PUPAL EMERGENCE INHIBITION ACTIVITY OF Acalypha Indica Leaf Extract against Dengue Vector, Aedes Albopictus Mosquito

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ABSTRACT

Objective: To investigate the larvicidal activities of six varying extracts of Acalypha indica (A. indica) leaves from family Euphorbiaceae against the dengue mosquito vector, Aedes albopictus (Ae. albopictus) in laboratory.

Methods: Leaves from the study plants were separated, air dried in room temperature, grounded and extracted with different solvents (petroleum ether, chloroform, ethyl acetate, n-butanol, ethanol and aqueous) by solvent apparatus and aqueous extract by maceration method. The extra solutions were evaporated to obtain crude extracts by using rotary evaporator. The crude extracts of six different solvents were dissolved in dimethyl sulphoxide (DMSO) to prepare test dosages of 1000, 2000, 3000, 4000 and 5000 ppm. Third instar larvae of Ae. albopictus were exposed to 1000, 2000, 3000, 4000 and 5000 ppm concentrations of petroleum ether, chloroform, ethyl acetate, n-butanol ethanol and aqueous extracts of A. indica and compared with control to determine the larvicidal effects. Larval bioassays were carried out according to World Health Organisation (WHO) procedures. The rate of larval mortality was recorded after 24 h and 48 h of time exposure. Three duplicate trials were made for each tested dosage. LC50 and LC90 values were calculated by using probit analysis.

Results: Based on probit analysis result the 24 h and 48h LC50 and LC90 of petroleum ether extract of A. indica against Ae. albopictus was found to be 2805.43 ppm and 2376.11 ppm, 3825.14 ppm and 3327.8 ppm, respectively. An LC50 and LC90 value of chloroform extracts of A. indica against third instar larvae was found to be 2276.5 ppm and 4015.8 ppm (24h), 2213.36 ppm and 3430.43 ppm (48h), respectively. An LC50 value of 4472.17 ppm and 2469.61 ppm, and LC90 value of 4215.84 ppm was obtained on ethylacetate extract treatment against Ae. albopictus for 24 h and 48 h exposure, respectively. The 24h and 48 h LC50 and LC90 values of n-butanol extracts of A. indica was found to be 2777.88 ppm and 3628.19 ppm, 2225.61 ppm and 2518.86 ppm, respectively. In the present study, the larvicidal bioassays demonstrated that the n-butanol extract was most effective with 100% mortality against larvae of Ae. albopictus at 3000, 4000 and 5000 ppm compared to other extracts. All other extracts (petroleum ether, chloroform and ethyl acetate) of A. indica at high concentration (4000 ppm and 5000 ppm) manifested a significant (P<0.01 and 0.05) knock down effect of 100% mortality after 24h and 48h exposure. While the third instar larvae of Ae. albopictus were found to be most susceptible and produced no mortality to ethanol and aqueous extract at varying parts per million.

Conclusion: A. indica leaf extract was tested for the first time against dengue vector Ae. albopictus and the results revealed that A. indica can be used to control dengue vector. Further this extract needs to be evaluated under field conditions for proper exploitation of Ae. albopictus mosquito larvae. Thus, the present study provided a first report on A. indica as a prompting mosquito larvicidal activity and can be considered for further investigations such as formulation of bioinsecticides to control Ae. albopictus populations.

Keywords: Mosquito, Ae. albopictus, Larvae, Medicinal plants, Diseases, Insecticides

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INTRODUCTION

Mosquitoes, the most important agent as vector transmits several serious human diseases like malaria, filariasis, Japanese encephalitis, dengue fever, chikungunya and yellow fever [1-3] and several diseases as a major public health problem in the world. The current mosquito control strategies are mainly focussed on synthetic insecticides considered as the first line of action against mosquito vectors. Besides its toxic nature continuous usage of synthetic insecticides causes ecological imbalance, environmental pollution by contaminating soil, water and air [4], destruction of non-target organisms including humans and animals [5, 6] and development of insecticide resistance in target insects [7]. Because of the undesirable side effects of the synthetic chemical pesticides, natural insecticides development achieved as an alternative way in mosquito control programs. Plants acts as an excellent source for the reduction of mosquito population at all the stages due to their excellent larvicidal, pupicidal and adulticidal properties. The use of plant extracts has insecticide comprises single active ingredient. These properties has stimulated many investigators to investigate about natural insecticides and also many researchers have reported the effectiveness of various plant against mosquito larvae.

Previous studies have evaluated the potential use of plants such as C. obtusifolius[13], Trachyspermumammi [14] against An. stephensi, Piper longum and Piper nigrum [15,16], Cassia fistula [17], Coriandrum sativum [18] against Ae. aegypti, P. nigrum against Culex quinquefasciatus [19], Cassia siamea against Plasmodium berghei [20], Cassia auriculata against An. stephensi and Culex quinquefasciatus[21], Lantana camera against Ae. aegypti, Culex quinquefasciatus, An. culicifacies, An. fluvialis and An stephensi [22], L camera against Ae. albopictus and Ae. aegypti [23] as larvicidal activity.

