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INSECTICIDAL EFFECTS OF ETHANOLIC LEAF EXTRACT OF *MILLINGTONIA HORTENSIS* L.F. (BIGNONIACEAE) AGAINST *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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ABSTRACT

Mosquitoes are the most important group of insects in terms of public health importance, which transmit serious diseases. Vector control is a major challenge now a-days when they became resistance against commonly available insecticides and an adverse effect on non-target organisms and environment. Plants are potential source of bioactive agents which can be used in vector control and phytochemicals may serve as appropriate alternative to synthetic insecticides. The present work evaluated the mosquito larvicidal, pupicidal potential of ethanol leaf extract of *Millingtonia hortensis* against the larval (I-IV) and pupal stages of *Aedes aegypti*. The larvicidal and pupicidal assay test are carried out the instructions of WHO guidelines. The data were subjected to Finney's method of probit analysis. The $LC_{50,90}$ /24,48 hours values of ethanol leaf extract of *M. hortensis* to I instar larvae was 0.032 0.038; 0.021 0.031 % and this was found to gradually increase with the age of larvae. Pupae showed the highest resistance to the ethanol leaf extract of *M. hortensis* as evident from the relatively higher $LC_{50,90}$ /24,48 hours values 0.068, 0.071; 0059, 0.069 %. Therefore, ethanolic leaf extract of *M. hortensis* can be used as alternative to synthetic larvicides and it may become an important tool in mosquito control programs.

Keywords: *Aedes aegypti,* Ethanolic leaf extract of *M. hortensis,* Qualitative Phytochemical, GC/MS, Larvicidal, Pupicidal effects.

1. INTRODUCTION

Mosquitoes are the most important single group of insects in terms of public health importance, which are responsible for spreading a number of diseases such as dengue, zika, yellow fever, chikungunya, filariasis, malaria etc [1], that have affected people around the world especially those inhabiting tropical countries [2]. The worldwide distribution of dengue epidemics includes over 100 countries and upto 50-100 million infections are now estimated to occur annually [3]. The container breeding *Ae. aegypti* grow well in urban and domestic environments where it passes on the dengue virus to humans [4]. Dengue fever has developed an important public health problem as the number of recounting cases continue to increase, especially with more severe of the disease, dengue shock syndrome or with abnormal manifestations such as central nervous system involvement [5].According to the NVBDCP (National Vector Borne Disease Control Programme) number of dengue and chikungunya cases 2022 in India was 1,10, 473 and 86 deaths and in Tamil Nadu was 4771 and 4 deaths; in India was 5320 and no deaths and in Tamil Nadu was 149 and no deaths (till 31st October) respectively. Drug prophylaxis and vector control are the only options available in case of malaria but in case of dengue there is no medicine available, hence prevention by vector control strategies remains the only cure [6]. Owing to the harmful effect of the disease transmitted by mosquitoes, the control of these diseases is essential for public health which depends on the controlling methods of their larval stages through spraying larvicides because it is easy to handle their larval form of mosquitoes than the adult form [7].

The approach to combat these diseases largely relies on interruption of the disease transmission cycle by either destruction of the aquatic stages by killing adult mosquitoes using chemical insecticides. The drastic effects of chemical insecticide based intervention measures for the control of disease vector have received wide public apprehension and have caused many

problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans and other non-target organisms. Approximately 3,55,000 deaths per year are associated with pesticide poisoning globally [8]. There is currently a great deal of interest in alternative methods and selective principles for the control of mosquitoes with less environmental damage [9]. In this sense, substances extracted from plants present a great perspective for the control of *Ae. aegypti*. Plants contain a wide range of potential larvicidal phytochemicals that are target specific, rapidly biodegrablable, less toxic to human health [10]. Some of the plant leaves extracts are tested for their diverse insecticidal properties on the medically important mosquitoes: ethanolic leaf extract of *Cymbopogon citratus, Ricinus communis*, *Allium sativum*[11]; crude extract of *Phyllanthus acidus* [12]. As far as our literature survey concern there was no information available on the larvicidal and pupicidal effects of ethanol leaf extract of *M. hortensis* against *Ae.aegypti*.

