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Mosquito larvicidal properties of volatile oil from salt marsh mangrove plant of *Sesuvium portulacastrum* against *Anopheles stephensi* and *Aedes aegypti*

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PEER REVIEW

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Comments

The indiscriminate use of synthetic insecticides is creating multifarious problems like environmental pollution, insecticide resistance and toxic hazards to humans. The plants are proven to have rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential. The secondary metabolites derived from seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to nematocidal and insecticidal in lower form of animal.

Details on Page 70

ABSTRACT

Objective: To identify the larvicidal activity of the volatile oil from *Sesuvium portulacastrum* (*S. portulacastrum*) against *Anopheles stephensi* and *Aedes aegypti*.

Methods: Volatile oil extract of *S. portulacastrum* was prepared in a graded series of concentration. The test for the larvicidal effect of volatile oil against mosquitoes larvae was conducted in accordance with the WHO standard method. Batches of 25 early 4th instar larvae of two mosquitoes were transferred to 250 mL enamel bowl containing 199 mL of distilled water and 1 mL of plant extracts. Each experiment was conducted with triplicate with concurrent a control group.

Results: Volatile oil extract of *S. portulacastrum* showed toxicity against 4th instar larvae of *Aedes aegypti* and *Anopheles stephensi* with equivalent LC₅₀ value [(63±7.8) µL/mL, LCL–UCL=55.2–64.0] and LC₉₀ value [(94.2±3.9) µL/mL] in maximum activity with minimum concentration (200 µL/mL) of volatile oil and followed by maximum activity of 250 µL concentration showed LC₅₀ value=(68.0±8.2) µL/mL, LCL–UCL=66.26–69.2 and LC₉₀ value of (55.2±2.8) µL/mL, LCL–UCL=53.7–56.9, LC₉₀=(95.2±1.25) µL/mL and followed by 250 µL of oil extract against 4th instar larvae of *Aedes aegypti* respectively.

Conclusions: It is inferred from the present study that, the extracts from salt marsh mangrove plant of *S. portulacastrum* are identified as a potential source of safe and efficacious mosquito control agents for the management of vector borne diseases of malaria and dengue.

KEYWORDS

Aedes aegypti, *Anopheles stephensi*, Larvicidal, Oleic acid, *Sesuvium portulacastrum*

1. Introduction

Mosquito borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates. However, no part of the world is free from vector–borne diseases[1].

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc., causing millions of deaths every year. *Aedes aegypti* (*A. aegypti*), a vector of dengue is widely distributed in the tropical and subtropical zones. Dengue

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fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. In 1990, almost 30% of the world population, 1.5 billion people, lived in regions where the estimated risk of dengue transmission was greater than 50%^[2]. *Anopheles stephensi* (*An. stephensi*) is the major malaria vectors in India. With an annual incidence of 300–500 million clinically manifest cases and a death toll of 1.1–2.7 million. Malaria is still one of the most important communicable diseases. Currently about 40% of the world's population lives in areas where malaria is endemic^[3,4]. One can speculate that people controlled and killed mosquitoes and other domestic insect pests by physically removing them or by using plant parts and plant derivatives before the advent of synthetic chemicals. In all probability, some plants containing insecticidal phytochemicals that were predominantly secondary compounds were used to protect themselves against herbivorous insects. However, there is a little other than anecdotal, traditional or cultural evidence on this topic^[5]. The control of mosquito larvae worldwide depends primarily on continued applications of organophosphates such as temephos, fenthion and insect growth regulators such as diflubenzuron and methoprene^[6]. Effective, repeated use of these controlling agents has fostered several environmental and health concerns, including disruption of natural biological control systems, outbreaks of other insect species, widespread development of resistance and undesirable effects on non-target organisms^[7]. These problems have highlighted the need for new strategies for mosquito larval control.

Researchers are now looking for natural insecticides which do not have any ill effects on non-target population and are easily degradable. The search is underway to find out newer insecticides which will be effective and safe, and also easily available at lower cost. Due to spiraling costs of insecticides and labour, paucity of funds and resistance developed by plasmodia or anophelines to chemicals, diseases carried by mosquitoes have reappeared since 1980s.

