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# Bioefficacy of essential oil from *Toddalia asiatica* (L.) Lam. against dengue vector mosquitoes *Aedes aegypti* L. and *Aedes albopictus* Skuse

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The present investigation aimed to evaluate the bioefficacy of the essential oil from the leaves of *Toddalia asiatica* (L.) Lam. against the dengue vector mosquitoes, *Aedes aegypti* L. and *Aedes albopictus* Skuse. The essential oil was extracted using Clevenger apparatus and tested at different concentrations (62.5, 125, 250, 500, and 1000 µg/mL) against I, II, III, and IV instar larvae of *Ae. aegypti* and *Ae. albopictus*. The larval mortality was recorded after 24 h of treatment. The $LC_{50}$ values of the essential oil against I, II, III, and IV instar larvae of *Ae. aegypti* were 29.90, 31.75, 54.70 and 86.63 µg/mL, respectively and $LC_{90}$ values were 86.00, 110.97, 296.38 and 500.97 µg/mL, respectively. When tested against I, II, III and IV instar larvae of *Ae. albopictus*, the $LC_{50}$ values were 31.18, 34.40, 64.24, and 98.52 µg/mL, respectively and the $LC_{90}$ values were 99.61, 251.03, 383.49, and 542.93 µg/mL, respectively. No mortality was observed in control groups of the two mosquito species. The results suggest that the essential oil of *T. asiatica* has the potential to be used as an ecofriendly larvicide against *Ae. aegypti* and *Ae. albopictus*.

**Keywords:** *Aedes aegypti* L., *Aedes albopictus* Skuse, Dengue, Essential oil, Larvicidal activity, Lethal concentrations, *Toddalia asiatica* (L.) Lam.

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## Introduction

Mosquitoes (Diptera: Culicidae) are the greatest source for transmission of human diseases than any other arthropods. Among the 3,492 species of mosquitoes recorded worldwide, more than a hundred species are capable of transmitting various diseases in human and other vertebrates[1]. They have the ability to transmit pathogens causing diseases in humans like malaria, dengue, filariasis, and viral encephalitis. Mosquito, *Aedes aegypti* L. is the primary vector of dengue virus, although *Ae. albopictus* Skuse is also capable of transmitting the disease as well[2-3]. Both the species are also capable of transmitting chikungunya virus[4] and they are commonly found in habitats associated with human presence and activity[5]. A recent study estimated 390 million dengue infections per year (95 % credible interval, 284–528 million), of which 96 million (95 % credible interval, 67–136 million) manifest clinical symptoms (with any severity of disease)[6]. Another study estimated that 3.9 million people from 128 countries may be at the risk of infection with dengue virus[7]. The first outbreak in India was reported from Delhi in 1988[8]. In 2010–2012, outbreaks of dengue/chikungunya-like illnesses with severe clinical manifestations were reported from several districts of Tamil Nadu, India[9].

The continuous and inappropriate use of synthetic pesticides results in developing resistance in *Ae. aegypti* and *Ae. albopictus* populations, causes environmental pollution, animal and human toxicity, and low biodegradability. This has led to an increasing interest in the development of alternative and safer methods of mosquito control. The repeated use of the chemical insecticides also leads to disruption of natural biological control systems in mosquito populations[10]. The resistance mechanisms have raised awareness on the importance of a good understanding of the resistance mechanisms for effective vector control[11]. Many synthetic pesticides are immunosuppressants[12]. Hence, plant derived compounds have emerged as good candidates, not only as new effective tools in vector management but

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also as environmentally safer agents[13-17]. The use of plants essential oils in insect control is an alternative method for minimizing the noxious effects[18].

Phytochemicals are botanicals which are naturally occurring bio-insecticides obtained from floral resources. Application of phytochemicals in mosquito control is in use since the 1920s[19], but the discovery of synthetic insecticides (e.g. DDT in 1939) side tracked the application of phytochemicals in mosquito control programme. Problems arising due to injudicious use of synthetic insecticides led to refocus on phytochemicals that are biodegradable and have no ill-effects on non-target organisms. Since then, the search for new bioactive compounds from the plants, determination of their structures and their commercial production has been initiated.

