

Larvicidal activities of *Merremia dissecta* (Jacquin) Hallier f. and *Peltroforum pterocarpum* (DC.) Backer ex k. Heyne leaves against *Aedes aegypti* (Diptera: Culicidae)

ABSTRACT

Aim: *Aedes aegypti* is the principal vector of dengue, chikungunya, zika. The burden of these diseases is typically highest in tropical and subtropical areas, affecting the most impoverished populations. This laboratory study accessed the larvicidal and pupicidal activities of ethanol, methanol, acetone leaf extracts of *Merremia dissecta* and *Peltroforum pterocarpum* against the *Ae. aegypti*.

Study Design: Conventional bioassay.

Place and Duration of Study: Department of Zoology, Government Arts College, Coimbatore – 641018, Tamil Nadu, India, between July 2021 July 2022.

Methodology: Larvae were exposed to various concentrations of plant leaf extracts for 24 hrs. at the laboratory. The probit analyses were used to analyze the data.

Results: Results revealed that the plant extracts recorded the highest larval mortality at 0.421 % and 0.634% ethanol, acetone leaf extracts of *M. dissecta*; 0.713%. and 0.621% methanol and ethanol leaf extract of *P. pterocarpum* against fourth instar of *Ae. aegypti* after 24 hrs.

Conclusion: The results suggest that this plant extracts can be used as an good larvicidal and pupicide against the dengue and zika virus vector *Ae. aegypti*.

Keywords: *Merremia dissecta*, *Peltroforum pterocarpum*, *Aedes aegypti*, larvicidal, pupicidal.

1.INTRODUCTION

Vector and vector-borne diseases have become a challenging problem to public health in these days as it has social and economical impact. Insect transmitted diseases remain major cause of illness and death worldwide [1]. Mosquitoes are vectors of several diseases affecting humans and domestic animals around the world and are the major vectors for the transmission of malaria, dengue, yellow fever, filariasis, Japanese encephalitis, zika fever causing millions of deaths every year [2]. *Aedes aegypti* is the principal vector of dengue,

chikungunya, zika. In India, mosquito is frequently found due to poor drainage system especially during rainy sessions [3]. One estimated prediction indicates that 3.9 billion dengue virus infections occurred on an annual basis and from that 0.096 billion are clinically expressed with disease severity [4]. The burden of these diseases is typically highest in tropical and subtropical areas, affecting the most impoverished populations [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.

According to [6], to reduce the incidence of different mosquito borne diseases, regulation of mosquito population is very important. The approach for control of mosquito-borne diseases predominantly relies on the interception of disease transmission cycle by preventing mosquitoes from biting humans, Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults [7]. Many synthetic pesticides including pyrethroids, carbamates and organophosphates are available in the market for mosquito control and but most of them are pollutants and harmful to non-target animals [8]. Moreover, their repeated use has developed resistance in target animals [9].

This also fostered environmental and human health concern that initiates a search for alternative control measures. Plant based pesticides are popular due to their low economic cost, easy availability and eco-friendly nature. In fact, chemicals identified in the plant extracts have been reported for their activity against various medically important insects [10]. In addition many plant-based extracts/compounds induce changes in morphology, physiology, biochemical processes and behaviour of different stages of mosquitoes suggesting their importance in controlling mosquito population [11]. Some of the plant leaves extracts are tested for their diverse insecticidal properties on the medically important mosquitoes: acetone extract of *Lisea floribunda* [12]; acetone, chloroform, ethanol extracts of *Sphaeranthus indicus* and *Caesalpinia pulcherrima* [13]; crude extract of *Phyllanthus acidus* [14].

Merremia dissecta (Jacquin) Hallier f. (Morning glory) is belongs into family Convolvulaceae and common names are Alamo vine, bindweed, cut-leaf morning glory, noon flower, noyau vine, snake vine. *M.dissecta* grows as a perennial herbaceous vine covered with sparse pubescence. The leaves are alternately arranges with 10 cm in length. *M.dissecta*

is used in condiments, medicines and as ornamentals [15]. It will grow in sunny or partially shady location [16]. Leaves essence extracted from *M.dissecta* leaves was used for food flavour. The leaves used as tea for relieving cold flu [17]. Leaf juice is also applied on scabies and skin diseases. Cold infusion of leaves was remedy for giddiness and is given as a treatment for chest complaints in children [18]. Crushed leaves was used for chest problems, applied against inflammation and work as emollients and sedatives at the same time [19]. Hot infusion of leaves was given to relieve urinary infections [20]. *Peltophorum pterocarpum* (DC.) Backer ex K. Heyne belongs to the family Fabaceae, is a native species of Sri Lanka, the Andamans, the Malay Peninsula and North Australia, commonly called copper pod or yellow flame tree. It is a very attractive tree with its spreading crown of many branches consisting of feathery mimosa like leaves and abundance of bright yellow blooms and gives wonderful sight when the copper-red seedpods cover the tree in profusion. Thus the tree is having high ornamental value and planted as avenue trees. It is a deciduous tree growing to 15–25 m (rarely up to 50 m) tall, with a trunk diameter of up to 1 m. The leaves are bipinnate, 30-60 cm long, with 16-20 pinnae, each pinna with 20-40 oval leaflets 8-25mm long and 4-10 mm broad. [16]. Moreover, the leaves of the trees are used to feed the goats and the dead branches are collected by village people to use as fire wood. In terms of biodiversity it serves as a good nectar source for Hymenopteran insects including honey bees, bumble bees and several economically important wasps [21,22]. Apart from these it is also having potent medicinal value. Traditionally the bark of the tree is used to treat wounds among the Paliyar tribe [23] and dentifrice among the indigenous inhabitants of Tamil Nadu for oral healthcare practices [24]. Orang Asli tribe of Kampung Bawong, Malaysia is using the powdered bark of this plant to treat psoriasis [25]. Studies revealed that the plant bark and leaves has antimicrobial [26]; [27], antioxidant [28]; antifungal [29]; apoptotic [30]; haematological [31];

