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Assessment of Larvicidal Potential using *Pisonia umbellifera* Leaf Extracts against Vector Mosquitoes *Aedes albopictus*, *Anopheles subpictus* and *Culex tritaeniorhynchus* (Diptera: Culicidae)

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Abstract: Mosquito vectors in urban environments, including the invasive species as well as the local species *Aedes albopictus,Anopheles subpictus* and *Culex tritaeniorhynchus*, cause significant pain to people and serve as carriers of dangerous illnesses. In this study, we tested the larvicidal efficiency of *Pisonia umbellifera* crude extracts on vector mosquito larvae. Crude extracts of *P. umbellifera* (in four different solvents: methanol, chloroform, ethyl acetate, and hexane) at various doses were examined on early third-instar larvae of *Ae. albopictus, An. subpictus,* and *Cx. tritaeniorhynchus*. The methanol crude extract had excellent larval mortality, with LC_{50} values of 63.14, 69.81, and 78.43 µg/ml, respectively. Our findings demonstrated that mosquitoes were effectively controlled by the *P. umbellifera* extracts. This research contributes to the development of eco-friendly bio alternatives for mosquito control, emphasizing the potential of botanical extracts in combating mosquito-borne diseases.

Keywords: Vector mosquitoes, Deadly diseases, *Pisonia umbellifera*, Crude extracts, Larvicidal, Bio-alternatives

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Introduction

Mosquitoes have long been considered a severe hazard to human health. Mosquitoes transmitted various vector-borne illnesses, including malaria, dengue fever, yellow fever, lymphatic filariasis, zika virus, and Japanese encephalitis, in tropical and subtropical climates. Through international trade and travel, these insects have the ability to spread to new geographic areas and habitats which puts millions of individuals at risk for the illnesses they spread (Kilpatrick, 2011). World Health Organization estimated 229 million cases of malaria and 409 thousand deaths, as well as 56 million cases of dengue, were reported globally in 2019 (WHO, 2020). In 2015, the estimated global mortality burden of Japanese Encephalitis was 25,000 fatalities (Quan *et al*., 2020).

Synthetic pesticides are the most sophisticated mosquito control agents available today. However, the consequences of these pesticides on non-target animals, environmental threats, and insecticide-resistant mosquitoes have all risen during the last 50 years (Wattanachai and Tintanon, 1999). Before synthetic organic insecticides were discovered, many herbal items were employed as natural insecticides. It is imperative that new insecticides should be developed that are more biodegradable, environmentally benign, and specifically aimed at mosquitoes. In actuality, several studies have shown how effective plant extracts and essential oils are at keeping mosquito larvae away (Rahuman *et al*., 2009).

Plant-based pesticides, on the other hand, are ecologically friendly, biodegradable, and harmless to animals other than their intended target. Numerous research has demonstrated the effectiveness of various solvent crude extracts of plants as mosquito larvicides without affecting the environment or human health (Sogan *et al*., 2018; Johnson *et al*., 2018; Bouabida and Dris, 2022). According to Sukumar *et al.* (1991) variety of plants possessing larvicidal and adulticidal qualities have been employed to combat insects. Therefore, this study evaluates the larvicidal activity of different crude extracts from leaves of *P. umbellifera* against *Ae. albopictus, An. subpictus* and *Cx. tritaeniorhynchus* mosquitoes.

P. umbellifera (Nyctaginaceae family) is also known as the lettuce tree, bird-lime tree, or bird catcher tree. It is an evergreen tree that grows between 9 and 12 meters tall and is found in the Andaman Islands beach forests. It is also somewhat grown in South India and Ceylon. *P. umbellifera* leaves are edible and mostly used in traditional medicine to treat wound healing, rheumatism, ciguatera intoxication as well as to prevent phytophage nematodes (Sripathi *et al*., 2011; Kuo, 2011). It is also used in the treatment of ulcer, dysentery and snake bite. Phytochemical analysis and anti-inflammatory activity of *P. umbellifera* has been reported by Kaviya Priya *et al*. (2019). In this study, we tested the larvicidal efficiency of *Pisonia umbellifera* crude extracts on vector mosquito larvae. Crude extracts of *P. umbellifera* (in four different solvents: methanol, chloroform, ethyl acetate, and hexane) at various doses were examined on early third-instar larvae of *Ae. albopictus, An. subpictus,* and *Cx. tritaeniorhynchus*.