Millingtonia hortensis L.f is an important medicinal plant in Southern Asia ranging from India, Burma, Thailand and South China. It is commonly known as Cork tree, Tree Jasmine and Maramalli in Tamil and the sole species in the genus *Millingtonia*. The tree grows to height of between 18 to 25 metres and has a spread of 7 to 11 meters. The leaf is imparipinnate and resembles that of the neem. The white flowers come as large panicles which emit a pleasant fragrance. It is an ornamental tree with pleasant flowers, which make suitable as a garden tree. The leaves are used a substitute for tobacco in cigarettes [13].The leaves of *M. hortensis* are used as antipyretic [13], antiasthmatic [14], tonic in folklore medicine, antibacterial [15], larvicidal [16].

The aim of the present study is to perform qualitative phytochemical analysis of ethanol leaf extract of *M.hortensis,* GC-MS analysis of ethanol leaf extract of *M.hortensis* and to estimate the toxicity ($LC_{50, 90}$ /24,48 hours) of ethanol leaf extract of *M. hortensis Ae.aegypti*.

2. MATERIAL AND METHODS

The eggs of *Ae. aegypti* were collected from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore (Dt), Tamil Nadu, India. They were hatched, reared and have been still maintained for many generations in the laboratory. The larvae were reared in glass beakers (27±2°C, relative humidity at 70-80%) and 12:12h light and dark cycles provided with commercial fish food *ad libitum* until their metamorphosis to pupal stage [17]. The pupae were collected from culture trays and were transferred to glass beakers. The pupae containing glass beaker were kept inside mosquito cage for adult emergence. The adult female *Ae. aegypti* were fed by human arm [18, 19]. Both females and males were provided with 10% glucose solution on cotton wicks [20]. A plastic cups (200 ml)(ovitraps) lined with filter paper containing water was kept in the cage to collect the eggs.

2.1. Collection and preparation of plant extract

M. hortensis leaves were collected from Government Arts College campus, Coimbatore, Tamil Nadu, Southern India. The identification of the plant was authentified at BSI Coimbatore (NO: BSI/SRC/5/ 23/2015/Tech/2168). The leaves washed with distilled water and then they kept for drying under shade at room temperature (27± 2°C) for about 2 weeks till they dried completely. The dried leaves were finely powdered using electric grinder. Powdered plant material (100g) were soaked in ethanol (1000 ml) in airtight wide mouth bottle and kept separately for 4 days with periodic shaking. After that, the extracts were filtered using Whatman No.1filter paper and kept in Petri dishes for drying at room temperature [21]. Dried extract where then used for the preparation of stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae.

2.2. Qualitative phytochemical analysis of ethanol leaf extract of *M. hortensis*

Qualitative phytochemical analyses of the plant extract were carried out using the standard protocol [22, 23].

2.3. Gas Chromatography- Mass Spectrometry (GC-MS) analysis of ethanol leaf extract of *M.hortensis*

The GC-MS analysis was conducted at SITRA, Coimbatore, Tamil Nadu. The GC-MS analysis was conducted at South Indian Textile Research Association, Coimbatore. 1 µl of plant extract was injected into a Thermo GC-Trace ultra Ver: 5.0, Thermo MS DSQ 11.The chromatography was performed by using the DB 35- MS capillary standard non- polar column. Helium flow was 1ml/min. The oven temperature was increased at 70°C /min to 250°C.

2.4. Larvicidal, pupicidal assay test

Bioassay test are carried out for testing the efficacy of

ethanol leaf extract of *M. hortensis* on *Ae. aegypti* at different stages of development *viz.* I, II, III and IV instars and pupae. Instructions of World Health Organization guidelines [24] for laboratory testing of mosquito larvicides were carefully followed. Different concentrations of the test compound were prepared using unchlorinated filtered water. Clean plastic cups of 500 ml capacity were used as test containers. Batches of 20 larvae were exposed to 200 ml of particular concentration of test solution. The larvae of either I, II, III, IV instar stages and pupae were collected with an eye dropper placed onto filter paper strips and immediately transferred to test cup containing test solution [25]. Five or more concentrations of a test compound giving between 0 and 100% mortality for larvae at different instar stages were tested. The larval food *ad libitum* was added to each test cup. The test containers were held at 25-28°C and preferably a photoperiod of 12 hours light followed by 12h dark. Distilled water (200ml) (positive control) and ethanol (1.0 ml) (negative control) dissolved in distilled water (199 ml) maintained separately and run simultaneously. Three replicates were done at each concentration. Mortality rates of larvae were recorded after 24 and 48 hours exposure. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region whilst moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed [24]. The values of $LC_{50,90}$/24, 48 hours and their 95% confidence limit of upper confidence limit (UCL) and lower confidence limit (LCL), regression and chi- square values were calculated using probit analysis [26]. The SPSS 17.0 (Statistical Package of Social Sciences) was used for statistical analysis.