The aim of the present study is therefore to find out:

- Qualitative phytochemical analysis of ethanol, acetone leaf extracts of *M.dissecta* and methanol, ethanol leaf extracts of *P. pterocarpum*.
- GC-MS analysis of ethanol and acetone leaf extracts of *M.dissecta* and methanol, ethanol leaf extracts of *P. pterocarpum*.
- Estimate the toxicity (LC₅₀/24 hrs) of ethanol and acetone leaf extracts of *M.dissecta* and methanol, ethanol leaf extracts of *P. pterocarpum*.

2. METHODOLOGY

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 plastic cups ($27\pm 2^{\circ}\text{C}$, relative humidity at 70-80%) and provided with commercial fish food *ad libitum* [32]. 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 [33, 34]. Both females and males were provided with 10% glucose solution on cotton wicks [35]. A plastic cups (200 ml) (ovitrap) lined with filter paper containing water was kept in the cage.

2.1. Collection and preparation of plant extracts

M. dissecta and *P. pterocarpum* leaves were collected from Government Arts College campus, Coimbatore, Tamil Nadu, Southern India. The identification of the plants were authenticated at BSI Coimbatore (NO:BSI/SRC/5/23/2018/Tech/571 and NO:BSI/SRC/5/23/2021/Tech/09). The leaves washed with distilled water and then they kept for drying under shade at room temperature ($27\pm 2^{\circ}\text{C}$) for about 2 weeks till they dried completely. The dried leaves were finely powdered using electric grinder. Powdered plant materials (100g) was soaked in ethanol, acetone, methanol (1000 ml) in airtight wide mouth bottle and kept separately for 4 days with periodic shaking. After that, the extracts was filtered using Whatman No.1 filter paper and kept in Petri dishes for drying at room temperature [36]. Dried extracts were 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, acetone leaf extracts of *M. dissecta* and methanol, ethanol leaf extracts of *P. pterocarpum*

Qualitative phytochemical analyses of the plant extracts was carried out using the standard protocol [37, 38].

2.3. Gas Chromatography- Mass Spectrometry (GC-MS) analysis of ethanol, acetone leaf extracts of *M. dissecta* and methanol, ethanol leaf extracts of *P. pterocarpum*

The GC-MS analysis was conducted at SITRA, Coimbatore, Tamil Nadu. The GC-MS analysis was conducted at South Indian Textile Research Association, Coimbatore. $1\ \mu\text{l}$ of plant extracts 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 tests are carried out for testing the efficacy of ethanol, acetone leaf extracts of *M. dissecta* and methanol, ethanol leaf extracts of *P. pterocarpum* on *Ae. aegypti* at different stages of development viz I, II, III, and IV instars and pupae. Instructions of World Health Organization guidelines [39] for laboratory testing of mosquito larvicides were carefully followed. Different concentrations of the test compound were prepared using unchlorinated filtered tap 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 stage and pupae were collected with an eye dropper placed onto filter paper strips and immediately transferred to test cup containing test solution [40]. 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* added to each test cup. The test containers are held at 25-28°C and preferably a photoperiod of 12 hr light followed by 12h dark. Distilled water (200ml) (positive control) and ethanol, acetone and methanol (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 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 raising to the surface or not showing the characteristic diving reaction when the water is disturbed (WHO, 2005). The values of $LC_{50}/24hrs$ 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 [41]. The SPSS 17.0 (Statistical Package of Social Sciences) used for statistical analysis.

3. RESULTS

3.1 Qualitative phytochemical analysis of ethanol, acetone leaf extracts of *M. dissecta* and methanol, ethanol leaf extracts of *P. pterocarpum*.

Qualitative phytochemical analysis revealed the presence of different phytochemicals such as carbohydrates, saponins, flavonoids, quinones, cardiac glycosides, terpenoids, triterpenoids and coumarins (ethanol); carbohydrate, tannins, saponins, flavonoids, quinones, cardiac glycosides, terpenoids, phenol, coumarins, phytosteroids (acetone); carbohydrate, cardiac glycosides, terpenoids, steroides (methanol); cardiac glycosides, terpenoids, steroids (ethanol) (Tables 1 & 2).