There is a need for plant derived insecticides which are target specific, eco-friendly, readily biodegradable, and cost-effective. In general, essential oils from plants have been recognized as important natural resource of insecticides. Many researchers have reported the control of mosquito larvae using the plant extract and the essential oils obtained from different parts of the plants[20-22].

*Toddalia asiatica* (L.) Lam., syn. *T. aculeata* (Sm.) Pers., *Paullinia asiatica* L., *Scopolia aculeata* Sm., is a medicinal plant belonging to the family Rutaceae. *T. asiatica* is commonly known as Wild Orange-tree, Lopez tree in English; *Jangli-kalimirch* in Hindi; *Kada-todali* in Bengali; *Kondakashinda*, *Yerakashida*, *Mirapakandra* in Telgu; *Milagarnai*, *Kattumilagu* in Tamil; *Kaara-mullu* in Malyalam; and *Tundpora* in Oriya[23]. It is a sprawling woody liana, which can reach a height of 15 m. It has pale brown bark and shiny green citrus scented leaves. Native to tropical Asia, it grows in the tropical and the subtropical areas. The plant has been widely used in traditional herbal medicines. The alkaloids from *T. asiatica* have anti-inflammatory[24] as well as antinocieptive and anti-inflammatory effects in rats[25]. *T. asiatica* also possesses anti-malarial and anti-leukimatic properties[26,27]. It is used to treat malaria and coughs[28]; roots are used to treat indigestion and influenza[29]. Studies on chemical constituents revealed the presence of many coumarins, alkaloids, sterols, N-cyclohexyl, triterpine acids, etc[30-33]. Earlier, researchers have studied the plant for its mosquitocidal effects[34-36] as well as the repellent and fumigation toxicity against the stored product pests[37]. The present research aimed to investigate the larvicidal properties of essential oil from *T. asiatica* against I, II, III, and IV instar larvae of *Ae. aegypti* and *Ae. albopictus*.

## Materials and Methods

### Plant material

The leaves of *T. asiatica* were collected from Poondy village, Thiruvalur District, Tamil Nadu, India. The plant specimen was identified by Dr. P Pandikumar, Plant Biologist, Entomology Research Institute, Loyola College, Chennai and a voucher specimen (ERIH-107) was deposited in the herbarium of Entomology Research Institute, Loyola College for future reference.

### Isolation of essential oil

Fresh leaves of *T. asiatica* (150-200g) were washed with water to remove all the unwanted impurities and subjected to hydro distillation in a Clevenger apparatus with condenser for 3-5 h at 100 $^{o}$C and the volatile compounds containing the water soluble fractions were allowed to settle for 30 min. The essential oil thus obtained was separated and purified from the aqueous phase through micro filtering. The essential oil was stored in airtight container at 4 $^{o}$C for larvicidal bioassay.

### GC-MS analysis of essential oil

GC-MS analysis of essential oil was carried out on Shimadzu QP- 2010 system and gas chromatograph interfaced to a mass spectrometer equipped with a VF- 5ms fused silica capillary column of 30 m length, 0.25 mm diameter, and 0.25 µm thickness. Helium was used as a carrier gas at a constant flow of 1.51 mL/min. The identification of components was based on comparison of their mass spectra with those of NIST08s, WILEY8 and FAME databases.

### Mosquito culture

Larvae of *Ae. aegypti* and *Ae. albopictus* were collected from various places in stagnant water bodies within Salem District, Tamil Nadu. They were colonized and maintained at 27±2 ºC, 75-85 % RH under a photoperiod of 14:10 h (light/dark) continuously for generations in the laboratory free of exposure to pathogens, insecticides, and repellents. Under these conditions, full development from egg to adult lasted about 3-4 weeks. Larvae were fed on finely ground dog biscuit and yeast extract in the ratio of 3:1. Water was changed every day to avoid scum formation that might have created toxicity. Pupae

were transferred from the trays to a cup containing tap water and placed in screened cages (30 x 30 x 30 cm dimension) for adult emergence. The adults were reared in respective glass cages (30 x 30 x 30 cm dimension). The adult colony was provided with 10 % sucrose solution and it was periodically blood-fed on restrained rats. After three days, ovitrap was kept in the cages for egg laying and the eggs were collected and transferred to enamel trays. Two developmental stages, larvae and adult females, were continuously available for the experiments. They were maintained in the same conditions as above.

