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Bio-efficacy of Karu Nochi, *Vitex negundo* L. (Lamiaceae) against the dengue vector, *Aedes aegypti* (L.)

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Abstract

The dengue vector, *Aedes aegypti* (L.) is one of the world's deadliest species and it spreads diseases like Zika, chikungunya, dengue, and yellow fever. In this present investigation, we attempt to evaluate the larvicidal toxicity of different solvent extracts and essential oil of Karunochi, *Vitex negundo* (L.) on third instar larvae of the dengue vector, *A. aegypti*. The crude extracts were prepared by using soxhlet apparatus and rotary vacuum evaporator technique. The highest mortality was observed in essential oil and ethanolic extract followed by hexane and acetone extracts, the lethal concentrations LC_{50} (LC_{90}) values were obtained from noticed, for Vn-EO- 218.46 (517.10), Vn-EE 268.37 (592.56), Vn-HE 309.59 (667.33), and Vn-AE 327.88 (748.47), respectively and nil mortality observed in the control treatment. Karunochi, *V. negundo* essential oil, and ethanolic extract (Vn-EE) were more efficient against *A. aegypti* than crude extracts of Vn-AE (acetone) and Vn-HE (hexane). Therefore, from our studies the formulations are promising for a low-cost larvicide without isolating an active ingredient, EO's were tested. The goal of this study is to promote the use of plant-based larvicides in public health programmes.

Keywords: Vector Control; Biopesticides; *Aedes aegypti*; *Vitex negundo*; Larvicidal activity

1. Introduction

Mosquitoes have long indeed been a health threat and they could spread diseases to millions of people at risk throughout the worldwide trade and travel. Dengue fever has risen rapidly globally in recent decades. The great majority of dengue cases are asymptomatic or moderate and self-managed, hence the true numbers are underestimated. Misdiagnosed as other febrile illnesses [1], this disease is caused by Dengue Viruses (I, II, III, and IV) and transmitted by *Aedes aegypti* (Linnaeus, 1762) female mosquito bite. According to WHO, 390 million dengue infections per year are estimated (95 percent credible range 284–528 million), of which 96 million (67–136 million) are clinical (any severity of disease), another research on dengue prevalence predicts 3.9 billion people at risk of infection with DV. Even though there is a risk of infection in 129 countries, 70% of the burden is in Asia [2, 3, 4].

Pesticides have become a daily occurrence and are heavily used to increase crop yields, and repeated use of some synthetic insecticides has caused many harmful issues [5, 6][5]. For example, many pesticide residues found in foods

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are beyond permissible limits, posing health risks The impact of pesticides on non-target organisms is very significant [7, 8]. Finally, disease and insect populations that are resistant to one or more synthetic pesticides are a major issue. These issues culminated in the second half of the 20th century, leading to current attempts to reduce pesticide use. Biopesticides (BPs) are botanical pesticides made from plant metabolites [9][10][11]. EO-based BPs take advantage of the oils' toxicity. The active ingredients in EOs have insecticidal, nematicidal, ovicidal, fungicidal, and bactericidal effects on pathogens and pests, which are key attributes in agricultural yield and vector control programs [9][12][13]. EOs also inhibit growth, food intake, and oviposition in a variety of pests [14].

Vitex negundo L., a member of the Verbenaceae family, used as a traditional medicine from ancient times, is an erect, aromatic shrub with a height of 2.5–4.5 m that is widely distributed in warm regions, moist areas mainly in India China, Sri Lanka, Pakistan, Burma, Bangladesh, Malaysia, Tropical Africa, Indonesia and Nepal [15]. It has been used traditionally used to treat microbial infections, cancer, inflammation, oxidative stress, hyperglycemia, rheumatism, expectorant, tonic, diuretic, and diabetes, among other things. Almost all parts of *V. negundo* have medicinal value, but the bark, leaves, and roots are particularly useful and are sold as drugs on the market [15] [16]. It has been found that the leaves of *V. negundo* have analgesic, antihistamine, antifilarial, antimalarial, antioxidant, hepatoprotective, and antibacterial properties[17] [18] [19]. *V. negundo* L. contains several polyphenolic compounds as active ingredients [20].

Mosquitoes are the world's deadliest animals and significant disease vectors. The two main mosquito control methods are source reduction and emergent control with chemical pesticides. Due to health concerns, chemical insecticides are being replaced by plant-based repellents and insecticides. Alternative mosquito larval control agents include plant-based EO's. Despite their medical potential, little research has been done on their larvicidal efficacy. The present study evaluated the several solvent extracts and essential oils obtained from the *Vitex negundo* herbal plant, as well as the larvicidal activity of its EO's and crude extracts against the dengue vector, *Aedes aegypti*.

2. Material and methods

2.1. Collection of plant material

Fresh leaves and stems from *V. negundo* were collected from in and around Bharathiar University Campus, Coimbatore, Tamilnadu, India. Species identification (BSI/VN/0921/2022) has been done in the Botanical Survey of India, Tamil Nadu Agricultural University Campus, Southern India, Coimbatore, TN. Voucher specimens were deposited and kept in our research laboratory for further reference at the Department of Zoology, Bharathiar University, Coimbatore-641 046, Tamilnadu, India.

