 48 hours for *Ae. aegypti*. The $LC_{50}$ and $LC_{90}$ values were 41.74 and 164.54 ppm, respectively at 24 hours and 24.35 and 83.78 ppm, respectively at 48 hours for *Ae. albopictus.*

A formulation of 300 mg active fraction per gram talcum powder used at 2 g/L concentration was effective in eliminating 100% of the larvae test subjects within a 24-hour period, showing its promise as a commercial larvicidal agent. Phytochemical screening of this active fraction revealed the presence of tannins, alkaloids, coumarins, indoles and steroids.

## REFERENCES

[1] Tayag, E. A., Roque, Jr., V. G., and Elfa, D. C. Disease surveillance report. Retrieved from: www.doh.gov.ph/sites/default/files/leptoup21.pdf, 201.
[2] World Health Organization. Dengue: Guidelines for diagnosis, treatment, prevention and control. Retrieved from: www.who.int/tdr/publications/documents/dengue-diagnosis.pdf, 2009.
[3] Negrelle, R. R. B., and Gomes, E. C. Cymbopogon citratus (DC.) Stapf: Chemical composition and biological activities. Rev. Bras. Pt. Med., 9(1), 80-92. Retrieved from: http://www.sbpmed.org.br/download/issn_07_1/revisao_v9_n1.pd,2007.
[4] Karunamoorthi, K., and Ilango, K. Larvicidal activity of Cymbopogon citratus (DC Stapf. and Croton macrostachyus Del. against Anopheles arabiensis Patton, a potent malarial vector. European Review for Medical and Pharmacological Sciences, 14, 57-62. Retrieved from: http://www.ncbi.nlm.nih.gov/pubmed/20184090, 2007.
[5] Cavalcanti, E., de Morais, S., Lima, M. A., & Santana, E. Larvicidal Activity of Essential Oils from Brazilian Plants against Aedes aegypti L. Mem. Inst. Oswaldo Cruz, 99(5), 541-544. Retrieved from: http://www.ncbi.nlm.nih.gov/pubmed/15543421, 2004.
[6] Quisumbing, E. Medicinal Plants of the Philippines. Quezon City, Philippines: JMC Press, Inc., 1978.
[7] Madhumathy, A., Aivazi, A. A., and Vijayan, V. Larvicidal efficacy of Capsicum annum against Anopheles stephensi and Culex quinquefasciatus. Journal of Vecotr Borne Diseases, 44, 223-226. Retrieved from: http://www.ncbi.nlm.nih.gov/pubmed/17896626, 2007.
[8] Vinayaka, K. S., Prakish Kekuda, T. R. and Nandini, K.C. Potent insecticidal activity of fruits and leaves of Capsicum frutescens (L.) var. longa (Solanaceae). Der Pharmacia Lettre, 2(4), 172-176. Retrieved from: http://scholarsresearchlibrary.com/DPL-vol2-iss4/DPL-2010-2-4-172-176.pdf, 2010.
[9] Batish, D. R., Singh, H. P., Kohli, R. K., & Kaur, S. Eucalyptus essential oil as a natu ral pesticide. Forest Ecology and Management, 256, 2166-2174. doi: 10.1016/j.foreco.2008.08.008, 2008.
[10] Kumar, S., Malhotra, R., and Kumar, D. Euphorbia hirta: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacgon. Rev., 4(7), 58-61. doi: 10.4103/0973-7847.65327, 2010.
[11] Patil, S., Patil, C., Salunkhe, R., & Salunke, B. Larvicidal activities of six plants extracts against two mosquito species, Aedes aegypti and Anopheles stephensi. Tropical Biomedicine, 27(3), 360-365. Retrieved from: http://www.ncbi.nlm.nih.gov/pubmed/21399575, 2010.
[12] Uche, F., and Aprioku, J. The Phytochemical Constituents, Analgesic and Anti-inflammatory effects of methanol extract of Jatropha curcas leaves in Mice and Wister albino rats. J. Appl. Sci. Environ. Manage., 12(4), 99-102. Retrieved from: http://www.ajol.info/index.php/jasem/article/view/55247, 2008.
