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doi:10.12980/JCLM.2.2014JCLM-2014-0029

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## Mosquitocidal properties of *Oxystelma esculentum* (Asclepiadaceae)-Indian medicinal plant tested against *Aedes aegypti* (Linn.) (Diptera: Culicidae)

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### PEER REVIEW

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#### Comments

This is an important research work in which authors have established mosquitocidal properties of *O. esculatum* against malarial vector *Ae. aegypti*. Further, these plants extracts/compounds may be useful to control the disease spreading vector *Ae. aegypti*. I suggest that this kind of novel approaches should be encouraged in future.

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### ABSTRACT

**Objective:** To evaluate the mosquitocidal activities of various solvent extract of *Oxystelma esculentum* (*O. esculentum*) against the medically important dengue vector mosquito, *Aedes aegypti* (*Ae. aegypti*) L.

**Methods:** A total of 25 early third instar larvae of *Ae. aegypti* were exposed to various concentrations (60-300 mg/L) and were assayed in the laboratory by using the protocol of World Health Organization, 2005; the 24 h LC<sub>50</sub> values of the *O. esculentum* leaf extract was determined by probit analysis. The ovicidal activity was determined against the freshly laid eggs of *Ae. aegypti* to various concentrations ranging from 50-300 mg/L under laboratory conditions. The pupicidal activity was determined against pupae of *Ae. aegypti* to various concentrations ranging from 70-280 mg/L after 24 h of exposure to the concern extract. The repellent efficacy was determined against adult female mosquito species at 1.0, 2.0 and 3.0 mg/cm<sup>2</sup> under laboratory conditions.

**Results:** The LC<sub>50</sub> value of methanol extract of *O. esculentum* against 3rd instar larvae of *Ae. aegypti* was 125.82 mg/L. The same extract showed 100% egg mortality at 250 mg/L and also pupicidal activity observed against the pupae of *Ae. aegypti* at 280 mg/L.

**Conclusions:** The present results suggest that the *O. esculentum* leaf extracts provided an excellent, potential phytopesticide for controlling *Ae. aegypti* mosquito.

### KEYWORDS

Larvicidal activity, Ovicidal activity, Pupicidal activity, Repellent activity, *Oxystelma esculentum*, *Aedes aegypti*

## 1. Introduction

The mosquito *Aedes aegypti* (*Ae. aegypti*) is a major vector of yellow fever, dengue and chikungunya viruses[1,2]. Mosquitoes cause substantial mortality and morbidity among people living in tropical and sub tropical zones[3,4]. It is arguably one of

most domestic mosquito vectors, feeding predominantly on man, mating and resting indoors and breeding in man-made containers in and around human habitations, especially in urban environments[5]. *Ae. aegypti* is highly anthropophilic and day-biter mosquitoes which reside in peridomestic habitats, serving as important vectors of arboviruses throughout the world[6]. The

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Foundation Project: Supported by University Grants Commission (UGC, New Delhi) [(Ref. No. 42-583/2013 (SR))] and Department of Science & Technology-Fast Track Young Scientist Project (DST, New Delhi) (Ref. NO. SB/FT/LS-356/2012).

Article history:

Received 3 Mar 2014

Received in revised form 12 Mar, 2nd revised form 17 Mar, 3rd revised form 24 Mar 2014