3.2 Gas Chromatography- Mass Spectrometry (GC-MS) analysis of ethanol, acetone leaf extracts of *M. dissecta* and methanol, ethanol leaf extracts of *P. pterocarpum*

Important compounds identified in the GC- MS analysis of plant leaf extracts. Betulin, Octadecanoic acid, ethyl ester (CAS), 1-Octadecene (CAS) 1-Hexdecanol, acetate (CAS), 2-Hexadecanol (CAS) (ethanol); Benzene,1,2,4,-trimethyl-(CAS), Benzene,1,3,5-trimethyl-(CAS), 2-Nonen-4-one,2-methyl-Furan,2-methoxy-(CAS), 1,5,5-Trimethyl-6-methylene-cyclohexane, 2,4-Heptadian-4-one-,2,6-dimethyl-(CAS) (acetone); Methane, nitro-Urea, 1-Butanol, 3-methyl-Pyridine, Pyridine (methanol); Methyl formate, Phenol,2-methoxy-, Indan,1-methyl-, Phenol,5-ethenyl-2-methoxy-, 1,1,5-Trimethyl-1,2-dihydronaphthalene (ethanol) (Tables 3,4,5 & 6).

3.3 Toxicity of ethanol, acetone leaf extract of *M.dissecta* and methanol, ethanol leaf extracts of *P. pterocarpum* on the developmental stages of *Ae.aegypti*

Bioassay test were conducted to find out the toxicity of plant leaf extracts of *M.dissecta* and *P. pterocarpum* 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} / 24 hour.

LC_{50} /24 hour values of ethanol and acetone leaf extracts of *M.dissecta* to I instar larvae was 0.115, 0.284, 0.367, 0.421% (ethanol) and 0.201, 0.311, 0.425, 0.634% (acetone) and this was found to gradually increase with the age of larvae. Pupae showed the highest resistance to the ethanol and acetone leaf extracts of *M.dissecta* as evident from the relatively higher LC_{50} / 24 hour value 0.610, 0.702% (Table -7).

LC_{50} /24 hour values of methanol and ethanol leaf extracts of *P. pterocarpum* to I instar larvae was 0.405, 0.513, 0.602, 0.713% (methanol) and 0.305, 0.426, 0.517, 0.621% (ethanol) and this was found to gradually increase with the age of larvae. Pupae showed the

highest resistance to the methanol and ethanol leaf extracts of *P. pterocarpum* as evident from the relatively higher LC₅₀/ 24 hour value 0.816, 0.806% (Table -8).

Table 1. Qualitative phytochemical analysis of *M.dissecta* ethanol and acetone leaf extracts

Phytochemical constituents	Ethanol	Acetone
Carbohydrates	+	+
Tannins	-	+
Flavonoids	+	+
Alkaloids	-	-
Quinones	+	+/-
Glycosides	-	-
Cardiac Glycosides	+	+
Terpenoides	+	+
Triterpenoides	+	-
Phenols	-	+
Coumarins	+/-	+
Steroides	+	-
Phytosteroides	-	+
Phlobatannins	-	-
Anthraquiones	-	-
Saponins	+	+

(+) : Present

(-) : Absent

Table 2. Qualitative phytochemical analysis of *P. pterocarpum* methanol and ethanol leaf extracts

Phytochemical constituents	Methanol	Ethanol
Carbohydrates	+	-
Tannins	-	-
Flavonoids	-	-
Alkaloids	-	-
Quinones	-	-
Glycosides	-	-
Cardiac Glycosides	+	+
Terpenoides	+	+
Triterpenoides	-	-
Phenols	-	-
Coumarins	-	-
Steroides	+	+
Phytosteroides	-	-
Phlobatannins	-	-
Anthraquiones	-	-
Saponins	-	-

(+) : Present

(-) : Absent

Table 3. Important compounds identified in the GC-MS analysis of ethanol leaf extract of *M.dissecta*

S.No	Retention Time	Area (%)	Compound Name	Molecular formula	Molecular weight
1.	31.51	12.48	Betulin	C ₃₀ H ₅₀ O ₂	442
2.	22.46	9.54	Octadecanoic acid, ethyl ester (CAS)	C ₂₀ H ₄₀ O ₂	312
3.	18.51	6.52	1-Octadacene (CAS)	C ₁₈ H ₃₆	252
4.	14.33	6.47	1-Hexadecanol,acetate (CAS)	C ₁₈ H ₃₆ O ₂	284
5.	26.15	4.93	2-Hexadecanol (CAS)	C ₁₆ H ₃₄ O	242

Table 4. Important compounds identified in the GC-MS analysis of acetone leaf extract of *M.dissecta*