Marine halophytes are a kind of salt tolerant plants having enormous diversity. Most of the plants have special adaptation to accumulate salts and some have the property to excrete salt through the leaves. The former are collectively called as salt intruders and the later are known as salt extruders. Previous studies on mangrove plant parts and its major chemical classes displayed various level of biological activities such as antibacterial^[8–10], antiplasmodial^[11,12], larvicidal^[13,14], hepatoprotective^[15–17], free radical scavenging activities^[15] and antifertility^[18]. Mangrove plant extracts have been used as a popular source to treat several health disorders for centuries. Plant-derived substances have recently become of great interest owing to their versatile applications. *Sesuvium portulacastrum* (*S. portulacastrum*) commonly known as “shoreline

purslane”, is a perennial herb that grows in coastal area throughout much of the world. *S. portulacastrum* contains fatty acid composition such as palmitic acid, oleic acid and linolenic acid. The plant extract showed antibacterial and anticandidal activities and moderate antifungal activity^[19]. Present study aimed to assess the larvicidal properties of volatile oil from salt marsh plant of *S. portulacastrum* against *Ae. aegypti* and *An. stephensi* mosquito larvae.

2. Materials and methods

2.1. Collection and extraction of *S. portulacastrum*

S. portulacastrum mangrove plants were collected from Pichavaram mangrove forest (latitude 11°27' N, longitudes 79°47'E) in south east coast of India. Shade dried mangrove plants samples were subjected to percolation by soaking in ethanol and water mixture (3:1, v/v). After 21 days of dark incubation, the filtrate was concentrated separately by Soxhlet apparatus evaporation (>45 °C) and then freeze-dried at –80 °C to obtain solid residue. The extracts of *S. portulacastrum* were screened for the presence of constitution by following method^[14].

2.2. Isolation of volatile oil from *S. portulacastrum*

A volume of 1 mL concentrated extract was dissolved in 20 mL petroleum ether and 2 mL 2 mol/L methanolic KOH was added. The mixture was shaken for 2 min and allowed to stand for 10 min. The upper layer was removed and washed with water and formed double layers. The upper layer was collected. This oil (as the methyl esters of the fatty acids) was analyzed by using GC-MS.

2.3. Mosquito larval culture

To satisfy the enormous number of mosquitoes need for the day to day bioassays, a colony is essential. The eggs and egg rafts of *Ae. aegypti* and *An. stephensi* were procured from National Centre for Communicable Diseases (NCCD), Mettupalayam, TamilNadu, India. Filter paper attached with eggs was dipped into a plastic tray containing 500 mL of dechlorinated water for 30–40 min, time enough to allow for eggs to hatch into larvae. They were reared indoors at (28± 2) °C and 14:10 light and dark period cycle. The larvae were fed with powdered mixture of dog biscuits and yeast powder in 3:1 ratio. After five days emergence, female mosquitoes were moved into a mosquito cage where the emergent adults were fed with a 100 g/L sucrose solution and allowed for blood feed using white albino rats for 2–3 h. A few days after having a blood meal, the gravid mosquito laid their eggs.

2.4. Larvicidal bioassay

The test of larvicidal activity of volatile oil derived from ethanol extract of *S. portulacastrum* plants against *An. stephensi*, and *Ae. aegypti* was conducted in accordance with the WHO, 2005 in standard ethanol [20]. *An. stephensi* and *Ae. aegypti* were transferred to a 100 mL of enamel bowl containing 50 mL of distilled water and 2 mL of volatile oil standard concentration of 100, 150, 200, 250, 300, 350 $\mu\text{L/mL}$ and then transferred to separate beakers respectively. The symptoms of treated larvae were observed and recorded immediately without time interval and the larvae were considered dead if, at the end of 24 h they showed lower concentration of volatile oil. Concentrations that had successfully produced 80% larval mortality rate was used in a toxicity test on a non-target probit analysis.

$$\% \text{ of mortality} = \frac{\% \text{ of total mortality} - \% \text{ of control mortality}}{100\% - \% \text{ of mortality in control}} \times 100$$

2.5. GC-MS analysis

The fatty acid composition was determined. Briefly 100 μL oil plus 1 mL 10% potassium hydroxide in methanol were heated for 45 min at 85 °C. Fatty acid was methylated with 1 mL boron trifluoride-methanol-complex (20% solution in methanol) plus 1 mL methanol for 45 min at 60 °C and then extracted from the methanolic phase with petroleum ether. A volume of 1 μL of the analyte was injected in the column equipped with column oven temperature of 60 °C and column flow of 0.99 mL/min. The compounds were identified by the GC-MS intensity of retention time and by comparison with those present in the Indian Institute of Technology, Chennai. The result was expressed as the relative percentage of each individual fatty acid present in each sample given by the corresponding retention time.