2.2. Preparation of Essential Oil

The essential oil preparation method was followed Pushpanathan et al [21]. The weighted fresh leaves were chopped into small pieces and hydro-distilled for 4 hours using a Clevenger device. The EOs were then dried over anhydrous sodium sulphate to remove the aqueous phase. Each extraction process was replicated three times. The resultant EO's were weighed and kept at 4°C until used in the bioassays.

2.3. Preparation of plant extracts

The plant extraction methodologies were followed by Subramaniam et al. [22]. The leaves of *V. negundo* were cleaned with tap water and dried in the shade at 282°C for 5–10 days. This was done with an electric blender (leaves). Using a Soxhlet apparatus, 300 g of plant material was extracted from the powder in 1 L of organic solvent (ethanol, hexane, and acetone) for 8 hours. A Buchner funnel with Whatman number 1 filter paper filtered the extracts. The plant extracts were dried in a rotary vacuum evaporator and stored at 4°C. One gram of plant residue was dissolved in 100 mL of acetone as a 1% stock solution. From this stock solution, concentrations of 100, 200, 300, 400 and 500 ppm were made respectively.

2.4. Collection of eggs and maintenance of larvae

The eggs of *A. aegypti* were collected using an "O"-type brush from the National Centre for Disease Control field station in Mettupalayam, Tamil Nadu, and India. These eggs were transported to the laboratory and placed in 18×13×4 cm enamel trays containing 500 mL water for hatching. The mosquito larvae were fed a 3:1 ratio of pedigree dog biscuits and yeast. The larvae were fed continuously until they turned into pupae.

2.5. Maintenance of pupae and adults

The pupae were collected from the culture trays and transferred using a dipper to plastic containers (12 x 12 cm) containing 500 mL of water. For adult emergence, the plastic jars were placed in a 90 90 90-cm mosquito cage. Mosquito larvae were maintained at a temperature of 27±2 °C, relative humidity of 75–85 %t, and a photoperiod of 14:10 (light/dark). Three days prior to blood-feeding, a 10% sugar solution was given.

2.6. Blood feeding of adult *A. aegypti*

Adult female mosquitoes were allowed to feed on the blood of a rabbit for two days (one rabbit per day, exposed on the dorsal side), ensuring adequate blood-feeding for five days. After a blood meal, enamel trays with water from the culture trays were put in the cage as a place for the mosquito vectors to lay their eggs.

2.7. Larval toxicity test

A laboratory-reared colony of *A. aegypti* larvae was used in the larvicidal bioassay. 25 third instar larvae were kept in a 500 mL glass beaker with 249 mL dechlorinated water and 1-mL of desired concentrations of *V. negundo* leaf extracts and/or EOs were added. For the test larvae, larval food was provided. Each concentration was examined in two to five trials, each with five replicates. 1 mL acetone in 249 mL dechlorinated water was used as a control. The control group consisted of larvae treated with dechlorinated water without acetone. They were corrected using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

The Probit analysis was used to calculate the LC_{50} and LC_{90} (Finney, 1971).

2.8. Statistical analysis

All data were analyzed using analysis of variance; means were separated using Alder and Rossler's Duncan's multiple range tests (1977). The average larval mortality data were subjected to probit analysis to get the LC_{50} and LC_{90} values using the Finney (1971) approach. SPSS (Statistical Package for the Social Sciences) 16.0 was utilized. Statistically significant results were determined as those with a $P< 0.05$.

3. Results

The evaluation method is a better way to assess through the WHO protocol to assess the larvicidal activity of various formulations (Vn-EE, VN-HE, Vn-HE, Vn-EO) derived from *V. negundo* leaves employed for this purpose. The essential oil and various solvent extracts of *V. negundo* were evaluated at different concentrations viz., 100, 200, 300, 400, and 500 ppm, and demonstrated activity against third-instar larvae of *A. aegypti* (Table 1).

The plant extracts and EO showed moderate larvicidal effects after 24 h; however, ethanolic extract, hexane extract, acetone extract, and essential oil from leaves of *V. negundo* against the third instar larvae of *A. aegypti*. The highest mortality was noticed at 500 ppm concentration of essential treatment and the lowest mortality value was observed in Vn-HE treatment. The LC_{50} values were noticed, 218.46, 268.37, 309.59, 327.88 ppm, respectively; LC_{90} values were assessed at 517.10 ppm for Vn-EO, 592.56 ppm for *Vn*-EE, 667.33 ppm for *Vn*-HE, and 748.47 ppm for *Vn*-AE, respectively. The Chi-square value was significant at $P>0.005$.

