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EVALUATION OF CHEMICAL COMPOSITION AND LARVICIDAL EFFICACY OF *PIPER LONGUM* L. LEAF EXTRACT AGAINST *AEDES AEGYPTI*

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ABSTRACT

Mosquito-borne diseases are major public health-associated diseases. The chemical insecticides are used for several decades to control vector mosquitoes. The large-scale application of chemical insecticides caused several problems like insecticide resistance, the resurgence of pest species, environmental pollution, toxic hazards to humans and other non-target organisms. Mosquito control with botanicals proved to be a good alternative to chemical insecticide. The essential point of this investigation was to screen non-poisonous and effectively accessible mosquito control of natural origin. The current investigation evaluated the role of larvicidal activity of crude hexane, petroleum ether, chloroform, and ethanol leaf extracts of *Piper longum* L. tested against the third instar of *Aedes aegypti* larvae at 24h exposure. Among the crude extracts, the highest larval mortality crude extract was undergone the GC-MS analysis for the identification of active phytocompouds. The highest larval mortality was observed in ethanolic extract followed by chloroform, petroleum ether, and hexane. The corresponding LC_{50} and LC_{90} values of hexane, petroleum ether, chloroform, and ethanol were 46.90 and 82.30; 39.82 and 75.22; 30.00 and 63.33; 22.55 and 52.63µg/dl respectively. Twenty-four compounds were identified by GC-MS analysis. Results of this study revealed that the ethanolic leaf extract of *P. longum* may be considered a potent source of mosquito larvicidal agents.

Keywords: *Aedes aegypti*, Crude extracts, GC-MS analysis, Larvicidal activity, *Piper longum*.

1. INTRODUCTION

Mosquitoes are the vectors and they transmit vector-borne diseases like malaria, dengue, filariasis, leishmaniasis, and chikungunya in tropical and sub-tropical regions [1, 2]. In many countries, vector-borne diseases are one of the major problems. The mosquitoes not only transmit pathogens, but is also the reason for causing allergic reactions like skin and systemic sensitivity. *Aedes aegypti* L. is the female day-biting mosquito, which transmits dengue to humans. Dengue fever is endemic in South East Asia, India, Bangladesh, and Pakistan [3]. Forty percent of the world's population, approximately three billion people, live in areas with a risk of dengue. Vector control is the fundamental part of the worldwide procedure for managing mosquito-borne diseases. Insecticide application is an important method to control vector-borne diseases. Larvicides kill the early life stages of mosquitoes before emerging them into adults. Larval control depends on the application of synthetic chemical insecticides. Continuous application of chemical insecticides leads to environmental pollution and resistance to mosquitoes [4-6], and this may lead to the search for new insecticides. Plant-based insecticides are the alternative sources for synthetic chemical insecticides.

Medicinal plants are the "backbone" of traditional medicine, which plays an important role in world health [7]. Plant-based phyto constituents are derived from whole plants like root, stem, bark, leaves, flowers, fruits, and seeds [8]. Plant extracts are recently evaluated as active agents for mosquito control [9-11]. The attainment of plant extracts in controlling mosquitoes is depending on the bioactive phyto-compounds, and they inhibit insect growth and metamorphosis [12-14]. The efficiency of essential oils and different solvent extracts of plants as mosquito larvicides without causing damage to human health and

the environment [15-18] has been identified. Various solvent extracts of *Nerium indicum* Mill leaves showed mosquitocidal activity [19]. Based on the previous study reports, *Piper longum* L. is selected for this study, because of the local availability and abundance.

P. longum is a slender, aromatic creeping and perennial herb [20]. It is used to cure bronchitis cough, stomach ache, and also to prevent cancer development [21]. *P. longum* plant extracts were tested for larvicidal activity [22, 23]. Compounds derived from *P. longum* possess a mosquito larvicidal activity [24]. This study reports that the larvicidal activity of *P. longum* leaf crude extract obtained from four different solvents namely hexane, petroleum ether, chloroform, and ethanol against the fourth instar larvae of *Ae. aegypti*. The higher mortality crude extract was undergone GC-MS studies for identifying the active phytocompounds. The present study could be useful in research at the development of a new agent for controlling mosquitoes with plant-based natural products.