**Larvicidal activity**

Bioassays were performed with I, II, III, and IV instar larvae of *Ae. aegypti* and *Ae. albopictus* using the essential oil concentrations of 62.5, 125, 250, 500, and 1000 µg/mL. A minimum of twenty five larvae were used for each concentration in all the experiments. Essential oil was dissolved in water with emulsifier (0.1 % Tween 80). Tween 80 was used as negative control whereas temephos (100 µg/mL) was used as positive control. The experiment was replicated five times. Mortality and survival rates were recorded after 24 h of the exposure period. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (after 1 h of treatment) or showing the characteristic diving reaction when the water was disturbed. Larvae were also observed for discoloration, unnatural positions, incoordination or rigor. $LC_{50}$ and $LC_{90}$were calculated using EPA Probit analysis software version 1.5.

## Results

The GC-MS profile of the essential oil of *T. asiatica* is given in Table 1 and Fig 1. The major components were: beta.-elemene (10.67 %), beta.-sesquiphellandrene (9.86 %), spathulenol (8.37 %), caryophyllene oxide (6.29 %), beta.-linalool (5.49 %), methyl methylanthranilate (5.45 %), and pogostol (5.20 %). Result of the larvicidal activity of the essential oil against the I, II, III, and IV instar larvae of *Ae. aegypti* and *Ae. albopictus* is given in Table 2. At 1000 µg/mL concentration, 100 % larval mortality was recorded in all the larval stages of two mosquito species (Table 2). The $LC_{50}$ value of essential oil from *T. asiatica* against the I, II, III, and IV instar larvae of *Ae. aegypti* was 29.90, 31.75, 54.70, and 86.63 µg/mL, respectively and $LC_{90}$ value was 86.00, 110.97, 296.38, and 500.97 µg/mL, respectively. When tested against the I, II, III, and IV instar larvae of *Ae. albopictus*, the $LC_{50}$ value was 31.18, 34.40, 64.24, and 98.52 µg/mL, respectively and the $LC_{90}$ value was 99.61, 251.03, 383.49, and 542.93 µg/mL, respectively. No mortality was observed in the control groups of the two mosquito species.

It was observed that immediately after exposure to essential oil, all the larvae were still active and exhibited a normal appearance with the siphon pointing up and head hung down, except for the I instar larvae. The processes of larval feeding, both collecting-filtering in the water column and collecting-gathering at the submerged surfaces, were clearly seen. After 1 h of treatment, all the larvae exhibited restless movements and were paralyzed. After 24 h, complete mortality was observed in all the larval instar at 1000 µg/mL concentration. The I instar larvae were highly susceptible at 1000 µg/mL followed by 500 and 250 µg/mL concentrations.

## Discussion

The essential oil of *T. asiatica* leaves showed prominent larvicidal activity against I, II, III, and IV instar larvae of *Ae. aegypti* and *Ae. albopictus*. The GC-MS analysis showed that beta-elemene, beta-sesquiphellandrene, spathulenol, caryophyllene oxide, and beta-linalool were the major constituents of the essential oil. These sesquiterpenes might have caused larvicidal activity. An earlier study has indicated that sesquiterpenes are highly toxic to *Ae. aegypti* mosquitoes[38]. In a previous study, the flindersine isolated from the leaf extract of *T. asiatica* showed $LC_{50}$ value of 2.90 and 4.19 µg/mL against the II and IV instar larvae of *Cx. quinquefasciatus* and 1.68 and 2.71 µg/mL for II and IV instar larvae of the *Anopheles stephensi*, respectively[35].