3. RESULTS

3.1. Qualitative phytochemical analysis of ethanol leaf extract of *M. hortensis*.

Qualitative phytochemical analysis revealed the presence of different phytochemicals such as carbohydrates, flavonoids, quinones, terpenoids, phenols, coumarins and phlobatannins (Table1).

3.2. Gas Chromatography- Mass Spectrometry (GC-MS) analysis of ethanol leaf extract of *M. hortensis*

Important compounds identified in the GC- MS analysis of ethanol leaf extract were 1-Pentanol,2-methyl; Saline, methyldimethoxyethoxy-; Benzenesulfonohydrazide, N2-(2-ethoxybenzylideno)-4-methyl; Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl; Cyclotrisiloxane, hexamethyl (Table 2)

Table 1: Qualitative phytochemical analysis of *M.hortensis* ethanol leaf extract

Phytochemical Compounds	Results
Carbohydrates	+
Tannins	-
Saponins	-
Flavonoids	+
Alkaloids	-
Quinones	+
Glycosides	-
Cardiac Glycosides	-
Terpenoids	+
Triterpenoids	-
Phenols	+
Coumarins	+
Steroids	-
Phytosteroids	-
Phlobatannins	+
Anthraquinones	-

(+)=Present, (-) =Absent

Table 2: Important compounds identified in the GC-MS analysis of ethanol leaf extract of *M. hortensis*

Retention Time	Compound Name	Chemical Formula	Component Area	Match Factor
4.6896	1-Pentanol, 2-methyl-	$C_6H_{14}O$	1674103.3	70.3
5.3254	Silane, methyl dimethoxy ethoxy-	$C_5H_{14}O_3Si$	2975834.0	78.5
5.3314	Benzene sulfonohydrazide, N2-(2- ethoxy benzylideno)-4-methyl	$C_{16}H_{18}N_2O_3S$	2314460.0	77.1
7.0377	Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl-	$C_8H_{22}O_3Si_2$	3360420.1	78.7
10.5455	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	1203753.8	69.4
18.1696	2-Propenamide, N,N-bis(1-methylethyl)-	$C_9H_{17}NO$	1622289.5	61.5
18.2016	2-Piperidinemethanamine	$C_6H_{14}N_2$	1200076.8	61.3
18.8097	1-Octene, 6-methyl-	C_9H_{18}	1379959.4	73.2
18.9992	4-Piperidinone, 2,2,6,6-tetramethyl-	$C_9H_{17}NO$	4986649.3	63.2
19.6458	N,4-Diethyl-4-octanamine	$C_{12}H_{27}N$	687467.9	62.9

3.3. Toxicity of ethanol leaf extract of *M. hortensis* on the developmental stages of *Ae. aegypti*

Bioassay test were conducted to find out the toxicity of ethanol leaf extract of *M. hortensis* to I, II, III, IV instars and pupae of *Ae. aegypti*. The data were subjected to Finney's method of probit analysis. The results expressed in terms of $LC_{50,90}$/ 24,48 hours. $LC_{50,90}$ /24, 48 hours values of ethanol leaf extract of *M. hortensis* to I instar larvae was 0.032 0.038; 0.021 0.031% and this was found to gradually increase with the age of larvae. Pupae showed the highest resistance to the ethanol leaf extract of *M. hortensis* as evident from the relatively higher $LC_{50,90}$/24,48 hours values 0.068, 0.071; 0.059, 0.069 % (Tables 3& 4).