2. MATERIAL AND METHODS

2.1. Plant collection and extraction

Mature fresh leaves of *P. longum* were collected from the college garden, Nagercoil, Tamil Nadu, India. The leaves were cleaned with tap water and shade dried at room temperature and powdered using an electric blender. The powdered samples were extracted in a soxhlet apparatus using four different solvents namely hexane, petroleum ether, chloroform, and ethanol individually. The extracts were concentrated in a rotary vacuum evaporator to collect the crude extract and preserved at 4°C in air tight bottles for conducting the further experiments.

2.2. Mosquito rearing

Egg cards of *Ae.aegypti* were procured from the Centre for Research in Medical Entomology (CRME), Madurai. The egg cards were placed in a tray containing ion-free water for hatching. The hatched out larvae were fed with powdered dog biscuit and yeast in the ratio 3:1.

2.3. Larvicidal bioassay

The larvicidal action of *P. longum* leaf crude extracts was assessed by the methods of WHO [25]. The fourth instar *Ae.aegypti*, larvae was raised in the laboratory and batches of ten larvae were exposed to the toxicants in 100ml glass beakers, the concentrations ranged from 15-75(µg/dl). Five replicates were maintained for each concentration. The control were set up simultaneously using tap water and 1ml of appropriate solvent. The exposed larvae were continuously monitored, the mortality was recorded after 24h.

2.4. Statistical analysis

The mortality values were indicated as mean±SD of five replicates. The larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90} values and other statistics at 95% confidence limits of the Upper and Lower Confidence Limit (UCL-LCL) were calculated using the dose-effect probit analysis [26].

2.5. GC-MS analysis of *P.longum* crude extract

The effective crude extract was analyzed by GC-MS for identification of active phytocompounds. GC-MS analysis was carried out in South Indian Textile Research Association (SITRA), Coimbatore. Thermo GC-Trace Ultra Ver: 5.0, Thermo MS DSQ II equipment with diamention 30 Mts, ID: 0.25 mm, FILM: 0.25 µm was used. Helium was used as a carrier gas at flow rate 0f 1.0ml/min. The oven temperature was 70°C raised to 260°C at 6°C/min. The spectra pertaining to each RT values were further characterized using mass-spectral analysis. CAS library reference was used to elucidate the structure of compounds.

3. RESULTS AND DISCUSSION

3.1. Larvicidal activity of crude extracts

In this study larvicidal activity of the crude hexane, petroleum ether, chloroform and ethanol extracts of *P. longum* leaves were studied. The results of the larvicidal bioassay are presented in table 1. Among the four crude extracts, ethanolic crude extract was found to have highest larvicidal activity against fourth instar *Ae.aegypti* larvae at 24h. Chloroform, petroleum ether and hexane resulted in moderate mortality. The LC_{50} and LC_{90} values of ethanol, chloroform, petroleum ether and hexane were 25.66 and 61.08; 30.00 and 63.33; 39.82 and 75. 22; 46.90 and 82.30 (µg/dl) respectively and given in the table 2 and fig. 1.

The high mortality might be due to the chemical constituents present in extracts that arrest the metabolic activities of the larvae. The variation in the susceptibility of the extracts to mosquito larvae may be due to variations in extraction solvents, mosquito species or exposure period [27]. The results of this study confirm the *P. longum* leaf capable for control the larval population of mosquitoes.

Table 1: Larvicidal activity of different solvent extracts of *P.longum* leaf against the fourth instar *Ae.aegypti* after an exposure of 24h

Conc. (µg/dl)	Solvent extracts			
	Hx	Pet	Chl	EtOH
	Percentage larval mortality ± SD			
15	17.74±1.74	18.4±1.85	27.4±1.74	38.21.83
30	26.8±1.93	38.8±1.60	47.8±1.60	58.61.95
45	37.6±1.49	57.0±1.78	68.2±1.93	78.4±1.49
60	55.6±1.20	66.8±1.93	87.6±1.49	100±00
75	85.8±1.16	88.4±1.49	96.8±1.93	-

Conc.-concentration, Hx- Hexane, Pet-Petroleum ether, Chl-Chloroform, EtOH-Ethanol, Mean±SD represents mean of five values, Control-Nil mortality

Table 2: Probit analysis of the mortality response of fourth instar *Ae.aegypti* to different crudes extract of *P. longum* after an exposure of 24h

Solvent extract	LC_{50} (µg/dl)	(LCL-UCL)	LC_{90} (µg/dl)	(LCL-UCL)
Hx	46.90	23.19-70.61	82.30	59.59-106.01
Pet	39.82	16.11-63.63	75.22	51.51-98.93
Chl	30.00	6.29-53.71	63.33	39.62-87.04
EtOH	22.55	3.19-41.91	52.63	33.27-71.99

LC_{50}-Lethal concentration that kills 50% of the exposed larvae, LC_{90}-Lethal concentration that kills 90% of the exposed larvae,LCL-lowerConfidence Limit, UCL-Upper Confidence Limit(95%).