Table 1

Larvicidal activity of volatile oil derived from *S. portulacastrum* mangrove plant against *An. stephensi*.

Concentration ($\mu\text{L/mL}$)	LC ₅₀ (Mean \pm SD) (LCL–UCL)	LC ₉₀ (Mean \pm SD) (LCL–UCL)	Regression equation	R ²	P value
100	22.0 \pm 6.2 (21.6–23.4)	78.5 \pm 2.5 (77.5–79.5)	Y=2.8+3.8x	1.130	0.850
150	32.0 \pm 3.4 (29.9–33.5)	81.2 \pm 3.6 (80.2–82.3)	Y=2.2+4.2x	1.980	0.960
200	63.0 \pm 7.8 (55.2–64.0)	94.2 \pm 3.9 (93.6–96.5)	Y=1.102+0.62x	0.991	0.045
250	68.0 \pm 8.2 (66.3–69.2)	110.2 \pm 2.5 (108.6–112.2)	Y=3.6+0.029x	2.950	0.890
300	72.0 \pm 3.5 (71.7–73.1)	121.2 \pm 2.6 (119.8–123.5)	Y=3.81+0.025x	1.820	1.020
350	79.0 \pm 6.6 (77.5–79.8)	125.3 \pm 6.3 (122.3–126.5)	Y=2.36+0.025	2.180	2.030

Table 2

Larvicidal activity of volatile oil derived from *S. portulacastrum* mangrove plant against *Ae. aegypti*.

Concentration ($\mu\text{L/mL}$)	LC ₅₀ ($\mu\text{L/mL}$) (Mean \pm SD) (LCL–UCL)	LC ₉₀ ($\mu\text{L/mL}$) (Mean \pm SD) (LCL–UCL)	Regression equation	R ²	P value
100	32.5 \pm 1.2 (30.9–34.9)	62.90 \pm 0.90 (57.50–64.90)	Y=3.67+4.2x	2.050	1.260
150	42.9 \pm 2.4 (41.9–43.5)	79.30 \pm 1.65 (78.20–80.12)	Y=2.65+3.5x	1.980	0.960
200	55.2 \pm 2.8 (53.7–56.9)	95.20 \pm 1.25 (94.30–96.80)	Y=0.995+0.56x	0.981	0.042
250	63.3 \pm 2.6 (62.2–64.2)	99.60 \pm 2.20 (98.50–101.23)	Y=3.58+0.02x	1.960	1.360
300	72.1 \pm 2.7 (69.7–74.2)	106.50 \pm 2.90 (105.60–107.50)	Y=2.81+0.25x	1.920	1.260
350	79.0 \pm 6.6 (77.5–79.8)	125.30 \pm 6.30 (122.30–126.50)	Y=1.9+0.2x	2.180	1.030

2.6. Statistical analysis

The average larval and pupal mortality data were subjected to probit analysis to calculate LC₅₀, LC₉₀ and 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression equation, Chi-square. Analysis variation values were calculated using the Stat plus 2009 software. Results with P<0.05 were considered to be statistically significant.

3. Results

The present study have tested with volatile oil from *S. portulacastrum* against the vector borne disease causing mosquitos like *Ae. aegypti* and *An. stephensi*. It revealed that, volatile oil extract of *S. portulacastrum* showed various ranges of larvicidal activities and the maximum percentage of larvicidal activity. LC₅₀ value of *S. portulacastrum* volatile oil extract ranged from 100 to 350 $\mu\text{L/mL}$ with different concentration. Table 1 shows the volatile oil extract 200 $\mu\text{L/mL}$ of *S. portulacastrum* showed minimum concentration of maximum activity level with LC₅₀ value (63.0 \pm 7.8) $\mu\text{L/mL}$, LCL–UCL=55.2–64.0, LC₉₀ value (94.2 \pm 3.9) $\mu\text{L/mL}$ and followed by maximum activity of 250 μL concentration showed LC₅₀ value at (68.0 \pm 8.2) $\mu\text{L/mL}$, LCL–UCL=66.26–69.20, LC₉₀ value of (110.2 \pm 2.5) $\mu\text{L/mL}$. The regression equation of *S. portulacastrum* for 4th instar larvae of *An. stephensi* were Y=1.102+0.62x (R²=0.991) and analysis of variation was significant at P<0.05 level respectively.