In the present study, essential oil pronounced minimal larvicidal effect on the IV instar larvae of *Ae. albopictus* ($LC_{50}$ value- 98.52 µg/mL). In an earlier study, essential oil of *T. asiatica* roots exhibited larval toxicity against the IV instar larvae of *Ae. albopictus* with the $LC_{50}$ value of 69.06 µg/mL and the isolated constituents, D-limonene, geraniol, and isopimpinellin also exhibited larvicidal activity against the *Ae. albopictus* mosquitoes with $LC_{50}$ values of 19.84, 30.13, and 32.05 µg/mL, respectively[36]. The different solvent extracts of *T. asiatica* leaves and fruits exhibited larvicidal

Table 1—Compounds identified in the essential oil of *Toddalia asiatica* (L.) Lam. leaves by GC-MS analysis

| Peak# | R. Time | Area | Area % | Name |
|---|---|---|---|---|
| 1 | 5.809 | 156253231 | 5.49 | beta.-Linalool |
| 2 | 6.168 | 9496613 | 0.33 | Allo-Ocimene |
| 3 | 6.425 | 23458241 | 0.82 | 6-Methyl-1-octanol |
| 4 | 6.866 | 26490197 | 0.93 | n-Nonanyl acetate |
| 5 | 7.036 | 9718970 | 0.34 | cis-3-Hexenyl Butyrate |
| 6 | 7.218 | 25536301 | 0.90 | 2-Cyclohexen-1-one, 4-isopropyl- |
| 7 | 7.296 | 20002324 | 0.70 | .alpha.-Terpineol |
| 8 | 7.414 | 120769230 | 4.24 | n-Decyl acetate |
| 9 | 7.985 | 28729546 | 1.01 | Linalyl acetate |
| 10 | 8.061 | 15642148 | 0.55 | 1-(1,2,2,3-Tetramethylcyclopentyl)Ethanone |
| 11 | 8.349 | 26148196 | 0.92 | 1-Dodecanol |
| 12 | 8.432 | 47361937 | 1.66 | Decyl acetate |
| 13 | 8.728 | 12120121 | 0.43 | 5,5-Dimethyl-6-methylenebicyclo[2.2.1]hept-2-yl acetate |
| 14 | 8.850 | 11843913 | 0.42 | Z-2-Octadecen-1-ol acetate |
| 15 | 9.122 | 77550347 | 2.72 | Myrtenyl acetate |
| 16 | 9.422 | 15412237 | 0.54 | .alpha.-Terpinyl acetate |
| 17 | 9.867 | 23348764 | 0.82 | Geraniol acetate |
| 18 | 10.096 | 303871744 | 10.67 | .beta.-Elemene, |
| 19 | 10.146 | 12970112 | 0.46 | .alpha.-Zingiberene |
| 20 | 10.289 | 70143147 | 2.46 | n-Decyl acetate |
| 21 | 10.454 | 155196962 | 5.45 | Methyl methylanthranilate |
| 22 | 10.522 | 71784040 | 2.52 | Caryophyllene |
| 23 | 10.568 | 24467205 | 0.86 | .gamma.-Elemene |
| 24 | 10.701 | 62760177 | 2.20 | .beta.-Sesquiphellandrene |
| 25 | 10.938 | 280563545 | 9.86 | .beta.-Sesquiphellandrene |
| 26 | 11.299 | 109411718 | 3.84 | Germacra-1(10),4(15),5-Triene, (-)- |
| 27 | 11.530 | 59095762 | 2.08 | .delta.-Guaiene |
| 28 | 12.163 | 67013496 | 2.35 | Sesquisabinene Hydrate |
| 29 | 12.253 | 29751732 | 1.05 | Germacrene B |
| 30 | 12.396 | 10560117 | 0.37 | 4aH-cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl- |
| 31 | 12.606 | 178994722 | 6.29 | Caryophyllene oxide |
| 32 | 12.925 | 57415309 | 2.02 | Humulene Oxide |
| 33 | 13.193 | 238369328 | 8.37 | Spathulenol |
| 34 | 13.492 | 148045240 | 5.20 | Pogostol |
| 35 | 13.620 | 18851310 | 0.66 | (+) Spathulenol |
| 36 | 13.734 | 42193873 | 1.48 | Alpha.-Bisabolol |
| 37 | 14.039 | 35675406 | 1.25 | 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol |
| 38 | 14.252 | 37140046 | 1.30 | PENT-1-YN-3-OL, 3-Methyl-5-(2,6,6-Trimethyl-1-Cyclohexenyl)- |
| 39 | 14.388 | 16301241 | 0.57 | 5.beta.,7.beta.H,10.alpha.-Eudesm-11-en-1.alpha.-ol |
| 40 | 14.507 | 14042161 | 0.49 | Alloaromadendrene oxide-(1) |
| 41 | 14.955 | 5116916 | 0.18 | Valerenol |
| 42 | 15.376 | 4818151 | 0.17 | Dihydro-Neoclovene-(I) |
| 43 | 16.598 | 38590051 | 1.36 | Palmitic acid |
| 44 | 16.692 | 4829193 | 0.17 | Methyl m-hydroxycinnamate |
| 45 | 17.912 | 98881840 | 3.47 | Phytol |
| | | 2846736860 | 100.00 | |