Accepted 1 Jun 2014

Available online 6 Mar 2015

incidence of dengue has grown dramatically around the world in recent decades[7]. Some 2.5 billion people - two-fifths of the world's population - are now at risk from dengue. World Health Organization (WHO) estimated there may be 50 million dengue infections worldwide every year. In 2007 alone, there were more than 890000 reported cases of dengue in the Americas, of which 26000 cases were dengue hemorrhagic fever. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific, the last two being the most seriously affected. Today, dengue is the most important mosquito-borne viral disease affecting humans[8]. Mosquitoes are responsible for the spread of more diseases than any other group of arthropods. Of particular interest is *Ae. aegypti* because of its role as a vector for the arboviruses responsible for yellow fever and dengue haemorrhagic fever, both of which are endemic to Central and South America, Asia and Africa[9]. Over the past 50 years, more than 2000 plant species belonging to different families and genera have been reported to contain toxic principles, which are effective against insects[10]. In India, there are various plants known for their insecticidal property and are popular as pesticides. Plant derived compounds (phytopesticides) in general have been recognized as an important natural resource of insecticides[11]. The extensive and indiscriminate application of synthetic chemical insecticides leads to environmental and health concerns, widespread development of resistance by mosquitoes and unwarranted toxic or lethal effects on non-target organisms[12]. These well known drawbacks with synthetic insecticides shifted the mosquito control programme to use of eco-friendly, bio-degradable plant compounds with mosquitocidal property. A variety of secondary metabolites in the extracts obtained from different parts of a whole range of plants have been found to kill adult mosquitoes or reduce/inhibit feeding, egg laying, growth and development of mosquito larvae and pupae[13,14]. The phytochemicals derived from plant resources can act as larvicides, adulticides, repellent and ovipositional attractants, having deterrent activities in different researchers and may be alternative sources of mosquito larval control agents[15-18]. The plant constitutes a rich source of bioactive compounds that are biodegradable into nontoxic products[19]. These botanical insecticides are believed to pose little threat to the environment or to human health and may provide a practical substitute for synthetic insecticides[20]. To date, a number of phytochemicals with biological activity against immature and adult mosquitoes have been described[21,22]. *Oxystelma esculentum* (*O. esculentum*) (family Asclepiadaceae), commonly known as 'Rosy Milkweed Vine'. This plant is also found in India, China and South Indonesia[23]. It has many potential therapeutic uses which are of vital importance in curing the diseases of the modern world such as cancer, hepatitis, kidney disorders, stress-related disorders and microbial infections. Many plants which are found commonly

and are mentioned in texts of traditional Indian medicine have not been investigated thoroughly[24,25]. Furthermore, a mosquitocidal property of *O. esculentum* has not yet reported. Therefore, in view of the recently increased interest in developing plant origin insecticides as an alternative to chemical mosquitocides, this study was undertaken to assess the larvicidal, ovicidal, pupicidal, and repellent efficacies of *O. esculentum* extracts against medically important species of dengue and chikungunya vector, *Ae. aegypti*.

## 2. Materials and methods

### 2.1. Plant material

*O. esculentum* leaves were collected from Yercaud hill station (11.7794° N 78.2034° E) Salem districts of the Tamilnadu during the growing season (August-December) of 2013. Bulk samples were air-dried in the shade at room temperature [(28±2) °C, relative humidity (75±5)%] and after drying the sample was ground to a fine powder. At the time of collection, two pressed voucher herbarium specimens were prepared and identified with the help of plant taxonomist, Department of Botany, Govt. Arts College (Autonomous), Nandanam, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

### 2.2. Extraction method

The shade dried leaf (100 g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with hexane, benzene, ethyl acetate and methanol (500 mL, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22-26 mmHg at 45 °C by 'Rotavapour' and the residue obtained was stored in an amber vials at 4 °C.

### 2.3. Mosquito rearing

*Ae. aegypti* colonies were maintained in the insectarium. The freshly laid eggs (0-6 h) were placed in white enamel trays (30 cm×24 cm×5 cm) each containing 2 L of tap water and kept at room temperature [(28±2) °C] with a photoperiod of 16:8 h (L:D) for larval hatching. The larvae of each mosquito species were maintained in separate trays under the same laboratory conditions and fed with a powdered feed containing a mixture of dog biscuit and baker's yeast (3:1 ratio). The trays with pupae of each mosquito species were maintained in separate mosquito cages at (28±2) °C and relative humidity of (85±3)% under a photoperiod of 16:8 h (L:D) for adult emergence. Cotton soaked in 10% aqueous sucrose solution admixed with few drops of multivitamin drops in a Petri

dish to feed adult mosquitoes was also placed in each mosquito cage. An immobilized young chick (head covered with a black cloth, wings and legs were tied) was placed for 3 h inside the cage in order to provide blood meal especially for female mosquitoes. A plastic tray (11 cm×10 cm×4 cm) filled with tap water with a lining of partially immersed filter paper was then placed inside each cage to enable the female mosquitoes to lay their eggs. The eggs obtained from the laboratory-reared mosquitoes were immediately used for toxicity assays or allowed to hatch out under the controlled laboratory conditions described above. Only the newly moulted larvae/pupae of *Ae. aegypti* were used in all bioassays.