Table 3: $LC_{50}/_{90}$ 24hour values of ethanol leaf extract of *M.hortensis* to the pre-adult stages (I, II,III, IV instars and pupae) of *Ae. aegypti*

Stages of development (Instars)	Number of Larvae /trials	$LC_{50/90}$ 24 hour (%)	95% Confidence interval		Regression Equation	R values	Slope	Chi-Squarevalues	Degrees of Freedom
			LCL	UCL					
I	20	0.032 (0.038)	0.028 (0.035)	0.036 (0.041)	y = 2300x - 17 (y = 2300x - 41)	0.9925 (0.9944)	112.56 (112.56)	0.215* (0.215*)	3(16.26) 3(16.26)
II	20	0.039 (0.042)	0.036 (0.037)	0.042 (0.045)	y = 2200x - 38 (y = 2200x - 59)	0.9878 (0.9837)	97.21 (96.21)	0.278* (0.287*)	3(16.26) 3(16.26)
III	20	0.047 (0.053)	0.044 (0.050)	0.051 (0.057)	y = 1059.3x (y = 942.11x)	0.6806 (0.6273)	157.13 (157.13)	0.613* (0.613*)	3(16.26) 3(16.26)
IV	20	0.059 (0.069)	0.056 (0.066)	0.062 (0.072)	y = 2150x - 78 (y = 2250x - 106.5)	0.9898 (0.9792)	214 (214)	0.731* (0.751*)	3(16.26) 3(16.26)
Pupae	20	0.068 (0.071)	0.064 (0.068)	0.072 (0.073)	y = 2250x - 105.5 (y = 2300x - 131)	0.9966 (0.9925)	189.67 (193.67)	0.813* (0.815*)	3(16.26) 3(16.26)

*$LC_{50/90}$ - lethal concentration that kills 50 % ,90% of the exposed larvae and pupae, LCL - lower confidence limit, UCL - upper confidence limit, R-value – regression value *P< 0.001 level of significance of chi-square values, Values in brackets represents LC_{90}/24hour.*

Table 4: $LC_{50}/_{90}$ 48 hour values of ethanol leaf extract of *M.hortensis* to the pre-adult stages (I, II, III, IV instars and pupae) of *Ae. aegypti*

Stages of development (Instars)	Number of Larvae/ trial	$LC_{50/90}$ 48 hour (%)	95% Confidence interval		Regression Equation	R values	Slope	Chi-Square values	Degrees of Freedom
			LCL	UCL					
I	20	0.021 (0.031)	0.018 (0.028)	0.024 (0.034)	y = 2346x .6116 (y = 2200x – 6)	0.9939 (0.9979)	112.56 (125.56)	0.215* (0.207*)	3(16.26) 3(16.26)
II	20	0.027 (0.039)	0.024 (0.036)	0.030 (0.042)	y = 2200x – 15 (y = 2150x – 3)	0.9837 (0.9773)	96.21 (102.21)	0.295* (0.293*)	3(16.26) 3(16.26)
III	20	0.037 (0.047)	0.033 (0.044)	0.041 (0.050)	y = 1338.9x (y = 1070.4x)	0.8143 (0.7109)	163.13 (147.13)	0.612* (0.613*)	3(16.26) 3(16.26)
IV	20	0.049 (0.059)	0.046 (0.056)	0.052 (0.062)	y = 2150x - 6.5 (y = 2300x – 5)	0.9898 (0.9925)	214 (212)	0.751* (0.745*)	3(16.26) 3(16.26)
Pupae	20	0.059 (0.069)	0.055 (0.065)	0.062 (0.073)	y = 2300x – 86 (y = 2300x –10)	0.9925 (0.9944)	193.67 (195.67)	0.815* (0.815*)	3(16.26) 3(16.26)

*$LC_{50/90}$ - lethal concentration that kills 50 % ,90% of the exposed larvae and pupae, LCL - lower confidence limit, UCL - upper confidence limit, R-value – regression value *P< 0.001 level of significance of chi-square values, Values in brackets represents LC_{90}/48hour.*

4. DISCUSSION

Resistance of vector mosquitoes to conventional chemical insecticides paves the way towards the development of new insecticides. The most reported insecticidal group of compounds from plants with larvicidal activity against various species of mosquitoes are steroids, flavonoids, phenols, tannins, terpenoids, carbohydrates and saponins [27]. The secondary metabolites are known to be effective against a wide range of insect pests as well as mosquitoes vectors [28]. This may be due to a variety of phytochemicals in plants working synergistically to produce such responses.