S.No	Retention time	Area (%)	Compound Name	Molecular Formula	Molecular Weight
1	4.43	43.64	Benzene,1,2,4,-trimethyl-(CAS)	C ₉ H ₁₂	120
2	4.96	20.23	Benzene,1,3,5-trimethyl-(CAS)	C ₉ H ₁₂	120
3	3.39	15.24	2-Nonen-4-one,2-methyl-Furan,2-methoxy-(CAS)	C ₁₀ H ₁₈ O	154
4	5.83	4.18	1,5,5-Trimethyl-6-methylene-cyclohexane	C ₁₀ H ₁₆	136
5	6.69	2.83	2,4-Heptadian-4-one-,2,6-dimethyl-(CAS)	C ₉ H ₁₄ O	138

Table 5. Important compounds identified in the GC-MS analysis of methanol leaf extract of *P.pterocarpum*

S.No	Retention time	Compound Name	Molecular Formula	Compound Area	Match Factor
1	2.8134	Methane,	CH3NO2	36735936.6	91.0
2	2.9235	nitro-Urea	CH4N2O	23329021.3	91.4
3	3.4496	1-Butanol,	C5H12O	13600414.4	90.8
4	3.5629	3-methyl-Pyridine	C5H5N	34959580.5	98.4
5	3.6004	Pyridine	C5H5N	31702515.2	95.1

Table 6. Important compounds identified in the GC-MS analysis of ethanol leaf extract of *P.pterocarpum*

S.No	Retention time	Compound Name	Molecular Formula	Compound Area	Match Factor
1	3.6988	Methyl formate	C2H4O2	31137064.3	92.6
2	10.1126	Phenol,2-methoxy-	C7H8O2	46043453.2	93.4
3	10.9945	Indan,1-methyl-	C10H12	46380942.9	93.3
4	16.5331	Phenol,5-ethenyl-2-methoxy-	C9H10O2	89417624.8	91.1
5	17.5277	1,1,5-Trimethyl-1,2-dihydronaphthalene	C13H16	114283405.5	95.1

Table 7. LC₅₀/24 hour values of ethanol and acetone leaf extracts of *M.dissecta* to the pre-adult stages (I, II, III, IV, Pupae) of *Ae. aegypti*.

Stages of development (Instars)	Number of Larva /trial	LC ₅₀ / 24 hour (%)	95% confidence interval		Regression Equation	R - Value	Slope	Chi-Square	Degrees of Freedom
			LL	UL					
I	20	0.115(e)	0.103(e)	0.127(e)	y=244.6x+9.18(e)	0.8604(e)	107.27(e)	0.163*(e)	3(16.26)
		0.201(a)	0.191(a)	0.239(a)	y=242.65x-3.3824 (a)	0.8897(a)	96.37(a)	0.251* (a)	
II	20	0.284(e)	0.269(e)	0.312(e)	y=218.46x+11.87(e)	0.9514(e)	95.50(e)	0.275*(e)	3(16.26)
		0.311(a)	0.275(a)	0.347(a)	y = 210x - 12 (a)	0.9866(a)	103.51(a)	0.386* (a)	
III	20	0.367(e)	0.338(e)	0.396(e)	y = 183.64x(e)	0.9519(e)	133.59(e)	0.492*(e)	3(16.26)
		0.425(a)	0.402(a)	0.465(a)	138.89x (a)	0.8479(a)	139.42(a)	0.495* (a)	
IV	20	0.421(e)	0.392(e)	0.459(e)	y = 205x - 33(e)	0.9842(e)	186.40(e)	0.641*(e)	3(16.26)
		0.634(a)	0.612(a)	0.667(a)	y = 215x - 78 (a)	0.9898(a)	187.16(a)	0.817* (a)	
Pupae	20	0.610(e)	0.582(e)	0.647(e)	y = 215x - 78(e)	0.9898(e)	174.83(e)	0.820*(e)	3(16.26)
		0.702(a)	0.671(a)	0.743(a)	y = 185x - 78.5 (a)	0.7329(a)	176.28(a)	0.962* (a)	

LC₅₀ – lethal concentration that kills 50% 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.

e – ethanol leaf extract

a – acetone leaf extract

Table 8. LC₅₀/24 hour values of methanol and ethanol leaf extracts of *P.pterocarpum* to the pre-adult stages (I, II, III, IV, Pupae) of *Ae. aegypti*.

Stages of development (Instars)	Number of Larva /trial	LC ₅₀ /24 hour (%)	95% confidence interval		Regression Equation	R - Value	Slope	Chi-Square	Degrees of Freedom
			LL	UL					
I	20	0.405(m)	0.363(m)	0.449(m)	y = 235x - 46(m)	0.9638(m)	102.56(m)	0.205*(m)	3(16.26)
		0.305(e)	0.272(e)	0.360(e)	y = 235x - 18.5(e)	0.9897(e)	111.23(e)	0.217* (e)	
II	20	0.513(m)	0.473(m)	0.581(m)	y = 225x - 64.5(m)	0.941(m)	98.21(m)	0.298*(m)	3(16.26)
		0.426(e)	0.329(e)	0.439(e)	y = 235x - 42 (e)	0.9866(e)	96.45(e)	0.345* (e)	
III	20	0.602(m)	0.523(m)	0.542(m)	y = 87.632x(m)	0.5986(m)	167.13(m)	0.603*(m)	3(16.26)
		0.517(e)	0.473(e)	0.548(e)	y = 109.63x (e)	0.7029(e)	147.36(e)	0.529* (e)	
IV	20	0.713(m)	0.642(m)	0.765(m)	y = 225x - 106.5(m)	0.9792(m)	204(m)	0.741*(m)	3(16.26)
		0.621(e)	0.543(e)	0.718(e)	y = 225x - 84(e)	0.9792(e)	209(e)	0.703* (e)	
Pupae	20	0.816(m)	0.751(m)	0.858(m)	y = 230x - 132(m)	0.9952(m)	191.67(m)	0.810*(m)	3(16.26)
		0.806(e)	0.783(e)	0.852(e)	y = 220x - 127(e)	0.9938(e)	181.76(e)	0.926* (e)	