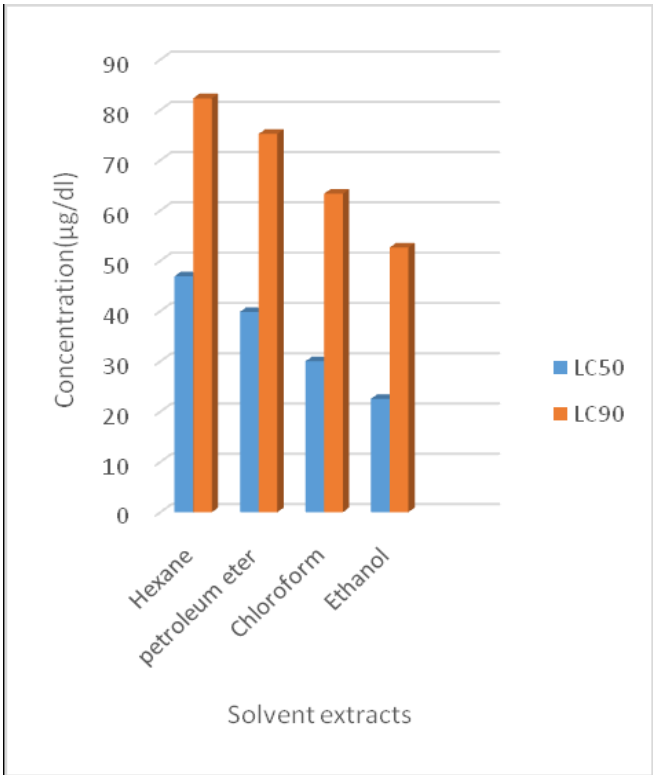

Fig. 1: Graph showing the LC_{50} and LC_{90} values of different solvent extracts of *P. longum* leaf against *Ae. aegypti*.

Mosquitoes cause nuisance and are most dangerous insects, since they transmit pathogens. Vector mosquitoes are well established in tropical and subtropical regions and they have also developed resistance to chemical insecticides. Hence biological control methods would be a good approach in mosquito control program. Recent awareness on harmful effects of chemical pesticides has directed the public to look for ecofriendly pesticides and non-chemical methods of pest control. Botanical pesticides are universally accepted alternate pesticides. The results of present study quite analogous with previous reports of [28] who have studied the larvicidal activity of methanol, chloroform, ethylacetae, acetone and petroleum ether leaf extracts of *Elaeagnus kologa*. Among all the extracts, methanol, ethyl acetate and acetone extracts recorded 100% mortality in 15 and 20mg/ml against second instar larvae of *Ae.aegypti*. The results of the study by Pohlet [29] showed that larvicidal efficacy of ethanol, methanol and aqueous crude extracts of *P. aduncum* leaf against the third instar *Ae.aegypti* larvae, in which ethanol and methanol extracts were active against mosquito. Govindarajalu [30] reported that the larvicidal effect of aqueous, ethanol and methanol leaf extracts of *A. reticulate* against third instar larvae of *Ae.aegypti* (24h), in these extracts ethanol leaf extract (LC_{50}=132.636; LC_{90}= 390.731 mg/L) showed highest larval activity followed by aqueous (161.447, 411.225mg/L) and methanol (162.156;511.771 mg/L) respectively. In another study Krishnappa et al. [31] screened different

solvent extracts of *Gliricidia sepium* (Jacq.) for larvicidal activity and they found that the ethanol extract of *G. sepium* was highly effective with LC_{50} and LC_{90} values of 121.79 and 231.98 ppm respectively against third instar larvae of *Anopheles stephensi*. Similarly, Kovendan [32] have reported that the hexane, chloroform, ethyl acetate, acetone and methanol crude leaf extracts of *O. thymiflorus* were tested for larvicidal activity against *An. Stephensi*, *Cx.quinquefasciatus* and *Ae.aegypti*. The maximum larvicidal activity was observed in methanolic extract followed by acetone, ethyl acetate, chloroform and hexane. The LC_{50} values were 201.39, 178.76, 158.06, 139.22 and 118.74ppm for *An. Stephensi*, 228.13, 209.72, 183.35, 163.55 and 149.96ppm for *Cx. quinquefasciatus*, 215.65, 197.91, 175.05, 154.80 and 137.26ppm for *Ae. aegypti*.