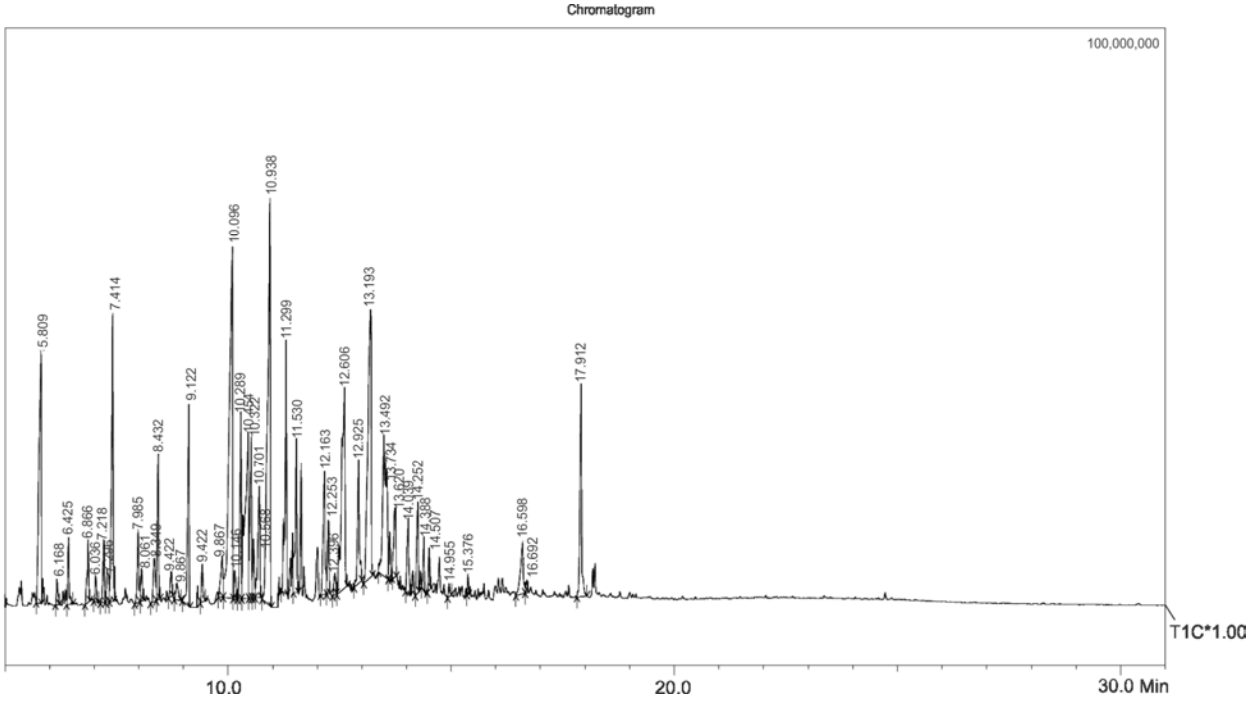


Fig. 1—GC-MS spectrum of *Toddalia asiatica* (L.) Lam. essential oil

Table 2 — Larvicidal activity of essential oil from *Toddalia asiatica* (L.) Lam. against *Aedes aegypti* L. and *Aedes albopictus* Skuse.