LC₅₀– lethal concentration that kills 50 % 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.

m – methanol leaf extract

e – ethanol leaf extract

4. DISCUSSION

Mosquitoes are the most annoying and dangerous insects because they transmit pathogens. Synthetic pyrethroids are extremely effective against mosquito vectors and have been used to control mosquito vectors in the field. However, these insecticides had a number of unintended consequences of humans and the environment. Because of the consistent use of insecticides for mosquito control, multiple insecticide resistance has developed [42]. Many studies have found that plant extracts have the potential to control mosquito-borne diseases [43, 44] due to active ingredient synergisms, plant crude extracts may be more effective than individual bioactive compounds in managing resistant mosquito populations [45]. The findings agree with some of the previous reports. The LC₅₀ and LC₉₀ for *Leucas aspera* ethanolic leaf extract against the 4th instar larvae of *An. stephensi* after 24 h of incubation were 24.08 ppm and 168.96 ppm respectively and after 48 h of exposure 51.67 ppm and 189.46 ppm respectively [46]; ethanol leaf extract of *Dioscorea sansibarensis* was evaluated against all the four instar larval stages of *An. gambiae* and *Cx. quinquefasciatus* susceptible laboratory colonies. The highest larvicidal potency was shown against the 4th instar stages of *Cx. quinquefasciatus* and *An. gambiae* with the LC₅₀ / 24 h values 55.432 of 60.915 ppm and 80.700 ppm respectively. The respective LC₉₅ / 24 h values for *Cx. quinquefasciatus* and *An. gambiae* were 168.898 ppm and 249.295 ppm [47]; aqueous leaf extracts of *Ammi majus* was used as larvicidal agent. The least larvicidal mortality was observed the leaf extract at an exposure time of 6 hours against *Ae. aegypti* [48]; methanol solvent leaf extract of *Colocasia esculenta* and *Wrightia tinctoria* were tested against second, third, fourth instars and pupa of *Ae. aegypti*. The results indicate that the LC₅₀/24 hour values of second, third, fourth instars and pupa of *Ae. aegypti* for *Colocasia esculenta* was 101.17 ppm, 126.02 ppm, 161.60 ppm and 189.28 ppm; likewise *Wrightia tinctoria* LC₅₀/24 hour values are 126.33 ppm for II instar, 149.90 ppm for III instar, 183.97 ppm for IV instar and 228.20 ppm for pupa respectively [49]; LC₅₀/24 hour of *Malvastrum coromandelianum* methanol leaf extract against *Ae. aegypti* larvae and pupae were 378.59 ppm (I), 404.42 ppm (II), 415.35 ppm (III), 486.01 ppm (IV) and 509.59 ppm (pupa) [50]; probit analyses of mortality rates of crude extracts of *Tropaeolum majus* show LC₅₀ and LC₉₀ values as 1.19% and 7.27% in leaf extract against 3rd instar larvae of *Cx. quinquefasciatus* within 24 hours of exposure [51]; LC₅₀ and LC₉₀ values recorded upon 24hour exposure to *Vepris soyauxii* methanol-based extracts were 203.92 ppm and 241.46 ppm in third instar larva *An. gambiae* and 215.01 ppm and 270.87 ppm *An. coluzzii* respectively [52].

The effectiveness of this plant could be attributed to the presence of phytochemical compounds that act as insecticides [53]. The phytochemical compounds observed in the present study, were previously reported to have mosquito larvicidal activity [54, 55]. These compounds may jointly (or) independently contribute to larvicidal activity against *Ae. aegypti*. The phytochemicals interfered with functioning of mitochondria [56] and primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae [57, 58]. 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 [59]. Cardiac glycosides are known to inhibit the action of membrane-bound enzyme $\text{Na}^+/\text{K}^+\text{-ATPase}$ [60]. Saponin interacted with the cuticle membrane of the larvae, ultimately disarranging the membrane causing larval death [61]. In this study, darkening of the larvae possibly occurs due to the overlap of cuticle of thoracic segments.