#### 2.4. Larvicidal activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO[26]. From the stock solution, five different test concentrations viz., 60, 120, 180, 240 and 300 mg/L were prepared and they were tested against the freshly moulted (0-6 h) third instar larvae of *Ae. aegypti*. The larvae of test species (25) were introduced in 500 mL plastic cups containing 250 mL of aqueous medium [249 mL of dechlorinated water + 1 mL of emulsifier, dimethyl sulfoxide (DMSO)] and the required amount of plant extract was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The LC<sub>50</sub> value was calculated by using probit analysis[27].

#### 2.5. Ovicidal activity

The method of Su and Mulla was slightly modified and used to test the ovicidal activity[28]. The various concentrations (50, 100, 150, 200, 250 and 300 mg/L) as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs of *Ae. aegypti* were counted individually with the help of hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to DMSO in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment.

#### 2.6. Pupicidal activity

Batches of 10 pupae were introduced into 500 mL of the test medium containing particular concentration of the crude extract in a plastic cups in five replications. In control, the same number of pupae were maintained in 500 mL of dechlorinated water containing appropriate volume of DMSO. All containers

were maintained at room temperature [(28±2) °C] with naturally prevailing photoperiod (12:12 h/L: D) in the laboratory. Any pupa was considered to be dead if did not move when prodded repeatedly with a soft brush. Mortality of each pupa was recorded after 24 h of exposure to the extract following the Abbott formula[29].

#### 2.7. Repellent activity

The repellent activity was studied by following the methods of WHO[30]. The 3-4 day-old blood-starved female *Ae. aegypti* mosquitoes (100) were kept in a net cage (45 cm×45 cm×40 cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of the test person were cleaned with isopropanol. After air drying the arm only 25 cm<sup>2</sup> of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves. The plant extract was dissolved in isopropanol and this alcohol served as control. The *O. esculentum* leaf extract at 1.0, 2.0 and 3.0 mg/cm<sup>2</sup> concentration was applied. The control and treated arms were introduced simultaneously into the cage. The number of bites was counted over 5 min every 30 min, *Ae. aegypti* was tested during the day time from 7:00 h to 17:00 h. The experiment was conducted five times. It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula.

$$\text{Repellency (\%)} = [(T_a - T_b) / T_a] \times 100$$

Where T<sub>a</sub> is the number of mosquitoes in the control group and T<sub>b</sub> is the number of mosquitoes in the treated group.

#### 2.8. Determination of lethal concentrations

Lethal concentration (LC<sub>50</sub>) represents the concentration of the test material that caused 50% mortality of the test organisms within the specified period of exposure, and it was determined by exposing the larvae of the mosquito to different concentrations of the extracts. Based on the mortality of the test organisms recorded in these bioassays, LC<sub>50</sub> was calculated along with their fiducial limits at 95% confidence level by probit analysis using SPSS software package.

#### 2.9. Phytochemical analyses

The preliminary phytochemical screening was carried out by following the standard procedures[31-33].

### 3. Results

The *O. esculentum* extract have been studied for use as natural insecticides instead of organic phosphorous materials or other

synthetic agents. Results on the larvicidal, ovicidal, pupicidal and repellent effects of leaf extract were reported in the present study, confirming their potential for control of the mosquito populations (Table 1 and 2 and Figure 1 and 2).

**Table 1**

Larvicidal activity of *O. esculentum* crude extracts against freshly moulted (0-6 h old) third instar larvae of *Ae. aegypti*.

Extract	Concentration (mg/L)	Mortality (%)	LC <sub>50</sub> (mg/L) (LCL-UCL)	LC <sub>90</sub> (mg/L) (LCL-UCL)	df	χ <sup>2</sup> value
Hexane	Control	0.0±0.0 <sup>a</sup>			5	15.124
	60	22.0±2.2 <sup>b</sup>				
	120	35.2±0.6 <sup>c</sup>	163.29	291.62		
	180	51.8±1.4 <sup>d</sup>	126.28-201.18	241.23-393.32		
	240	73.6±1.8 <sup>e</sup>				
Benzene	Control	0.0±0.0 <sup>a</sup>			5	19.221
	60	24.8±1.4 <sup>b</sup>				
	120	43.2±0.8 <sup>c</sup>	149.64	269.65		
	180	56.4±1.6 <sup>d</sup>	16.29-190.28	221.41-378.96		
	240	73.2±1.4 <sup>e</sup>				
Ethyl acetate	Control	0.0±0.0 <sup>a</sup>			5	14.226
	60	26.3±0.4 <sup>b</sup>				
	120	41.8±1.8 <sup>c</sup>	143.35	271.48		
	180	63.2±1.4 <sup>d</sup>	109.12-170.64	224.24-350.62		
	240	78.6±1.4 <sup>e</sup>				
Methanol	Control	0.0±0.0 <sup>a</sup>			5	15.832
	60	31.6±1.4 <sup>b</sup>				
	120	42.4±1.8 <sup>c</sup>	125.82	243.81		
	180	68.2±2.6 <sup>d</sup>	93.64-151.18	205.16-326.82		
	240	83.5±2.8 <sup>e</sup>				
	300	97.8±2.4 <sup>f</sup>				