| Plant | instars | Concentrations (µg/mL) | | | | | $LC_{50}$ | 95 % Confidence Limit | | $LC_{90}$ | 95 % Confidence Limit | | Slope |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1000 | 500 | 250 | 125 | 62.5 | | LFL | UFL | | LFL | UFL | |
| *Aedes aegypti* | I | 25±0 | 25±0 | 25±0 | 23.6±0.54 | 20.6±0.54 | 29.90 | 15.02 | 41.26 | 86.00 | 71.85 | 104.21 | 2.79 |
| | II | 25±0 | 25±0 | 24.6±0.54 | 22.4±0.54 | 19.2±0.83 | 31.75 | 18.37 | 43.10 | 110.97 | 93.23 | 136.39 | 2.35 |
| | III | 25±0 | 23.6±0.54 | 21.6±0.54 | 17.8±0.44 | 14.2±0.44 | 54.70 | 39.70 | 68.93 | 296.38 | 243.20 | 384.48 | 1.74 |
| | IV | 25±0 | 21.8±0.44 | 18.6±0.89 | 14.4±0.54 | 11.4±0.54 | 86.63 | 34.12 | 136.57 | 500.97 | 299.99 | 1692.11 | 1.68 |
| *Aedes albopictus* | I | 25±0 | 25±0 | 25±0 | 22.6±0.54 | 20±0 | 31.18 | 17.36 | 42.42 | 99.61 | 83.78 | 121.62 | 2.54 |
| | II | 25±0 | 24.2±1.09 | 21.4±0.54 | 19.2±0.83 | 17.4±0.54 | 34.40 | 20.13 | 48.58 | 251.03 | 201.50 | 334.94 | 1.48 |
| | III | 25±0 | 22.8±0.44 | 20.4±0.54 | 16.2±0.44 | 13.4±0.89 | 64.24 | 47.79 | 80.03 | 383.49 | 310.00 | 509.24 | 1.65 |
| | IV | 25±0 | 21.2±0.44 | 18.2±0.44 | 13.8±0.44 | 10.2±0.44 | 98.52 | 42.65 | 153.16 | 542.93 | 322.64 | 1874.52 | 1.72 |

Values are mean±SD of five replicates; LFL – Lower fiducial limit; UFL - Upper fiducial limit

activity against several mosquitoes such as *Cx. quinquefasciatus, An. arabiensis* and *Ae. aegypti*[39-42]. The essential oils from various plants such as *Eucalyptus urophylla* ($LC_{50}$=95.5 µg/mL against *Ae. aegypti* and 285.8 µg/mL against *Ae. albopictus*)[43]; *Achillea millefolium* ($LC_{50}$=211.3 µg/mL), *Helichrysum italicum* ($LC_{50}$=178.1 µg/mL), *Foeniculum vulgare* ($LC_{50}$=142.9 µg/mL) against the larvae of *Ae. albopictus*[44]; and *Hyptis suaveolens* mortality percentage rates ranging from 98.33 and 93.33 % against the larvae of *Ae. albopictus*[45]; citrus limonoids, nomilin and limonin from citrus plants exhibited larvicidal activity against *Ae. albopictus* with the $LC_{50}$ values 305.83, 176.08, and 136.07 µM for nomilin and 850.09, 600.72, and 407.09 µM for limonin after 24, 48, and 72 h, respectively[46]. The chloroform extract of *Knema attenuata* ($LC_{50}$=141 ppm) showed larvicidal activity against *Ae. albopictus*[47]. In a previous study, Geraniol, D-limonene, and isopimpinellin exhibited strong larvicidal activity against *Ae. albopictus* with $LC_{50}$ values of 30.13, 19.84, and 32.05 µg/mL, respectively, while the essential oil of *T. asiatica* had an $LC_{50}$ value of 69.09 µg/mL[36]. Several coumarin derivatives have been demonstrated to possess larvicidal activity against *Ae. aegypti*[48]. Isopimpinellin, a compound isolated from the fruit of *Cnidium monnieri* has shown larvicidal activity

against *Ae. aegypti* and *Cx. pipiens pallens* with $LC_{50}$ values of 6.82 and 5.89 µg/mL, respectively[49]. The essential oil and isolated compound confertifolin from *Polygonum hydropiper* exhibited highest larvicidal activity against the II and IV instar larvae of on *Ae. albopictus*[50] and *Ae. aegypti*[51].

## Conclusion

The essential oil and its compounds from the leaves of *T. asiatica* may be used as a potential larvicide or insecticide. Further research is needed to establish their human and environmental safety. Moreover, field evaluation along with further investigations on the mode of action of the essential oils and their constituents on mosquito larvae and non-target organisms are necessary.

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