The result of the present study is in agreement with the earlier findings on the larvicidal and pupicidal effect of different plant origin. The maximum larval mortality was detected in ethanol leaf extract of *Scutellaria violacea*

against 4th instar larvae of *An. stephensi* and *Cx. quinquefasciatus* (LC_{50}/24 h 47.6 and LC_{95}/24 h 225.3) and *Cx. quinquefasciatus* (LC_{50} / 24 h 51.8 and LC_{95}/24 h 218.4) [29]; at 24 hrs LC_{50} and LC_{90} values of the methanolic leaf extract of *Lansium domesticum* were 0.22 % and 0.32 %, whereas at 48 hrs LC_{50} and LC_{90} values were 0.7 % and 1.2 % against 3rd instar larvae of *Ae. aegypti* [30]; the mosquito larvicidal activity of *Hyptis suaveolens* water leaf extract purified fraction was tested against 4th instar larvae of *Ae. aegypti* and *An. stephensi,* water extract of *Hyptis suaveolens* then applied against larvae of both the species, it was noticed that the maximum efficacy was observed with 83.33% mortality of the extract reported in triplicates at 100% concentration [31]; at the end of 24 h and 48 h exposure of *An. stephensi* larvae mortality rate increased in ascending order of concentrations. The 1000 mg/L concentrations methanol leaf extract of *Hyptis suaveolens* recorded the highest mortality rate of *An. Stephensi* larvae of 100% [32]; the highest larvicidal activity was observed against 4th instar larvae *Cx. quinquefasciatus* in crude leaf extract of *Olax scandens* with corresponding LC_{50} and LC_{90} values of 0.354%, 0.572% respectively after 72h of exposure. While mortality rates of *Ae. Albopictus* in crude leaf extract of *Olax scandes* is much higher than *Cx. quinquefasciatus* with corresponding LC_{50} and LC_{90} values of 0.496%, 0.879% respectively[33]; crude aqueous leaf extract of *Momordica foetida* showed strong larvicidal activity against 4th instar larvae of *An. stephensi* having LC_{50}/24hr value of 34.61ppm and LC_{90}/24hr value of 57.61ppm followed by *Zehneric scabra* (LC_{50}/24hr=35.85 ppm, LC_{90}/24hr =68.26ppm) and *Calpurnia aurea* (LC_{50}/24hr = 38.69ppm; LC_{90}/24hr = 108.28ppm[34]; the n-hexane extract of *Murraya paniculata* leaves was toxic to the first instar larvae of *Ae. aegypti* at all concentrations, toxicity increased with increasing concentrations (LC_{50}/24hr = 92.848ppm, LC_{90}/24hr= 792.310ppm) respectively [35].

The effectiveness of this plant could be attributed to the presence of phytochemical compounds that act as insecticides [36]. The phytochemical compounds observed in the present study, were previously reported to have mosquito larvicidal activity [37, 38]. These compounds may jointly (or) independently contribute to larvicidal and pupicidal activity against *Ae. aegypti*. The phytochemicals interfered with functioning of mitochondria [39] and primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae [40, 41]. Flavonoids are compounds which are also toxic to insects. It works as a strong inhibitor of respiration or as a respiratory toxin. Flavonoids have a way of working that is by entering into the body of the larvae through the respiratory system which will then cause wilting on the nerves as well as damage to the respiratory system and cause the larvae cannot breathe and eventually die. The position of the larval body that changes from normal can also be caused by flavonoid compounds due to its way through the siphon causing damage so that the larvae must be by its position on the surface of the water to facilitate the taking of oxygen [42].

5. CONCLUSION

The investigation established the potential of ethanol leaf extract of *M.hortensis* against the larval and pupal stage of *Ae.aegypti* and results clearly reveals that ethanol leaf extract of *M.hortensis* could serve as a potential larvicidal, pupicidal against *Ae.aegypti*. The plant is tested in this study *M. hortensis* is easily available, cheap and the result of its bioassay is encouraging for future research. It can be used as an eco-friendly, alternative agent in place of synthetic insecticides. Since, this present investigation was undertaken under laboratory conditions, field application of this plant for mosquito vector control should be tested and further investigation should also be done on the effect of the extract on non-target organisms.

Conflict of interest

None declared

6. REFERENCES

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