Mosquito mostly belongs to genera Anopheles, Aedes and Culex as vectors for pathogens of various diseases. Among the mosquitoes Ae. albopictus (Diptera: Culicidae), a competent vector of Aedesaegypti causes chikungunya and dengue viruses, highly dangerous to human health [24]. Very few studies have been carried out on Ae. albopictus mosquitoes over a long period [25-28] hence the present study is focused on this species. Keeping these points in view, in this context, the purpose of the present investigation is to explore the larvicidal
activity of A. indica leaf extract against Ae. albopictus under the laboratory conditions. Therefore, this study provides the first report on the mosquito larvicidal activity effect of A. indica leaf extract against third instar larvae of Ae. albopictus as target species.

**MATERIALS AND METHODS**

**Chemicals and reagents**

All the solvents and other reagents used in the present study are of analytical grade and purchased from Sigma-Aldrich Co.

**Collection of plant materials**

The leaf of A. indica (family: Euphorbiaceae) was collected in and around Velapadi (12°56'5.8"N and 79°8'48.77"E), Vellore district, India. The collected materials were identified by using the standard taxonomic key. The Voucher specimen (No. 1316) was deposited and kept in our Herbarium for further reference.

**Preparation of plant extract**

A. indica plant was washed with tap water and then followed by distilled water to remove the sand particles. Leaves were separated, air dried in shade for 20–30 d at environmental temperature. The shade dried materials were ground into fine powder using a electrical stainless steel blender and stored in air tight bottles until further use.

**Extraction method**

From the A. indica leaf powder 30g of weight quantity was extracted with petroleum ether (250 ml, Qualigens chemicals, India), Chloroform (400 ml, Qualigens chemicals, India), Ethyl acetate (300 ml, Qualigens chemicals, India), n-Butanol (350 ml Qualigens chemicals, India) and ethanol (500 ml Qualigens chemicals, India) as solvent by employing a soxhlet apparatus separately until exhaustion. The pooled extract were concentrated under reduced pressure in a Vaccum evaporator at 40 °C to get a semi solid residues. Twenty grams of A. indica leaf powder was macerated in 200 ml of distilled water on 250 ml Erlemeyer flasks which were continuously shaken on a rotary at 180 rpm/min for 24 h at room temperature. The suspension was filtered using a fine muslin cloth and then through a whatman No: 1 filter paper via a Buchner funnel. The residue was further macerated twice under the same condition. The obtained filtrate of aqueous extract were mixed and concentrated under vacuum, and then dried by using lyophilizers. The obtained extracts was stored at 4 °C in air tight bottle until required for a further analysis.

**Larvicidal activity**

**Stock solution**

0. lg of the crude extract (petroleum ether, chloroform, ethyl acetate, n-Butanol, ethanol and aqueous) was dissolved in 1 ml of particular solvent and added 2 drops of Tween 80. Tween 80 (Qualigens) was used as an emulsifyer. This was made up 100 ml using distilled water.

This solution was considered as stock solution (0. lg/100 ml, 1000 ppm). From the stock solution of all the six solvent extracts different concentration were prepared with dechlorinated water ranging from 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm respectively, and then subjected to Larvicidal bioassay screening.

**Collection of larvae**

The larvae were collected from fresh stagnant water (waste pot, plastic container, plant pot) near D. K. M. College, Garden, Vellore. The larvae was collected in a plastic container and transferred to the Laboratory immediately and identified. Preliminarily, the mosquito larvae were collected from fresh stagnant water (waste pot, plant pot) near D. K. M. College, Garden, Vellore.

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The larval mortality of *Ae. albopictus* after the treatment of ethylacetate extract of *A. indica* was observed. Table 1, 2 provides the result of larval mortality of *Ae. albopictus* (third instar) after the treatment of *A. indica* ethyl acetate at different concentration (1000 ppm-5000 ppm). No mortality was noted by treatment of *A. indica* at 1000 ppm, 10% mortality was noted at 24h at 2000 ppm, whereas it has increased to 30% at 48h. Similar trend has been noted for 3000 ppm(30%,24h,70%,48h) and 4000 ppm(40%,24h,50%,48h) and 3000 ppm(60%,24h,100%,48h). The LC50 and LC90 values were represented as followed LC50 values of third instar larva was 4472.24 ppm in 24h and the LC90 values of 3rd instar larva was 2469.61 ppm in 24h and 4215.84 ppm in 48h. The chi-square value significant at 24h (P≤0.01)48h(P ≤ 0.01).