Mosquitoes play a predominant role in transmission of several life-threatening diseases to the human population all around the world. They are the principle vector of many vector-borne diseases affecting human beings and animals, in addition to nuisance. In present study, the result clearly reveals that the ethanol, acetone leaf extracts of *M. dissecta* and methanol, ethanol leaf extracts of *P. petrocarpum* could serve as a potential larvicidal, pupicidal effects against dengue vector *Ae. aegypti*. Larvicidal and pupicidal efficacy of this plant extracts under the field conditions should be scrutinized and determined. Besides, further investigation regarding the effect on non-target organism and synergism with biocides are extremely important.

REFERENCES

1. Pavela R. Larvicidal effects of some Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). *Parasitology Research*. 2009; 105:887-92.
2. James AA. Mosquito molecular genetics: the hands that feed bite back. *Science*. 1992; 257: 37-8.
3. Center for disease control (CDC), Northern Territory Government.2014.
4. Waggoner JJ, Gresh L, Vargas MJ, Ballesteros G, Tellez Y, Soda KJ, Sahoo MK, Nunez A, Balmaseda A, Harris E, Pinsky BA. Viremia and clinical presentation in Nicaraguan patients infected with zika virus, chikungunya virus, and dengue virus. *Clinical Infectious Diseases*. 2016; 63: 1584-2159. <https://doi.org/10.1093/cid/ciw589>.
5. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*, *elife*. 2015; 4, e08347. [CrossRef]
6. Singha S, Siddharthasankar B, Goutam C. Synergistic effect of *Croton caudatus* (fruits) and *Tiliacora acuminata* (flowers) extracts against filarial vector *Culex quinquefastciatus*. *Asian Pacific Journal of Tropical Biomedicine*. 2011; 1: 159-164.
7. Corbel V, Duchon S, Zainm M, Hougand JM. Dinotefuran: A potential neonicotinoid insecticide against resistant mosquitoes. *Journal of Medical Entomology*. 2004; 41: 712-7.
8. Benelli G, Govindarajan M, Rajeswary M, Senthilmurugan S, Vijayan P, Alharbi NS, Khaled JM. Larvicidal activity of *Blumea eriantha* essential oil and its components against six mosquito species, including zika virus vectors: the promising potential of (4E,6Z)-allo-ocimene, carvotanacetone and dodecyl acetate. *Parasitology Research*. 2017; 116(4), 1175–1188. <https://doi.Org/10.1007/s00436-017-5395-0>.
9. Ignacimuthu S, David B. Ecofriendly insect pest management. Delhi: Elite Publishing House. 2009;
10. Wachira SW, Omar S, Jacob JW, Wahome M, Alborn HT, Spring DR, Torto B. Toxicity of six plant extracts and two pyridine alkaloids from *Ricinus communis* against the malaria vector *Anopheles gambiae*. *Parasites & Vectors*. 2014; 7(1): 312. <https://doi.org/10.1186/1756-3305-7-312>.

11. Lija-Escaline J, Senthil-Nathan S, Thanigaivel A, Pradeepa V, Vasantha-Srinivasan P, Ponsankar A, Abdel-Megeed A. Physiological and biochemical effects of botanical extract from *Piper nigrum* Linn (Piperaceae) against the dengue vector *Aedes aegypti* Liston (Diptera: Culicidae). *Parasitology Research*. 2015; 114(11), 4239–4249. <https://doi.org/10.1007/s00436-015-4662-1>.
12. Roja HR, Jeyabalan D. Evaluation of *Litsea floribunda* leaf extract on mosquitocidal potential against filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). *Journal of Emerging Technologies and Innovative Research*. 2022; 9(9):225-235.
13. Dhivya R. Screening of phytochemical composition, ovicidal and repellent activity of leaf extracts of *Sphaeranthus indicus* and *Caesalpinia pulcherrima* against the mosquito *Culex quinquefasciatus* (Diptera: Culicidae). *International Journal of Mosquito Research*. 2022; 9(5): 01-06. DOI:<https://doi.org/10.22271/23487941.2022.v9.i5a.624>.
14. Arpita Gope, Anjali Rawani. Evaluation of mosquitocidal potency of leaves and fruits extracts of *Phyllanthus acidus* L. against filarial vector *Culex quinquefasciatus* Say. *International Journal of Mosquito Research*. 2022; 9(4): 49-56. DOI: <https://doi.org/10.22271/23487941.2022.v9.i5a.632>.
15. Austin DF. *Merremia dissecta* (Convolvulaceae) Condiment, medicine, ornamentals and weed- a reviews. *Economic Botany*. 2007; 61:109-120.
16. Huxley A. *New RHS dictionary of gardening*. Macmillan Press, 1992; ISBN.0-333-47494-5.
17. Shahin SM, Ei-Sayed BA, Tayeb HF. Improving growth and quality of *Merremia dissecta* (Jacq) H. G. Hallier twiner by some fertilization treatments. *Alexandria Journal of Agricultural Research*. 2014; 59: 43-49.
18. Dalziel JM. *The useful plants of West Tropical Africa*. (Being an appendix to *The Flora of West Tropical Africa*). Crown agents for the colonies, London. (2nd Rep) (1st reprint, 1948; end reprint, 1955) 612. 1937.
19. Roig JT, Mesa Y. *Plantas Medicinales, Aromaticas O Venenosas De Cuba Medicinal, Aromatic, or poisonous plants of Cuba*; Cuba Ministerio De Agricultura, Cuba Ministry of Agriculture, Havana, Cuba: 1945; 872.
20. Morton JF. *Atlas of Medicinal plants of middle America Bahamas to Yucatan*. Charles C. Thomas, Springfield, Illinois, 1981; 78-80.