Materials and Methods

Plant Collection

Fresh leaves of *P. umbellifera* were gathered from in and around the Kumbakonam, Thanjavur district, Tamil Nadu, India. A plant taxonomist from the Department of Botany at the Government College for Women (Autonomous) verified its authenticity. A voucher specimen has been placed in our research laboratory for future reference.

Preparation of crude extract

Fresh leaves were cleaned with tap water and allowed to dry in the shade at room temperature (28 ± 2°C), and then ground into fine powder using an electronic blender. Using four solvents methanol, chloroform, ethyl acetate, and hexane (1L) the powder (250 g) was extracted for 8 h using the Soxhlet apparatus following the standard procedure (Vogel, 1978). A Buchner funnel was used to filter the extracts using Whatman No. 1 filter paper. Using a rotary evaporator, the filtrate was dried out under low pressure, producing a viscous, dark greenish extract. Crude extract yields in methanol, chloroform, ethyl acetate, and hexane were 15.6, 14.4, 12.6, and 11.2 g, respectively. For the bioassays, acetone was used to dissolve the residues and create standard stock solutions at 1%.

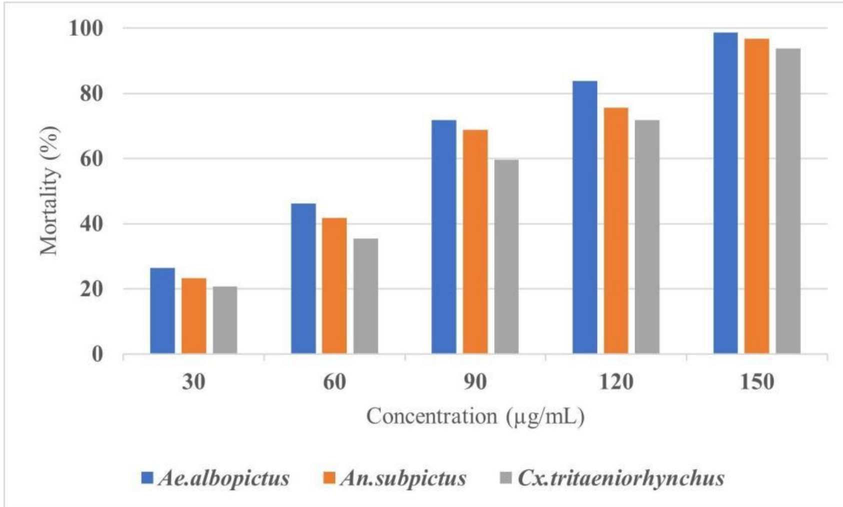

Fig. 1: Mortality of *P. umbellifera* methanol crude extract against mosquito vectors at various concentration.

Test Organisms

Laboratory-bred pathogen-free strains of mosquitoes (*Ae. albopictus, An. subpictus* and *Cx. tritaeniorhynchus*) were reared in our research laboratory, Department of Zoology, Government College for Women (A). The larvae were fed 3:1 mixture of yeast powder and dog biscuits. At the time of adult feeding, these mosquitoes were 3–4 days old after emergences and were starved for 12 h before feeding. A one-week-old chick blood was given as meal, and adults were given a 10% sucrose solution. The mosquitoes were held at 28 ±2 °C, 70%–85% relative humidity, with a photoperiod of 12 h light and 12 h dark (Govindarajan and Sivakumar, 2015).