Another study reports by Kovenden [33] showed the different solvent crude extracts such as hexane, chloroform, ethyl acetate, acetone and methanol of *A. alanifolia* tested against the fourth instar *An. Stephensi* larvae had values of LC_{50} and LC_{90}= 197.37 and 477.60; 178.75 and 459.21; 164.34 and 435.07; 149.90 and 416.20; 125.73 and 395.30ppm; *Ae.aegypti* had values of 202.15 and 476.57; 182.58 and 460.83;160.35 and 440.78; 146.07 and 415.38; 128.55 and 381.67ppm; *Cx. quinquefasciatus* had values of 198.79 and 458.73; 172.48 and 430.66; 151.06 and 418.78; 140.69 and 408.83; 127.98 and 386.26ppm, respectively. In another experiment, Govindajan and Sukumar [34] studied the larvicidal, ovicidal and adulticidal potential of the crude hexane, benzene, chloroform, ethyl acetate and methanol solvent extracts from the *E. indica* against the *An. Stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. All extracts showed moderate larvicidal effects; however the highest larval mortality was found in methanol extract with the LC_{50} and LC_{90} values of 69.43, 75.13 and 91.41, 125.49 and 134.31, 167.14 ppm, respectively. Now-a-days, mosquito control is mostly directed against larvae and only against adults when necessary. This is because the fight against adults is temporary, unsatisfactory and polluting the environment, while larval treatment in more localized in time and space resulting in less dangerous outcomes. Larval control can be an effective control tool due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are manmade and can be easily identified [35].

Previous study reports showed that ethanolic fruit endocarps extracts of *M. azedarach* and *A. indica*, (Meliaceae), were found to have lethal effects on *Ae. Aegypti* larvae, with LC_{50} values ranging from 0.017 to 0.034 g% [36]. Tennyson [37] evaluated the methanolic crude leaf extracts of *P. betle* against *Ae. aegypti* larvae had LC_{50} values for 24h were 236.73ppm respectively. Chloroform leaf extract of *P. fimbriulatum* showed LC_{100} values<30 µg/ml against *Ae. Aegypti* larvae by Calderson [38]. Pohlit et al [29] reported that *P. tuberculatum* crude leaf extracts were active against *Ae. Aegypti* larvae .

3.2. Identification of chemical components of *P. longum* ethanolic leaf extract by GC-MS

The chemical components of ethanolic leaf extract of *P. longum* was analysed by GC-MS. The twenty-four chemical components are identified and listed in table 3 and fig. 2.