21. Kiruba S, Ruba Gnana Solomon S, Israel Stalin S, Jeeva S, Sam Manohar Das S. Commonly used medicinal plants of costal belts of Kanyakumari district and their role in conservation of butterfly diversity. In: Vikram Reddy R. (eds.). 2008a. Wildlife Biodiversity and Conservation. New Delhi: Daya Publishing House.
22. Kiruba S, Ruba Gnana Solomon S, Israel Stalin S, Jeeva S, Sukumaran S, Prakash JW. Diversity of larval host plants of Kanyakumari district. Journal of Basic Applied Biology. 2008b; 2: 16-30.
23. Duraipandian V, Ayyappan M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamilnadu, India. BMC Complementary and Alternative Medicine. 2006; 6:35.
24. Ganesan S. Traditional oral care medicinal plant survey of Tamilnadu. Natural Product Research. 2008; 7:166-172.
25. Samuel AJSJ, Kalusalingam A, Chellapan DK, Gopinath R, Radhamani S, Husain H. Ethanomedical survey of plants used by the Orang Asli in Kampung Bawong, Perak, West Malaysia. Journal of Ethnobiology and Ethnomedicine. 2010; 6:5.
26. Voravuthikunchai S, Lortheeranuwat A, Jeju W, Srinrak T, Phongpacicht S, Supawita T. Effective medicinal plants against ethnohaemorrhagic *Escherichia coli* O157:H7. Journal of Ethnopharmacology. 2004. 94; 49-54.
27. Ravikumar R, Rathinam KMS. Toxic effects of *Peltophorum pterocarpum* on haematological and biochemical parameters of rats. International Journal of Chemical Science. 2009a; 7: 1136-1142.
28. Ling LT, Radhakrishnan AK, Subramanian T, Cheng HM, Palanisamy DP. Assessment of antioxidant capacity and cytotoxicity of selected Malaysian plants. Molecules. 2010; 15: 2139-2151.
29. Sathish S, Mohana DC, Raghavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sps. Journal of Agricultural Science and Technology. 2007; 3: 109-119.
30. Jabbir MSM, Husain AM, Afsar TS, Kumar SS. Study on the apoptotic properties of methanolic extracts of *Peltophorum pterocarpum*, *Cassia auriculata*, *Cassia alata* and *Lamprachaenium microcephalum*. Biomedical and Pharmacology Journal. 2009; 2(2): 381-385.
31. Ravikumar R, Rathinam KMS. Antibacterial activity of hexane and acetone extracts of *Peltophorum pterocarpum*, *Calvillea racemosa* and *Bauhinia purpurea*. International Journal of Chemical Science. 2009b; 7: 1751-1756.

32. Lymio EOW, Koella JC. Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of *Anopheles gambiae*. *Entomologia Experimentalis et Applicata*. 1992; 63: 265-271,
33. Judson CL, Alternation of feeding behavior and fertility in *Aedes aegypti* by the chemosterilant apholate. *Entomologia Experimentalis et Applicata*. 1967; 10: (3-4) 387-394. DOI:10.1111/j.1570-7458.tb02458.x.
34. Briegel A. Metabolic relationship between female body size, reserves and fecundity of *Aedes aegypti*. *Journal of Insect physiology*. 1990; 36(3): 165-172. DOI: 10.1016/0022-1910(90)90118-Y
35. Villani F, White GP, Curtis CF, Miles SJ. Inheritance and activity of some estrases associated with organophosphate resistance in mosquitoes to the complex of *Culex pipiens* L. (Diptera: Culicidae). *Bulletin of Entomological Research*. 1983; 73(1):153-170. DOI: <https://doi.org/10.1017/S0007485300013882>.
36. Kongkathip N. Chemistry and extraction method of neem- Bangkok: 3rd Workshop in the using neem leaf extracts for control and eradicate insects 1994.
37. Harbone JB. *Phytochemical methods. A guide to modern technique of plant analysis*. Chapman and Hall, London, 1984; 49-188.
38. Trease GE, Evans WC. Ed. *Pharmacognosy*, 11th Edition, Brailliar Tridel and Macmillan Publishers, London. 1989.
39. WHO. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organizaton Communicable Disease Control, Prevention and Eradication. WHO Pesticide evaluation Scheme. 2005; 1-41.
40. Cilek JW, Webb JD, Knapp FW. Residual concentration and efficacy of three temphos formulations for control of larval *Aedes aegypti*. *American Journal of Mosquito Control Association*. 1991; 7: 310-312.
41. Finney DJ. *Probit Analysis*. Cambridge University, London. 1971; 68-78pp.
42. Muthusamy R, Shivakumar MS. Resistance selection and molecular mechanisms of cypermethrin resistance in red hairy caterpillar (*Amsacta albistriga* Walker). *Pesticide Biochemistry and Physiology*. 2014; 117: 54-61.
43. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research*. 2012; 135: 581-598.
44. Kamaraj C, Rahuman AA, Mahapatra A, Asokan B, Elango G. Insecticidal and larvicidal activities of medicinal plant extracts against mosquitoes. *Parasitology Research*. 2010; 107: 1337-1349.