Larvicidal Bioassay

Larvicidal activity of the crude extract from *P. umbellifera* was evaluated according to WHO protocol (WHO, 2005). 25 late III instar larvae were placed for 24 h in a 250 ml water cup filled with 200 ml of dechlorinated water plus 1 ml of the desired concentration of *P. umbellifera* crude extract. In all experiments, each concentration was replicated five times. After 24 h, the lethal concentration (LC_{50}) values were determined using Probit analysis (Finney, 1971). In control treatments, 25 larvae were kept in the 200 ml of dechlorinated water.

Data analysis

Probit analysis was applied to get LC_{50} and LC_{90} values using SPSS statistical data analytical tool. The significance level was set at $p<0.05$.

Results and Discussion

The toxicity of *P. umbellifera* crude extracts was tested against the larvae of *Ae. albopictus*, *An. subpictus*, and *Cx. tritaeniorhynchus*. The methanolic extract of *P. umbellifera* showed excellent larval mortality (Fig. 1) on mosquito larvae than other solvent crude extracts. LC_{50} values for methanol extract were 63.14, 69.81, 78.43 µg/ml and LC_{90} values were 125.74, 137.64, 149.07 µg/ml, respectively (Table 1). Chloroform crude extract of *P. umbellifera* exhibit high mortality rate (Fig. 2) with LC_{50} and LC_{90} values were 88.13, 96.82,105.73 and 169.08, 181.56, 194.83 µg/ml, respectively (Table 2).

Moderate larval death (Fig. 3) was caused by ethyl acetate crude extract. LC_{50} and LC_{90} values were 105.26, 117.65, 128.33 and 215.19, 230.23, 245.26 µg/ml, respectively (Table 3). Hexane crude extract exhibited moderate larval mortality(Fig. 4). LC_{50} and LC_{90} values were 128.70, 142.57, 156.45 and 267.35, 287.00, 299.23 µg/ml, respectively (Table 4). No mortality was observed in control. The chi-square values were

Table 1: Larvicidal activity of *P. umbellifera* methanol crude extract against third instar of *Ae. albopictus, An. subpictus* and *Cx. tritaeniorhynchus*

Species	LC_{50} (µg/ml) (LCL - UCL)	LC_{90} (µg/ml) (LCL – UCL)	Regression equation	χ^2*
Ae. albopictus	63.14 (55.86 – 69.57)	125.74 (116.53 – 137.87)	y=10.76+0.6067x	3.250*
An. subpictus	69.81 (62.43 – 76.47)	137.64 (127.32 – 151.44)	y=11.39+0.921x	6.213*
Cx. tritaeniorhynchus	78.43 (71.27 – 85.17)	149.07 (137.72 – 164.40)	y=1.560+0.6080x	3.621*

* $p \leq 0.05$, Level of Significance

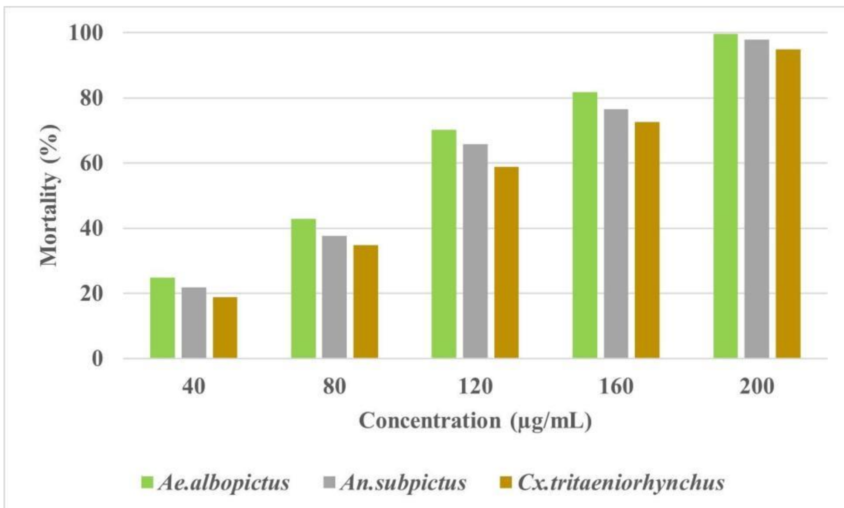

Fig. 2: Mortality of *P. umbellifera* chloroform crude extract against mosquito vectors at various concentration.