Table 1 Larvicidal activity of essential oil and plant crude extracts of *V. negundo* leaves against third instar larvae of dengue vector, *A. aegypti*

Target	Treatment	Concentration (µg/mL)	Mortality (%)	Regression equation	LC_{50} (LC_{90})	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	X^2 *df* =4
III Instar	(*Vn-EE*) ethanolic extract	Control	0.00±0.00	X=0.004 -1.061Y	268.37 592.56	236.56 297.83	532.43 682.69	3.218 n.s.
		100	27.80±0.83^{e}					
		200	38.80±0.44^{d}					
		300	53.20±1.78^{bc}					
		400	64.60±1.51^{b}					
		500	86.60±1.14^{a}					
III Instar	(*Vn-HE*) hexane extract	Control	0.00±0.00	X=0.004 -1.109Y	309.59 667.33	276.98 343.16	591.92 785.46	0.302 n.s.
		100	23.60±1.14^{e}					
		200	34.80±1.64^{d}					
		300	46.40±0.89^{c}					
		400	62.80±1.30^{ab}					
		500	76.20±1.30^{a}					
III Instar	(*Vn-AE*) acetone extract	Control	0.00±0.00	X=0.003 -0.999Y	327.88 748.47	290.41 369.25	648.08 919.03	0.388 n.s.
		100	26.00±1.00^{e}					
		200	33.40±1.81^{de}					
		300	45.80±1.64^{d}					
		400	57.80±1.09$_{bc}$					
		500	71.40±1.67^{a}					
III Instar	(*Vn-EO*) Essential oil	Control	0.00±0.00	X=0.004 -0.937Y	218.46 517.10	184.39 247.02	468.64 587.34	1.835 n.s.
		100	32.40±1.14^{e}					
		200	46.20±0.83^{d}					
		300	62.80±1.48^{c}					
		400	74.60±1.67^{b}					
		500	91.60±1.14^{a}					

Vn-EE- *V. negundo* ethanolic extract; Vn-HE- *V. negundo* hexane extract; Vn-AE- *V. negundo* acetone extract; Vn-EO- *V. negundo* essential oil; LC50- Lethal concentration that kills 50% of exposed larvae; - LC90-Lethal concentration that kills 90% of exposed larvae; LFL-Lower confidence limit; UFL-Upper confidence limit; x2- Chi Square Value; d.f.- degrees of freedom; n.s. – not significant (α = 0.05).

4. Discussion

Mosquitoes are represented as a serious threat to society as they are carriers of deadly diseases. These arthropod pests are controlled mainly through the application of chemical insecticides on a routine basis. Researchers are becoming more interested in developing low-cost, environmentally friendly, and efficient pesticide alternatives because of the well-known health and environmental effects of chemical pesticides, the high cost of controlling pests, and the reported development of insecticide resistance in important disease vectors.

In this present study, we attempt to evaluate the ethanol, hexane, and acetone extracts and essential oils obtained from the leaves of *V. negundo* and its larvicidal activity against the dengue vector, *Aedes aegypti* (Table 1), and our results showed that the test plant oils and their major fractions could be developed as natural pest control agents to control *A.*

aegypti. Similarly, Mavundza et al [21] have reported that the bark of *O. dissitiflora* has the ability to employed as a larvicides for *A. arabiensis* and also compare with other plant extracts of *C. anisata*, *C. menyaarthii*, *L. javanica*, *O. dissitiflora* and *T. emetica*. The larvicidal activity of *O. dissitiflora* bark extract was highest, with an LC_{50} of 25.24 mg/ml.

Regnault-Roger et al [9] have reported that the essential oils (EOs) are volatile oils with strong aromatic components that give an aromatic plant a unique smell, flavour, or fragrance. More than 17,500 aromatic plant species produce EOs, including Lamiaceae, Rutaceae, Myrtaceae, Zingiberaceae, and Asteraceae [22]. The essential oil of *V. negundo* has the highest larvicidal activity against *A. aegypti* due to containing various polyphenolic compounds as active ingredients [20] and active constituents of *V. negundo* L. are polyphenolics, flavonoids, that are usually active antioxidants [23, 24]. Rana [24] has studied that, the inhibition efficiency of active principal compound 4,5-diethyl-3′-ethoxy-pyro-flavone, derived from a methanolic extract of *V. negundo* L., has demonstrated promising macrofilaricidal activity against the adult filarial worm *Setaria cervi*.

5. Conclusion

In conclusion, Karunochi, *V. negundo* essential oil, and ethanolic extract (Vn-EE) have shown to be more effective than crude extracts of Vn-AE (acetone) and Vn-HE (hexane) against *A. aegypti* under laboratory conditions. EO's had the notable bio-efficacy, indicating the need of manufacturing a low-cost larvicide without isolating an active ingredient. These formulations will be needed to be evaluated in the field trial assays to control *A. aegypti* larvae in the field. This study demonstrates the necessity for standardization of larvicides assessment methods against *A. aegypti* and is carried out with the ultimate intention of promoting plant-based larvicides for use in public health programs.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors are declaring that there is no conflict of interest.

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