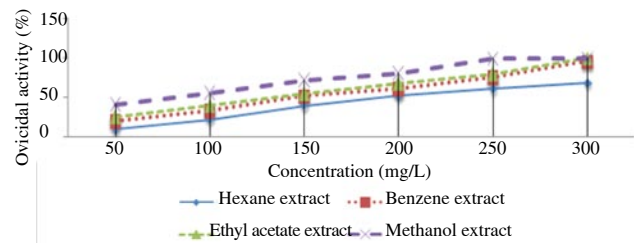
Values are represented as mean±SD of five replications. \*Mortality of the larvae observed after 24 h of exposure period. Values in the column with a different superscript alphabet are significantly different at P<0.05 level DMRT test. LC<sub>50</sub>=lethal concentration brings out 50% mortality and LC<sub>90</sub>=lethal concentration brings out 90% mortality. LCL=lower confidence limit; UCL=upper confidence limit.

**Table 2**

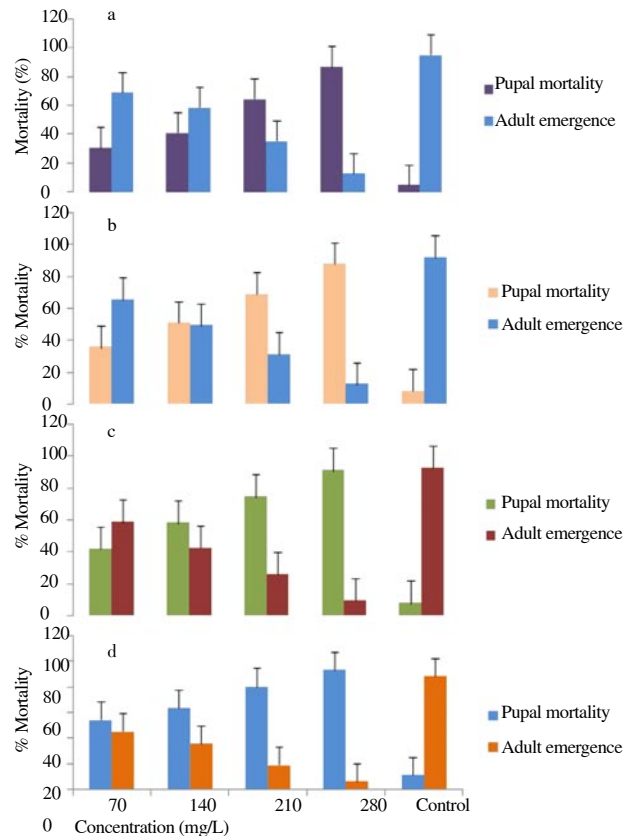
Repellent activity of *O. esculentum* crude extract against *Ae. aegypti*.

Solvent	Concentration (mg/cm <sup>2</sup> )	Percentage of repellency							
		30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Hexane extract	1.0	100.0±0.0	100.0±0.0	100.0±0.0	86.2±1.6	74.4±1.8	63.8±1.6	56.3±1.4	45.6±1.2
	2.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.6±1.3	75.8±1.6	67.4±1.3	53.8±1.6
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.6±1.8	72.4±1.2
Benzene extract	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.4±1.6	72.8±1.3	68.3±1.6	54.8±1.6
	2.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.6±1.2	71.6±1.6
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.6±1.8	82.6±2.3
Ethyl acetate extract	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	74.2±2.4	69.6±1.2	60.2±1.1
	2.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.6±3.2	81.4±3.4
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.9±1.8	78.4±1.3
Methanol extract	1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.6±1.4	74.6±1.6	69.8±2.4
	2.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	79.4±1.4	73.4±1.2
	3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	78.2±1.6

Values are represented as mean±SD of five replications.