Table 2: Larvicidal activity of *P. umbellifera* chloroform crude extract against third instar of *Ae. albopictus, An. subpictus* and *Cx. tritaeniorhynchus*

Species	LC_{50} (µg/ml) (LCL - UCL)	LC_{90} (µg/ml) (LCL – UCL)	Regression equation	χ^2*
Ae. albopictus	88.13 (78.98– 96.37)	169.08 (157.02 – 184.89)	y=7.260+0.4715x	6.421*
An. subpictus	96.82 (87.78 – 105.17)	181.56 (168.59 – 198.67)	y=7.21+0.005x	6.191*
Cx. tritaeniorhynchus	105.73 (96.69 – 114.32)	194.83 (180.65 – 213.76)	y=0.9800+0.4745x	3.675*

*$p \leq 0.05$, Level of Significance

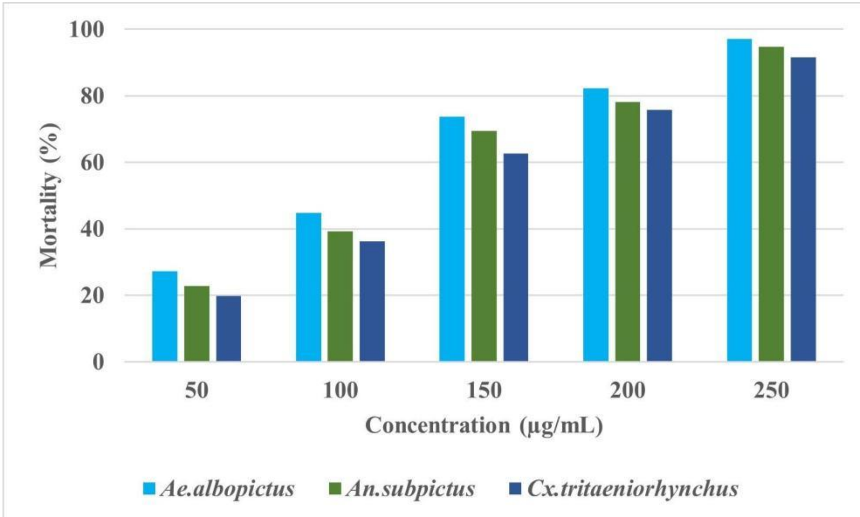

Fig. 3: Mortality of *P. umbellifera* ethyl acetate crude extract against mosquito vectors at various concentration.

Table 3: Larvicidal activity of *P. umbellifera* ethyl acetate crude extract against third instar of *Ae. albopictus, An. subpictus* and *Cx. tritaeniorhynchus*

Species	LC_{50} (µg/ml) (LCL - UCL)	LC_{90} (µg/ml) (LCL – UCL)	Regression equation	χ^{2*}
Ae. albopictus	105.26 (92.47 – 116.46)	215.19 (199.03 –236.66)	y=11.82+0.3548x	3.437*
An. subpictus	117.65 (105.45– 128.68)	230.23 (213.12 –253.03)	y=5.02+0.003x	3.112*
Cx. tritaeniorhynchus	128.33 (116.32 – 139.53)	245.26 (226.77 –270.13)	y=2.240+0.3664x	1.002*

*$p \leq 0.05$, Level of Significance

significant at $p<0.05$ level.

