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ANTI-LARVAL ACTIVITIES OF ESSENTIAL OIL OF PSIDIUM SP. AGAINST DENGUE CAUSING MOSQUITO *AEDES AGYPTI* L. (DIPTERA: CULICIDAE)

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ABSTRACT: Mosquito borne diseases such as dengue, ebola, zika, chikungunya, malaria and filariasis etc. pose a serious threat worldwide in terms of public health. They are responsible for a significant economic burden for the African and Asian continent. Development of resistance, cross-resistance, rising cost and possible toxicity hazards associated with synthetic insecticides are some of the reasons for revival of interest in plant-based products in recent years. Search for cost-effective, safe and highly potent plant-based insecticides for the control of mosquitoes requires the preliminary screening of plants to evaluate their effectiveness in mosquito control and selecting the plants with high potency for further study. Essential oil of *Psidium guajava* L.variety Navalur Light pink was studied for its chemical composition and larvicidal potential against *Aedes aegypti* larve. Totally 84.33%compounds were identified in species by GC-MS (Gas Chromatography-Mass Spectrometry). The major chemical compounds were 1-(1-Ethylvinyl)-1-(-2-methylene-3-butenyl) cycloproane of 53.34% in Navalur Light. Preliminary bioassay test was carried out by using modified WHO method for determination of larvicidal activity against *Aedes aegypti* larve. Essential oils of *Psidium guajava* extract showed good mortality rate in *Aedes* larvae. Experimental results suggest that essential oil of *Psidium guajava* species are one of the inexpensive and eco friendly sources of natural mosquito larvicidal agent to control or to reduce the population of *Aedes aegypti* mosquito. Further isolation and identification of active ingredients in essential oil of *Psidium guajava* are under process.

KEYWORDS: *Psidium guajava* L., Essential oil, GC-MS, larvicidal activity.

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1.INTRODUCTION

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions in recent years. Dengue is an acute viral infection with potential fatal complications [1, 2, 3]. Dengue virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and to a lesser extent *Aedes albopictus*. Dengue is widespread throughout the tropics, with local variations in risk influenced by rainfall, temperature and unplanned rapid urbanization. It estimates that 3.9 billion people, in 128 countries, are at risk of infection with dengue viruses [2, 3]. The *Aedes aegypti* mosquito is the primary vector that transmits the dengue virus disease. The immature stages of *Aedes aegypti* mosquito are found in water-filled habitats, mostly in artificial containers closely associated with human dwellings and often indoors. This means that people, rather than mosquitoes, rapidly move the virus within and between communities and places World Health Organisation (WHO) [4, 5, 2, 3]. Dengue disease continues to involve newer areas, newer populations and is increasing in magnitude, epidemic after epidemic. Every aspect of dengue viral infection continues to be a challenge; the pathogenesis of severe dengue disease is not known, no vaccine is yet available for protection and the vector control measures are inadequate World Health Organisation (WHO) [6, 7, 8, 9, 10, 11, 4, 5, 2, 3]. Search for new anti-dengue agents from medicinal plants has assumed more urgency than in the past. Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases. So there is a need to find out novel drugs from indigenous medicinal plants to combat dengue transmitting vectors and demand for plant-based medicines is growing as they are generally considered to be safer, cheaper, non-toxic and less harmful than synthetic drugs. Medicinal plants have been used widely to treat a variety of vector World Health Organisation (WHO), [4, 5, 2, 3]. The larvicidal activity of hydrodistillate extracts from *Mentha piperita* L. *Ocimum basilicum* L. *Curcuma longa* L. and *Zingiber officinale* L. *Cymbopogon citrates* L. *Lippi asidoides* L. *Ocimum americanum* L. *Ocimum gratissimum* L. were investigated against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae). The results indicated that the mortality rates at 80, 100, 200 and 400 ppm [12,13]. *Psidium guajava* belongs to the family Myrtaceae; the majority of these species are essential oil bearing plants. The family consists of about 75 genera and nearly 3000 species of mainly tropical evergreen trees and shrubs [14, 15]. The chemical composition of the essential oils can vary widely in different regions, principally because of environmental factors, as well as genetic factors that can induce modifications in the secondary metabolism of the plant [16, 17, 18]. In India guava is considered as the fifth most important fruit after the production of mango, citrus, banana and apple. Guava is a hardy, prolific bearer and highly remunerative fruit [19, 20]. The guava fruit growers found wide commercial market due to its wide adaptability and higher return per unit area. Guava fruit is grown in the most parts of India particularly in the kitchen gardening, near the well and tube-well premises on a commercial scale. Nature has endowed it liberally to tolerate the drought and flood condition and adaptability to a wide range of soil and climatic conditions. Its cultural requirement is also very limited. Besides other factors of crop production, varieties play an important role. The local commercial varieties of guava viz., Allahabad Safeda, Sardar, Chittidar, Dharidar and Red Fleshed are commonly grown in different agro-climatic regions. The performance of a particular variety in one agroclimatic region may not prove suitable for other regions due to their inherent characters [21, 22]. Pink pulped guava varieties supply a carotenoid called as lycopene. It has been considered as a potential agent for the prevention of some types of cancers, particularly prostate cancer. Lycopene is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene and xanthophylls. Thus, nutritionally guava fruit is a good choice for better health [23, 24]. Guava fruit has high demand in preservation industry for preparation of jelly, candy, nectar and mixed jam due to its qualitative physico-chemical attributes and or-ganoleptic scores[15, 25, 26].The present study deals with chemical composition of essential oil and larvicidal activity.