Table 2 shows the toxic effect of tested volatile oil of *S. portulacastrum* on early 4th instar larva *Ae. aegypti*. Volatile oil extract at 200 $\mu\text{L/mL}$ showed minimum concentration with maximum activity level with LC₅₀ value of (55.2 \pm 2.8) $\mu\text{L/mL}$, LCL–UCL=53.7–56.9, LC₉₀=95.20 \pm 1.25 and followed by 250 μL

potential larvicidal activity of volatile oil popularly used for this purpose. The evidence for the precise use of marine flora in treatment of human ailments is extensive. Many studies on plant extracts against mosquito larvae have been conducted around the world. Ethanolic extract of *S. portulacastrum* were used for antimalarial activity, antitumor activity, anti-human immunodeficiency viruses and anti-diabetic activities^[12,24]. *S. portulacastrum*, a salt marsh halophyte, is a rich source of an array of aminoacids^[25]. An unusual secondary metabolite, 2-nitro-4-(2-nitroethenyl) phenol has been isolated from the salt marsh plant of *S. portulacastrum*. The present study also aimed to identify the fatty acid chemical classes of the active volatile oil of *S. portulacastrum*. The results suggest that, a total of 12 peaks fatty acid compounds were identified in volatile oil of *S. portulacastrum* respectively with different retention time intervals with unique peak area. Fatty acid profile revealed the presence of 2,7-dimethyl-eicocene, 1-octanol, 2-butyl-dodecane, 2-tridecyl ester, 4-dimethylcholesta-8,24-dien-3-yl, oleic acid, and hexadecanoic acid which could be further used as a chemical marker for authentication and checking adulteration in the finished products of insecticides of marine origin. The results of this study clearly showed that the oil extract of *S. portulacastrum* that contain fatty acid, such as oleic acid demonstrate a high larval mortality. The volatile oil extract of *S. portulacastrum* have potential to be developed as natural larvicidal agent. In this context, the highly bioactive compounds of *S. portulacastrum* which is being grown widely in most areas of India, offer an opportunity for developing alternatives to replace rather expensive and environmentally hazardous organic insecticides. Furthermore, the findings of the high correlation of compound and larval mortality would also open the door for using fatty acid like, oleic acid as natural larvicidal agents. It is inferred from the present study that, the extracts from salt marsh mangrove plant of *S. portulacastrum* are identified as a potential source of safe and efficacious mosquito control agents for the management of malaria and dengue.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

According to WHO International Travel and Health, the Philippines reported 77 012 dengue cases from January 1 to September 4 of 2010, twice the number of cases reported during the same period last year. Dengue fever is caused by dengue virus, a mosquito-borne virus that is prevalent in Philippines, this virus victimize children mostly below the age of 15. In some cases dengue develop into a severe disease called dengue hemorrhagic fever that is fatal and at times deadly. This study attempts to develop a way to prevent dengue virus carrying mosquitoes from harming children.

Research frontiers

The studies are carried out to investigate the mosquito larvicidal activity of the volatile oil from *S. portulacastrum* against *Ae. aegypti* and *An. stephensi* vectors among different extracts.

Related reports

Compared with all the concentrations of volatile oil of *S. portulacastrum* showed minimum concentration of maximum activity. Previously Syed Ali evaluated ethanol extracts from seaweeds, seagrass and mangrove extracts for mosquito larvicidal activity against *Ae. aegypti*.

Innovations & breakthroughs

The study showed that the volatile oil of *S. portulacastrum* have some phytochemicals which are responsible for the activity against the mosquito larvicidal activities.

Applications

S. portulacastrum which were collected from South East coast of India, showed great potential of mosquito larvicidal activities. Further studies on synergistic combinations and isolation of bioactive fraction/constituent are needed.

Peer review

The present resurgence of vector-borne diseases is due to the higher number of breeding places in today's society. Further the indiscriminate use of synthetic insecticides is creating multifarious problems like environmental pollution, insecticide resistance and toxic hazards to humans. The plants are also proven to have rich source of structurally diverse bioactive compounds with valuable pharmaceutical

potential. The secondary metabolites derived from seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to nematicidal and insecticidal in lower form of animal.

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