**Figure 1.** Ovicidal activity of *O. esculentum* crude extracts against freshly laid eggs (0-6 h old) of *Ae. aegypti*.



**Figure 2.** Pupicidal activity of *O. esculentum* crude extracts against the pupae of *Ae. aegypti*.  
a: Hexane extract, b: Benzene extract, c: Ethyl acetate extract, d: Methanol extract.

The LC<sub>50</sub> value of hexane, benzene, ethyl acetate, and methanol extract of *O. esculentum* against early third instar larvae of *Ae. aegypti* were 143.35, 163.29, 149.64 and 125.82 mg/L, respectively. Maximum larvicidal activity was observed in the methanol extracts followed by ethyl acetate, benzene and hexane. No mortality was observed in control groups (Table 1). Among four tested solvents, the methanol extract was found to be most significant ovicidal activity observed against selected mosquito and same extract provide 100% egg mortality (zero hatchability) at 250 mg/L (Figure 1). Furthermore, methanol extract found to be a most significant pupicidal activity observed against *Ae. aegypti* and a higher concentration of same extract 280 mg/L produced 28.12% pupicidal activity recorded (Figure 2). Similarly, methanol extract was found to be the most effective for repellent activity and a higher concentration of the same extract 3.0 mg/cm<sup>2</sup> provide 100% protection up to 210 min (Table 2). This study show that leaf extract of *O. esculentum* may be a potent source of natural larvicidal, ovicidal, pupicidal and repellent activities against *Ae. aegypti*. The preliminary phytochemical analyses of *O. esculentum* showed that the presence or absence of various phytochemical constituents (Table 3).

**Table 3**Phytochemical analysis of *O. esculentum*.

Phytochemical constituents	Results
Alkaloids	+
Cardian glycosides	+
Flavanoids	+
Phlorotannins	-
Quinones	-
Saponin	-
Steroids	-
Tanins	+
Terpenoids	+
Total alkaloid content	0.07%

+: presence, -: absence.

#### 4. Discussion

The results of the present investigation clearly showed that the crude extract of *O. esculentum* had significant larvicidal, ovicidal, pupicidal and repellent activities against *Ae. aegypti* mosquito. These results are comparable with an earlier report by Hossain *et al.* who reported that the mortality rate was higher in 50 mg/L doses of methanolic extracts of *Dregea volubilis* and *Bombax malabaricum*, both the plants against *Culex quinquefasciatus* (*Cx. quinquefasciatus*)[34]. The corresponding LC<sub>50</sub> values were 56.97 mg/L and 48.85 mg/L. Recently, Govindarajan *et al.*

have reported that the crude extract of *Ervatamia coronaria* (*E. coronaria*) exerted zero hatchability (100% mortality) at 250, 200 and 150 mg/L for *Cx. quinquefasciatus*, *Ae. aegypti* and *Anopheles stephensi* (*An. stephensi*), respectively[35]. The crude extract of *Caesalpinia pulcherrima* zero hatchability (100% mortality) at 225 mg/L for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. The methanol extract of *E. coronaria* found to be more repellent than *Caesalpinia pulcherrima* extract. A higher concentration of 5.0 mg/cm<sup>2</sup> provided 100% protection up to 150, 180 and 210 min against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. The methanol extract of *E. coronaria* showed promising larvicidal and ovicidal activity against *An. stephensi*. The direct and indirect contributions of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for management of *An. stephensi*[36].