2. MATERIALS AND METHODS

Leaves of P*sidium gujava* Navalur light pink were collected in 2017 during summer season, from University of Agricultural Sciences Plantation, Dharwad, and Karnataka, India. The plant was identified by Dr.G.Mulugund with taxonomic keys. Herbarium specimens were prepared and lodged at the Ward Herbarium (RKC/KUD-BOT/02) at Department of Botany, Karnatak University, Dharwad, Kranataka India.

2.1 Extraction of essential oil

Psidium gujava collected leaf sample were washed with tap water and allowed to shade dry for 5 days. The leaf sample was powdered and stored for further use. The essential oils were obtained by hydrodistillation method using a Clevenger-type apparatus. Further after 6 hours of extraction oil sample was collected and stored in air tight containers [27, 28, 29].

2.2 Gas chromatography-mass spectrometry (GCMS)

Gas chromatography was performed using system of a model QP5050 in Karnataka, mass spectrometer equipped with a Shimadzu AOC 5000 series auto-injector. Using a column RTX-5MS (30M*0.25mm), 0.25 μm film thickness the temperature program was set from 60-29degree in 1-3 min the injection temperature was 250°C and the injection volume was 1μl. The inlet pressure was 68.6 kPa was used as a carrier gas. With a linear velocity (*u*) of 39.2 cm/s. Injection mode was split. MS interface temperature was 290°C. MS mode was TIC, detector voltage was 1.4 Kv, mass range was 100-920m/z, scan speed was 1000 scan/s and interval was 0.5 sec.

2.3 Mosquito rearing

Eggs of *Aedes aegypti* were collected from ICMR centre, Bengaluru, Karnataka, India. The egg rafts were then brought to the laboratory. The eggs were placed in enamel trays (30 cm×24 cm×5 cm) each containing 2 L of R.O water and kept room temperature (28 ± 2) 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 (26±2) 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 in a Petri dish to feed adult mosquitoes was also placed in each mosquito cage. An immobilized mice 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 larvicidal assays or allowed to hatch out under the controlled laboratory conditions described above. Only the newly hatched larvae of *Aedes aegypti* were used in all bioassays.

2.4 Larvicidal activity

The larvicidal activity of plant essential oil extract was assessed by using the standard method as prescribed by World Health Organisation (WHO) [30]. From the stock solution, five different test concentrations (viz., 25, 30, 35, 50, 75, 100ppm) were prepared and they were tested against the late third instar larvae of *Aedes aegypti*. The larvae of test species (25) were introduced in 500 ml plastic cups containing 100 ml of aqueous medium (99 mL of dechlorinated water + 1 mL of emulsifier) and the required amount of plant essential oil extract was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, three replicates were maintained at a time [4, 31, 32].