Our results are in agreement with earlier findings of Govindarajan and Angelina (2010) who have showed larvicidal efficacy of *Ficus benghalensis* extract against early second, third and fourth larvae *of Cx. quinquefasciatus, Ae. Aegypti* and *An. stephensi* as 41.43, 58.21 and 74.32 ppm; 56.54, 70.29 and 80.85 ppm; and 60.44, 76.41 and 89.55 ppm, respectively. *Aloe vera* leaf extract exhibited excellent larval toxicity against I, II, III and IV instar larvae of *Ae. aegypti* with LC_{50} values as 162.74, 201.43, 253.30 and 300.05 ppm and LC_{90} values as 442.98, 518.86, 563.18 and 612.96 ppm, respectively (Subramaniam *et al*., 2012).

Methanolic extract of *Persea americana* unripe fruit peel revealed good larval mortality against vector mosquitoes with LC_{50} and LC_{90} values of 6.65 and 71.62 ppm *(An. stephensi*), 7.12 and 86.59 ppm (*Ae. aegypti*) and 10.78 and 69.39 ppm (*Cx. quinquefasciatus*), respectively (Louis *et al*., 2020). Methanol crude extract from *Rosmarinus officinalis* and *Melissa officinalis* plants revealed excellent larvicidal toxicity on *Cx. pipiens* mosquito with

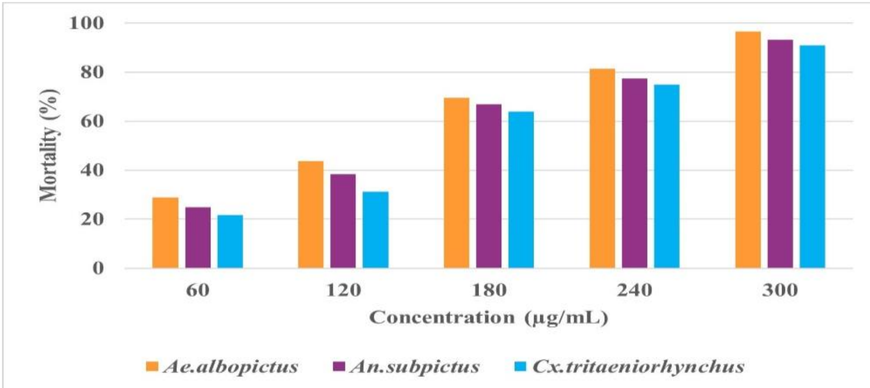

Fig. 4: Mortality of *P. umbellifera* hexane crude extract against mosquito vectors at various concentration.

Table 4: Larvicidal activity of *P. umbellifera* hexane crude extract against third instar of *Ae. albopictus, An. subpictus* and *Cx. tritaeniorhynchus*

Species	LC_{50} (µg/ml) (LCL - UCL)	LC_{90} (µg/ml) (LCL – UCL)	Regression equation	χ^{2*}
Ae. albopictus	128.70 (112.81 – 142.58)	267.35 (246.67 – 295.13)	y=11.98+0.2890x	2.697*
An. subpictus	142.57 (127.09 – 156.51)	287.00 (264.63 – 317.29)	y=6.940+0.6033x	2.217*
Cx. tritaeniorhynchus	156.45 (141.94 – 170.05)	299.23 (276.40–330.05)	y=1.840+0.3033x	3.491*

*$p \leq 0.05$, Level of Significance

LC_{50} values as 9.795 and 26.505 µg/ml, respectively (Asmaey *et al*., 2024).

Conclusion

The present study clearly indicated that crude extracts from *P. umbellifera* could be used as natural mosquito larvicide against the three mosquitoes species (*Ae. albopictus*, *An. subpictus* and *Cx. tritaeniorhynchus*). Therefore, the mosquito vectors control program needs to support the current approach. The mode of action and larvicidal efficiency of *P. umbellifera* under the field condition should be scrutinized and determined. Furthermore, it is essential and crucial that more research be carried out on the impact of plant extracts on non-target organisms.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar 608002, Tamil Nadu, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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