[13] Kovendan, K., Murugan, K., Vincent, S., & Kamalakannan, S. Larvicidal efficacy of Jatropha curcas and bacterial insecticide, Bacillus thuringiensis, against lymphatic filarial vector, Culex quinquefasciatus Say (Diptera:
[14] Culicidae). Parasitology Research, 109, 1251-1257. doi: 10.1007/s00436-011-2368-6, 2011.
[15] Handral, H. K., Pandith, A., and Sd, S. A review on Murraya koenigii multipotential medicinal plant. Asian Journal of Pharmaceutical and Clinical Research, 5(4), 5-14. Retrieved from: http://www.ajpcr.com/Vol5Suppl4/1356.pdf, 2012.
[16] Patil, S., Patil, C., Salunkhe, R., & Salunke, B. Larvicidal activities of six plants extracts against two mosquito species, Aedes aegypti and Anopheles stephensi. Tropical Biomedicine, 27(3), 360-365. Retrieved from: http://www.ncbi.nlm.nih.gov/pubmed/21399575, 2010.
[17] Harve, G., & Kamath, V. Larvicidal activity of plant extracts used alone and in combination with known synthetic larvicidal agents against Aedes aegypti. Indian Journal of Experimental Biology, 42, 1216-1219. Retrieved from: http://www.ncbi.nlm.nih.gov/pubmed/15623234, 2004.
[18] Kaushik, R., and Saini, P. Screening of some semi-arid region plants for larvicidal activity against Aedes aegypti mosquitoes. Journal of Vector-Borne Diseases, 46, 244-246. Retrieved from: http://www.ncbi.nlm.nih.gov/pubmed/19724091, 2009.
[19] Suryawanshi, R., Patil, C., Borase, H., Narkhede, C., and Patil, S. Screening of Rubiaceae and Apocynaceae extracts for mosquito larvicidal potential. Natural Products Reseach, 29 (4) 1-8. doi: 10.1080/14786419.2014.941362, 2014.
[20] Herath, H., & de Silva, S. New Constituents from Gliricidia sepium. Fitoterpia, 71 (6), 722-724. Retrieved. from: http://www.ncbi.nlm.nih.gov/pubmed/11077187, 2000.
[21] World Health Organization. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. World Health Organization Press. Retrived from: http://whqlibdoc.who.int/hq/2005/who_cds_whopes_gcdpp_2005.13.pd, 2005.
[22] Kaushik, R., and Saini, P. Screening of some semi-arid region plants for larvicidal activity against *Aedes aegypti* mosquitoes. Journal of Vector-Borne Diseases, 46, 244-246. Retrieved from: http://www.ncbi.nlm.nih.gov/pubmed/19724091, 2009.
[23] Liu, Z. L., Liuo, Q. Z., Du. S. S., and Deng. Z. W. Mosquito larvicidal activity of alkaloids and limonoids derived from Evodia rutaecarpa

unripe fruits against Aedes albopictus (Diptera: Culicidae). Parasitology Research, 111(3), 991-996. doi: 10.1007/s00436-012-2923-9, 2012.
[24] da Silva, H. H. G., da Silva, I. G., dos Santos, R. M. G., Rodrigues, E. R., and Elias, C. N. Larvicidal activity of tannins isolated of Magonia pubescens St. Hil. (Sapindaceae) against Aedes aegypti (Diptera, Culicidae). Revista da Sociedade Brasileira de Medicina Tropical, 396-399. Retrieved from: http://www.scielo.br/pdf/rsbmt/v37n5/21338.pdf, 2004.
[25] Ranganatha, V.L., Begum, A.B., Prashanth, T., Gurupadaswamy, H.D., Madhu, S. K., Shivakumar, S., & Khanum, S. A. Synthesis and larvicidal properties of benzophenone comprise indole analogues against Culex quinquefasciatus. Drug Intervention Today, 5(4), 1-6. doi: 10.1016/j.dit.2013.10.001, 2013.
[26] National Pesticide Center. Capsaicin technical fact sheet. Retrieved rom: http://npic.orst.edu/factsheets/Capsaicintech.pdf.