3. RESULTS AND DISCUSSION

The volatile oils of the dried, powdered leaves from *Psidium guajava* were a light yellowish liquid with a strong aromatic fragrance. The distribution of the different chemical groups of the compounds is shown in Table-1. The compounds from essential oils are grouped in Table 1 based on their chemical structures in which they are classified. The Gas chromatography-mass spectrometry (GC-MS) analysis of the oils of *Psidium gujava* Navalur light pink resulted in 84.33% from 9 compounds. The highest percentage of compounds were 1-(1-Ethylvinyl)-1-(-2-methylene-3-butenyl) cycloproane (53.34%), followed by 2-methyl-5,7-dimethylene-1,8-Nonadiene (7.72%), Santolinatriene (4.46%), Cis-alpha-bisabolene (4.16%), (5e,9E)-12-methyl-1,5,9,11-tridecatetraene (3.96%), Copene (2.97%), 1,11-Dodecadiyne (7.72%) alkyne, Beta-Linalool 0.01 phenolin the graph**.** In our present study Essential oil extraction from plant extracts confirmed their potential for control of the larvicidal populations (Table2). Similarly, a higher concentration of 100, 75, 50 ppm gave 100% protection within 6-8 hours. On the other hand the lower concentration of 35ppm gave the good result within 24 hours against *Aedes aegypti* larvae. This study showed that leaf essential oil of *Psidium* would be a potent source of natural larvicidal, activities against selected medically important vector mosquito species.

Table1: Chemical composition of *Psidium guajava* L. Navalur light pink.

Navalur light pink	Group	Molecular formula	Retention time	Percentage of compound
1-(1-Ethylvinyl)-1-(-2-methylene-3-butenyl)cycloproane	Alkanes	C12H18	11.05	53.34
Copene	Alkenes	C15H24	10.55	2.97
2-methyl-5,7-dimethylene-1,8-Nonadiene	Alkenes	C12H18	11.15	7.72
Santolinatriene	Alkenes	C10H16	11.27	4.46
Cis-alpha-bisabolene	Alkenes	C15H24	11.53	4.16
(5E)-2,5-Dimethyl-3-methylene-1,5-heptadiene	Alkenes	C10H16	11.59	0.01
(5e,9E)-12-methyl-1,5,9,11-tridecatetraene	Alkenes	C14H22	12.28	3.96
1,11-Dodecadiyne	Alkynes	C12H18	11.25	7.72
Beta-Linalool	phenol	C10H18O	11.99	0.01
TOTAL 84.33%				

Table 2:larvicidal activity of *Psidiumguajava*L. variety Navalur light Pink.

Concentration	No of larvae	No of active larvae	No Moribund larvae	No of dead larvae
Control	90	90	-	-
Temphos	90	-	-	90
100	90	-	-	90
75	90	-	-	90
50	90	-	1	89
35	90	-	5	85
30	90	-	15	75
25	90	3	22	65

4. CONCLUSION

Extracts of essential oil from *Psidium* sp. are safe for human life, with no side effect and found to be environmental friendly. The larvae of *Aedes aegypti* are susceptible to the composition of the essential oil under evaluation. The use of natural products might be considered as an important alternative insecticide for the control of *Aedes aegypti* larvae. Plant extracts constitute a rich source of bioactive compounds that are biodegradable, nontoxic, and potentially suitable for use in integrated larvae management programs. However, the cost of the essential oil might also be an important factor for its implementation, which depends on the availability of the plant and its yield/ha. In conclusion, essential oil might be used as an ecologically safe alternative larvicide. Furthermore, 1-(1-Ethylvinyl)-1-(-2-methylene-3-butenyl)cycloproane, is a major compound in the essential oil of *Psidium guajava* L. variety Navalur light Pink and small traces of other components, was found to be the active principle responsible for the larvicidal action.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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