45. Maurya P, Sharma P, Mohan L, Verma MM, Srivastava CN. Larvicidal efficacy of *Ocimum basilicum* extracts and its synergistic effect with neonicotinoid in the management of *Anopheles stephensi*. Asian Pacific Journal of Tropical Disease. 2012; 2: 110- 116.
46. Kiruthika J, Mathi EPE, Kanakalakshmi K. Chromatographic separation of bioactive compounds of *Leucas aspera* (L) and larvicidal activity of *Anopheles stephensi* larvae. Research Journal of Biotechnology. 2021; 16: 53-60.
47. Anitha P. Larvicidal potency of *Dioscorea sansibarensis* leaf extract against vector mosquitoes: *Anopheles gambiae* S.S. and *Culex quinquefasciatus*. Tanzania Journal of Science. 2021; 47: 655-663.
48. Dar AA, Jamal K. Evaluation of larvicidal effects of aqueous leaf and flower extracts of *Ammi majus* (Linn.) on mosquito larvae. Munis Entomology and Zoology. 2021; 16: 891-897.
49. Dass K, Prakash N, Mariappan P, Prabhu S, Sureshkumar J. Evaluation of larvicidal activity from selected plant extracts against *Aedes aegypti*. International Journal of Entomology and Research. 2022 b; 7: 147-153.
50. Irudayaraj, Mary Fabiola. Phytochemical analysis of *Malvastrum coromandelianum* and *Mimusops elengi*: mosquitocidal properties of their leaf extract against dengue vectors, *Aedes aegypti*. International Journal of Entomology Research. 2022; 7(3): 171-180.
51. Paramanik M, Paramanik S, Mukherjee S, Kapuri R, Dassmodak S. Larvicidal efficacy of the leaf and flower extracts of *Tropaeolum majus* L. (Tropaeolaceae) against the vector of lymphatic Filariasis. International Journal of Mosquito Research. 2022; 9: 26-29.
52. Njuabe MT. Larvicidal activity of *Momoedica foetida* (Cucurbitaceae), *Gnidia glauca* (Thymelaeaceae) and *Vepris soyauxii* (Rutaceae) extracts on *Anopheles gambiae* mosquitoes and their acute toxicity on rats. International Journal of Environmental Research and Public Health. 2022; 6:031-045.
53. Abayomi S. Historical review of traditional medicine in Africa. Ibadan: Spectrum Book Ltd. 1993; 3: 9-25.
54. Scherer R, Wanger R, Meireles MAA, Godoy HD, Durate MCT, Filho JT. Biological activity and chemical composition of hydrodistilled and supercritical

- extracts of *Xanthium strumarium* L. leaves. Journal of Essential oil Research. 2010; 22:424-429.
55. Farooq AM, Waseem B, Muzaffar R, Tirupathi J, Tharani M, Sharma M. A comparative study of phytochemical investigation of *Xanthium strumarium* medicinal plant. International Journal of Pharmaceutical Chemistry and Analysis. 2014; 4: 96-100.
56. Usta J, Kreydiyyeh S, Bakajian K, Chmaisse HN. In vitro effect of eugenol and cinnamaldehyde on membrane potential and respiratory complexes in isolated rat liver mitochondria. Food and Chemical Toxicology. 2002; 40: 935-940.
57. Rey D, Pautou MP, Meyran JC. Histopathological effects of tannic acid on the midgut epithelium of some aquatic dipteran larvae. Journal of Invertebrate Pathology. 1999; 73: 173-181.
58. David JP, Rey D, Pautou MP, Meyran JC. Differential toxicity of leaf litter to dipteran larvae of mosquito developmental sites. Journal of Invertebrate Pathology. 2000; 75: 9-18.
59. Cania EB, Setyaningrum E. Test the effectiveness of larvicidal leaf extract legundi (*Vitex trifolia*) against *Aedes aegypti* larvae. Medical Journal of Unila. 2013; 2: 52-60.
60. Horisberger JD, Lemas V, Kraehenbuhl JP, Rossier BC. Structure function relationship of Na⁺ K⁺-ATPase. Annual Review of Physiology. 1991; 53: 565-584.
61. Khanna VG, Kannabiran K. Larvicidal effect of *Hemidesmus indicus*, *Gymnema sylvestre* and *Eclipta prostrata* against *Culex quinquefasciatus* mosquito larvae. African Journal of Biotechnology. 2007; 6: 307-311.