The ethanol and aqueous extract of *A. indica* does not cause any mortality at varying concentrations against the third instar larvae of *Ae. albopictus*.

### Table 1: Percentage mortality at different time interval in larvicidal activity

| Extract                  | Concentration | % mortality at different time interval | Mortality percent means (±SE) at two intervals |
|--------------------------|---------------|---------------------------------------|------------------------------------------------|
|                          |               | 24h                                   | 48h                                            |
| **Larvicidal activity**  |               |                                       |                                                |
| Petroleum ether          | 1000 ppm      | 20                                    | 30                                             |
|                          |               |                                       | 20.33±0.57                                     |
|                          |               |                                       | 30.33±0.57                                     |
| Chloroform extracts      | 1000 ppm      | 20                                    | 40                                             |
|                          |               |                                       | 40.33±0.57                                     |
|                          |               |                                       | 40.66±0.57                                     |
| n-Butanol extracts       | 1000 ppm      | 20                                    | 40                                             |
|                          |               |                                       | 40.66±0.57                                     |
| Ethyl acetate extracts   | 1000 ppm      | 20                                    | 40                                             |
|                          |               |                                       | 40.66±0.57                                     |
| Ethanol and aqueous      | 1000 ppm-5000 ppm | 0                                     | 0                                              |
|                          |               |                                       | 0.00±0.00                                      |
| Control                  | 1000 ppm-5000 ppm | 0                                     | 0                                              |
|                          |               |                                       | 0.00±0.00                                      |

Values represent the mean of three replicates. Values are given as mean±Standard derivation, ppm indicatesParts per million, h-hour.

### Table 2: Larvicidal activity of Acalyphaindica leaf extract against aedesalbopictus

| Extract                  | 95% confidence limits | Regression equation | Chi-square | P value |
|--------------------------|-----------------------|---------------------|------------|---------|
|                          | LC50 (LCL-UCL)        |                     | 24h        | 48h     | 24h | 48h | 24h | 48h | P value |
|                           | 24h                   | 48h                 | 24h        | 48h     |      |      |      |      |         |
| Petroleum ether          | 2805.43               | 3825.14             | Y=2.815+0.005x | 3.36 | 6.18 | 0.05 | 0.01 |
|                          | (2253.13-3493.18)     | (3688.82)           | Y=2.713+0.054x | 5.36 | 6.18 | 0.05 | 0.01 |
| Chloroform extracts      | 2592.75               | 4015.8              | Y=2.087+0.127x | 3.8  | 7.29 | 0.01 | 0.01 |
|                          | (1835.23-2825.88)     | (3919.33)           | Y=1.452+0.041x | 3.8  | 7.29 | 0.01 | 0.01 |
| Ethyl acetate extracts   | 4472.14               | -                   | Y=5.285+0.017x | 4.42 | 8.93 | 0.01 | 0.01 |
|                          | (3831.54-5219.83)     | (4298.29)           | Y=3.715+0.094x | 4.42 | 8.93 | 0.01 | 0.01 |
| n-butanol                | 2777.88               | 3628.19             | Y=3.486+0.066x | 7.53 | 10.8 | 0.01 | 0.01 |
|                          | (2490.72-3098.15)     | (3320.22)           | Y=1.897+0.072x | 7.53 | 10.8 | 0.01 | 0.01 |

LCL-lower confident level, UCL-upper confident level, h-hours, P-Significant, LC50 lethal concentration that kills 50% of the exposed larvae, LC90 lethal concentration that kills 90% of the exposed larvae.

**DISCUSSION**

In the present study, effect of *A. indica* leaf extract on *Ae. albopictus* third instar larvae were compared in terms of relative potential with control. As the result indicated that *A. indica* extract with petroleum ether, chloroform, n-Butanol, ethylacetate, ethanol and aqueous, applied in different concentrations have a different larvicidal effect against third instar *Ae. albopictus* larvae under laboratory condition. The 3rd instar larva showed restless movement for short time and then settled at the bottom of the disposable cup and slowly dead.

In the laboratory after 24 h exposure, the n-butanol and chloroform extracts obtained from the leaf of *A. indica* at 5000 ppm have shown higher percentage mortality while the petroleum ether extract and ethyl acetate extract of *A. indica* had moderate percentage mortality.

The mean percentage mortality of 3rd instar larva on treatment with n-butanol, chloroform extract at 5000 ppm were 100% respectively (40 h). While petroleum ether extract with the sample concentration showed 100% (24 h and 48 h). Where as ethylacetate extract of *A. indica* showed 60% (24 h) and 100% (48 h). The

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percentage larval mortality of 3rd instar larvae of Ae. albopictus increased as time of exposure increased. Ethanol and aqueous extract of A. indica at 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm showed no mortality after 24 h and 48 h exposure under laboratory condition. Alwala et al., [29] reported that the methanolic extracts of Mangifera indica exhibited no toxicity while the other extract (aqueous and acetone extract) showed toxicity against A. aegypti, dengue vector. The result reveals that the toxic activity may be due to change in the selection of the solvent [30] Similarly, Adebayo et al., [31] examined the bioactivity of methanolic extracts of Mangifera indica leaf against A. aegypti and found to be more effective against 4th instar larvae.