Abdalla *et al.* have also reported that the *Anopheles arabiensis* extracts against *Cx. quinquefasciatus* that caused high, moderate and low larval mortality in the larvicidal experiment against 3rd instar larvae[37]. It was found that LC<sub>50</sub>-LC<sub>90</sub> values calculated were 273.53-783.43, 366.44-1018.59 and 454.99-1224.62 mg/L for 2nd, 3rd and 4th larval instars, respectively, of *Anopheles arabiensis* and 187.93-433.51, 218.27-538.27 and 264.85-769.13 mg/L for 2nd, 3rd and 4th larval instars, respectively, of *Cx. quinquefasciatus*. The methanolic extracts of *Acalypha indica* and *Achyranthes aspera* leaves against *Ae. aegypti*. Based on LC<sub>50</sub> values for 4th instar *Ae. aegypti*, the combined extracts showed the strongest larvicidal activity (277 mg/L). *Achyranthes aspera* and *Acalypha indica* extracts individually gave similar results (409 and 420 mg/L, respectively). Respective LC<sub>50</sub> values for pupae were 326, 456, and 467 mg/L[22]. Kweka *et al.* have reported that necessitated the search and development of environmentally safe, biodegradable, low-cost, and indigenous methods for vector control, which can be used without risk of harm to individuals and communities[38]. Tren and Roberts reported that the efficacy shown by *Schinus terebinthifolia* for knockdown time and 100% mortality after 24 h to adult mosquitoes from wild resistant population warrants further investigation of these compounds for indoor residual spraying small scale whether singly or in blends[39]. This essential oil may be of great value in complementing other compounds which are losing efficacy. In tropical countries, plants are known to possess larvicidal, ovicidal and adulticidal activities[40]. Recently Eliningaya *et al.* have been reported the mortality of *Cx. quinquefasciatus* ranged from 0.5% to 96.75% while for *Anopheles gambiae s.s.* it was from 13.75% to 97.91%[41]. The LC<sub>50</sub> and LC<sub>95</sub> value in the laboratory was similar for both species while in the semi-field they were different for each. The essential oil of *Blumea martiniana* against *Anopheles anthropophagus* and the oil



and linalool, germacrene D, borneol, terpinene exerted significant larvicidal activity with  $LC_{50}$  value of 46.86, 35.87, 44.61, 35.89, and 29.21 mg/L, respectively[42]. Since there is no previous record of literature available about the mosquitocidal activity of the selected plant *O. esculentum* these present investigations serve as first hand information. The finding of the present investigation revealed that the leaf extract of *O. esculentum* possessed remarkable larvicidal, ovicidal, pupicidal and repellent activities against medically important species of chikungunya vector, *Ae. aegypti*.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

Authors are gratefully acknowledged to Prof. N. Kalaichelvi, Head, Department of Zoology, Dr. K. M. Prabhu, Principal, Govt. Arts College (Autonomous) Nandanam for their support and laboratory facilities provided, and University Grants Commission (UGC, New Delhi) for their financial assistance (Ref. No. 42-583/2013 (SR) and Department of Science & Technology-Fast Track Young Scientist Project (DST, New Delhi) Ref. NO. SB/FT/LS-356/2012.

### Comments

#### Background

The mosquito *Ae. aegypti* is a major vector of yellow fever, dengue and chikungunya viruses. Mosquitoes cause substantial mortality and morbidity among people living in tropical and sub tropical zones. Extinguishing the mosquito with chemistry has a lot of side effects.

#### Research frontiers

The materials and methods adapted in the present study are reproducible and are universally accepted by several authors and also followed by WHO. The concentrations fixed in the present research are admissible to the non target organisms like aquatic organisms. The follow up of the methodology are easy even to a local people.

#### Related reports

One of the important innovative approach in the present investigation is methanol extracts of *O. esculentum* showing good results against the selected mosquito. Thus it paves the way for further exploration of possible utilization of the selected plant against other mosquito species and also it is first hand information.

#### Innovations and breakthroughs

This article stated the negative impacts of chemical pesticides

and also focused the importance of the plant compounds and their role in nature to save the environment. Thus, no doubt, this kind of research must be encouraged to mitigate the environmental pollution by chemical pesticides. The plant derived compounds could be possible utilized in the Vector Control Programme in the near future.

### Applications

*O. esculentum* extracts showed promising larvicidal, ovicidal, repellent and pupicidal activities against various life stages of *Ae. aegypti*. The results reported in this study open the possibility for further investigations of the efficacy of larvicidal activity, ovicidal activity, repellent activity and pupicidal activities of natural product extracts as a potential agent for controlling malarial vector mosquito *Ae. aegypti*. And also this plant is first hand information on *Ae. aegypti*.

### Peer review

This is an important research work in which authors have established mosquitocidal properties of *O. esculatum* against malarial vector *Ae. aegypti*. Further, these plants extracts/compounds may be useful to control the disease spreading vector *Ae. aegypti*. I suggest that this kind of novel approaches should be encouraged in future.

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