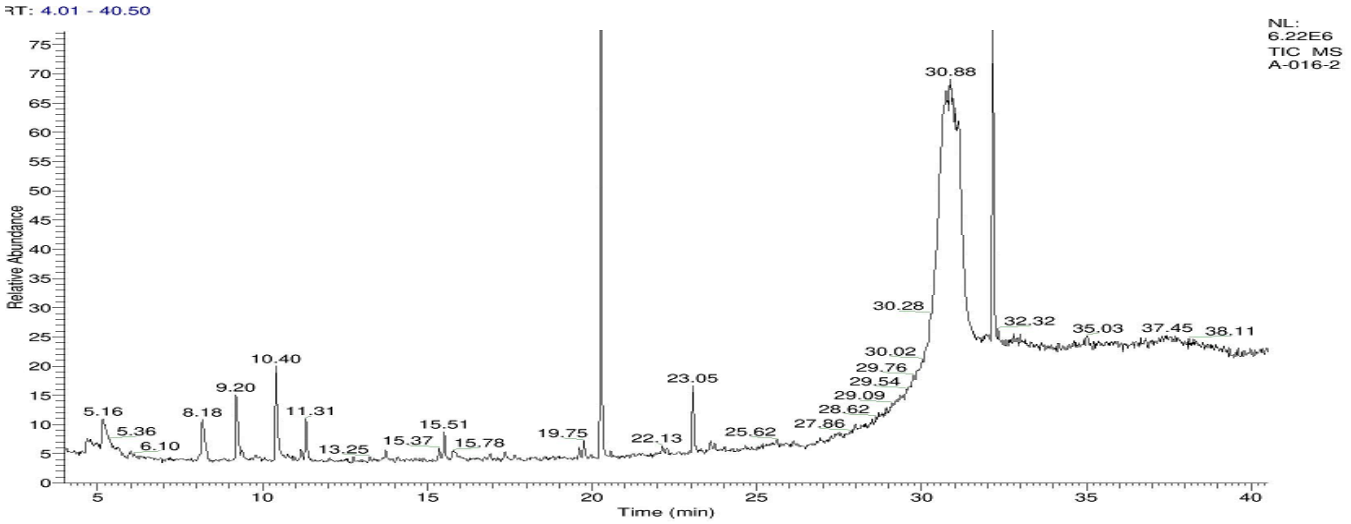

Fig. 2: GC-MS chromatogram of ethanolic leaf extract of *P.longum*

Table 3: Compounds detected in *P. longum*ethanolic leaf extract by GC-MS analysis

RT	Compound Name	MF	MW
5.18	Benzene, 1,3,5-trimethyl- (CAS)	C9H12	120
5.63	Pentadecylbenzene	C21H36	288
8.20	Dodecane (CAS)	C12H26	170
9.22	3,5-methyladamantan -1-ylamine	C12H21N	179
10.42	Benzene,1-(4-methyl-4-pentenyl)-4-(trifluoromethyl)-	C13H15F3	228
11.31	Docosane (CAS)	C22H46	310
13.74	3,4-Dihydro-2H-1,5-(3"-t-butyl) benzodioxepine	C13H18O2	206
15.51	Heneicosane (CAS)	C21H44	296
15.82	Decanoic acid, 2,3-dihydroxy propyl ester (CAS)	C13H26O4	246
18.59	n-Hexadecanoic acid	C16H32O2	256
19.75	Octadecane (CAS)	C18H38	254
20.26	Isopropyl myristate	C17H34O2	270
21.55	Octadecanoic acid	C18H36O2	284
22.13	Neronine,4a,5-dihydro (CAS)	C18H21NO6	347
23.07	Dibutyl phthalate	C16H22O4	278
25.60	2-Acetyl-3-(2-cinnamido) ethyl-7-methoxyindole	C22H22N2O3	362
27.98	Dotriacontane (CAS)	C32H66	450
30.88	5,11,17,23-Tetra-t-butyl-25,26,27,28-tetra hydroxycalix -4-arene	C44H56O4	648
32.16	1,2-Benzene dicarboxylic acid, mono (2-ethylhexyl) ester	C16H22O4	278
32.82	Nonacosane (CAS)	C29H60	408
35.03	Dotriacontane (CAS)	C32H66	450
36.77	Triacontane,1-bromo	C30H61Br	500
37.52	Heptacosane (CAS)	C27H56	380
39.17	Estra1,3,5(10)- Trien17-one,3,16 -bis (acetylory)-2 -methoxy-, (16a) (CAS)	C23H28O6	400

Isopropyl myristate act as antioxidant and antibacterial activity [39], and it is also used in mosquito repellent cream [40]. N-hexadecanoic acid as antimicrobial, nematicidal and mosquito larvicidal properties [41-43]. Dodecane as antiseptic and anesthetic [44]. Heneicosane is an insect pheromone and it is effective against *Ae. aegypti* mosquitoes [45]. Docosane has anti-inflammatory, antimicrobial and analgesic activities [46].

Previous studies determined the chemical profile of aqueous extracts of *P. longum* leaves using GC-MS [22]. Dhas et al [47] reported the presence of thirteen phytocompounds in chloroform leaf extracts of *P. longum*.

Synthetic chemicals have been indiscriminately used against vector mosquitoes and over the last few decades, mosquito control measures were mainly dependent on synthetic chemicals. In particular, larval and adult stages are being targeted and different groups of chemical insecticides such as organochlorines, organophosphates and carbamates are being used. The long term and continuous use of chemical insecticides resulted in many side effects including environmental pollution.

There is an urgent need for alternate mosquitocides to minimize the deleterious effects caused by chemical insecticides. Many scientists have evaluated plant extracts and plant compounds against mosquito life stages. Literature survey shows that plant compounds could be used for the control of mosquito vectors without any side effects to the environment and human health [48, 49].

4. CONCLUSION

In conclusion, the present study reports has shown that the ethanolic leaf extract of *P. longum* may have an efficacy in control the larvae of *Ae.aegypti*. The results could be useful in search eco-friendly and effective natural compounds as larvicide.

5. ACKNOWLEDGEMENTS

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Conflict of interest

None declared

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