From previous report it was found that hexane and chloroform extract from bark of A. squamosa showed 84 and 100% mortality against 4th instar A. stephensi after 24 h exposure. Kumaraj et al., [32-33] reported the variation in toxicity against the larval instar may be due to presence of phytochemical compound which varied with plant part, solvent used and the extract concentration.

According to Abbas et al., [34] the larvicidal activity of Tagetesminuta was possible due to the effective components like terpenoids, aglycon, flavanoids and saponin. Similarly, this study showed that the larvicial effect of A. indica was possibly because of alkaloids, flavanoids, Tannins and phenolic compounds, tosylate and terpenoids. Also Marcard et al., [35] reported that the larvicidal mortality was positively correlated with concentration and duration of exposure. Similarly in this study, has the concentration of extract increases from 1000 ppm to 5000 ppm, the percentage mortality also increases almost five fold, from initial concentration to final concentration. Several investigators also confirmed positive relationship between larval mortality and time factor [36-39]. As the time of exposure increases, the percentage larval mortality of 3rd instar larva of Ae. albopictus were also found to be increases. The mortality of petroleum ether and chloroform extract of A. indica at 4000 ppm was 100% after 48 h exposure. While mortality of n-Butanol extract at 3000 ppm was 100% after 48 h exposure. Whereas ethylacetate extract showed 100% mortality at 5000 ppm after 48 h exposure under laboratory condition.

This result also illustrated that all different active chemical compound in the leaf part of A. indica was responsible for diverse activity against Ae. albopictus. Singh and Prakash, [40] reported that larvicial activity was observed against A. stephensi when six different concentration were used (5,10,20,30,40 and 50 mg/l). Similarly in the present study six different concentration were used (1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm) against Ae. albopictus.

In the present study it can be pointed out that leaf of A. indica excerted larvicidal properties. LC50 ranged from 2777.88 ppm-4472.14 ppm when using n-butanol, chloroform and ethyl acetate as solvent within 48 h exposure period. Sakthivadivel and Thilagavathy, Tang et al., [41-42] has also observed LC50 range value between 30.47 mg/ml and 13.58 mg/ml when using petroleum ether extract at 24 h from seeds of A. mexicana. However our present investigation observed a low LC50 value (LC50 ranged from 2376.11 ppm (48 h)-2805.43 ppm (24 h)) when using petroleum ether as solvent from leaf of A. indica. Similar a low LC50 value (20 mg/ml-50 mg/ml) were observed by when using hexane extract from seeds of A. mexicana and stem bark of P. perniciosum at 48 h exposure respectively. Bilal et al., [43] successful, tested the larvicial activity of selected plant extracts against A. albopictus. All the extracts showed the moderate activity with a lowest LC50 value at the dose of 363.7, 377.5 and 403.5 mg/l respectively, for 24 h and the value get reduced to 263.95, 300.8 and 342.2 mg/l after 48 h respectively. As similar to previous research our report also documented a LC50 value of 2276.5, 2777.88, 2805.43 and 4472.14 for 24 h exposure and the value get reduced to 2225.61, 2213.36, 2376.11 and 2469.61 ppm for 48 h exposure. Among the tested plant extract n-butanol, chloroform, ethylacetate, and petroleum ether of A. indica (leaf) demonstrated remarkable larvicidal activity Ae. albopictus. Thus this plant extract provide the bases to act as alternative to synthetic insecticide in control programme of mosquito. To avoid the detrimental effects caused by chemical agents against mosquito vector, natural and nontoxic bioactive compound from plant origin can be used as an alternative control agent toward mosquito vectors [44]. Hereby, this study finally proposed a new alternative floral biopesticide in the mosquito management rather than using conventional chemical control. Chemical control is more expensive than the biological control (plant origin) and is also more effective and target specific[45] than conventional chemical control.

CONCLUSION

In conclusion, this study clearly reveals that A. indica leaf extract could be one of the most potential bio larvicidal against the vector Ae. albopictus. Therefore the present result also emphasized the need for further research and investigation to isolate and identify the most bioactive compound and there activity against mosquito vector Ae. albopictus.

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AUTHORS CONTRIBUTION

First Author act as a researcher, Second author did data analysis, Third author contributed some financial support for publication and Fourth author acted as supervisor for this work.

CONFLICTS OF INTERESTS

Declared none

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