

## Phytochemical and larvicidal investigations of *Turnera Ulmifolia*: A potential natural mosquito control agent

Jeyanthi R<sup>1</sup>, Kaleena P K<sup>1\*</sup>, Babu M<sup>2</sup>, Sudha Ravi<sup>1</sup>, Janaki A<sup>1</sup>, Velu K<sup>1</sup>

<sup>1</sup> Department of Zoology, Presidency College, Chennai, Tamil Nadu, India

<sup>2</sup> Department of Microbiology and Biotechnology, Faculty of Arts and Science, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India

### Abstract

Vector control programs are concerned about the resistance status of mosquito vectors to several kinds of insecticides used for public health. It is crucial to look for alternative compounds to enhance the current instruments in order to combat the pesticides' current resistance and persistence in the environment and vectors, respectively. Natural plant-based products with insecticidal qualities are the main focus of this study in order to control insect vectors. Extracts of *Turnera Ulmifolia* in aqueous, ethanol, methanol, hexane, and chloroform were tested against the fourth-instar larvae of three clinically significant mosquito species: *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. In the plant extracts, active phytochemicals like tannins, alkaloids, flavonoids, anthocyanin, quinines, triterpenoids, flavonoids, saponin, and steroids were detected by phytochemical screening. After a 24-hour exposure to varying concentrations of the plant extract, the percentage of larval death for each mosquito species was calculated. Due to its strong larvicidal effects, the plant's extracts could be worthy of investigation into further. GC-MS analysis was also performed for the plant extracts.

**Keywords:** *Turnera Ulmifolia*, phytochemicals, larvicidal activity, *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*

### Introduction

According to Khan (2015) <sup>[1]</sup>, mosquitoes are the most significant group of insects in terms of their impact on public health. They transmit a variety of diseases, including dengue, Japanese encephalitis, filariasis, and malaria, which kill millions of people year. Synthetic insecticides have been used repeatedly to control mosquitoes, which has upset natural biological control systems and caused mosquito populations to rebound. Additionally, it has led to the emergence of resistance, unfavorable impacts on organisms that are not the intended target, and heightened concerns about human and environmental health, which prompted the search for alternate control strategies (Dahmana and Mediannikov, 2020) <sup>[2, 13]</sup>. According to Priya *et al.*, (2024), plants are thought to be a rich source of bioactive compounds and could serve as a substitute supply of mosquito control agents.

More than seven million people die each year from vector-borne illnesses (World Health Organization, 2014) <sup>[4]</sup>, with mosquito-borne illnesses being the most dangerous because of their widespread incidence and, as a result, higher rate of disease transmission. Culicidae is a huge mosquito family that includes Toxorhynchitinae, Anophelinae, and Culicinae sub-families, with 3,300 service species over 41 genera (Senthil-Nathan, 2020) <sup>[5]</sup>. The most harmful of the 31 genera are *Aedes*, *Culex*, and *Anopheles*. Major life-threatening infections, including filariasis-transmitting agents like *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, as well as a few arboviruses, are carried by *Anopheles* species (Takken and Lindsay, 2019) <sup>[6]</sup>.

Due to their ease of control in breeding environments and poor mobility, larval mosquitoes make an appealing target for control operations (Carrasco-Escobar *et al.*, 2022). In underdeveloped nations, mosquito control measures are a crucial part of illness prevention initiatives. Lima *et al.*,

(2006) <sup>[7]</sup> have drawn attention to alternate chemicals for mosquito control due to the growing resistance of mosquitoes to pesticides.

Since mosquito control efforts had to be halted due to the discovery of DDT pesticides in the late 1930s and early 1940s and the subsequent development of organochlorine and organophosphate pesticides, the sale of biological pesticides was halted. Due to their restricted species diversity and ongoing population growth, mosquitoes pose a greater risk of infection (Ghayal *et al.*, 2010) <sup>[8]</sup>. Prior pesticides like malathion and permethrin, along with other organophosphates, were used in vector control systems until recently. The lack of novel pesticides, their high cost, concerns about environmental sustainability, their negative impact on human health, the number of unintended individuals, their persistence, the proliferation of "biological growth" in the ecosystem, and the emergence of insecticide-resistant organisms are the causes of this (Ester Innocent *et al.*, 2008) <sup>[9]</sup>.

Numerous issues with mosquito control methods arise from the development of DDT resistance in *Aedes* species. Malaria control programs manufacture mosquitocides in multiple phases (Wiseman *et al.*, 2005) <sup>[10]</sup>. The mosquito's higher concentration of harmful enzymes including monooxygenases (MFOs), glutathione-S-transferases (GST), and carboxyl-cholinesterase (CCE) allows it to evade the effects of these artificial substances. While GSTs are frequently linked to organochloride resistance, such as DDT, MFOs are frequently linked to metabolic resistance in pyrethroids, such as permethrin. Increased CCE activity is the cause of resistance to pyrethroids, organophosphates, and carbamates, including bendiocarb (Hemingway and Ranson, 2000).

In order to control mosquitoes, additional insecticides such as benzylphenyl urea and the larvicide *Bacillus thuringiensis*

israelensis (Bti) are utilized (Mazigo *et al.*, 2019) <sup>[11]</sup>. The framework of mosquito control measures is prevented by unexpected natural or man-made changes in nature that modify actual habitats. These changes have a positive impact on vector biology and, consequently, their presence and disease incidence. Investigating flora and fauna and going into the field to apply safe plant pesticides as an easy and efficient method of controlling mosquitoes are two of the best substitutes for biodiversity management (Diaz, 2016) <sup>[12]</sup>. Furthermore, pesticide-derived pesticides consist of a blend of plant compounds that interact in biological and behavioral processes, in contrast to traditional pesticides that are based on a single active ingredient. Therefore, the likelihood of insects being able to tolerate such things is extremely low. Further successful vector control depends on the identification of bio-insecticides that work as well as on their applicability and environmental adaptation. In addition to being widely used insecticides, botanicals will undoubtedly be a new weapon in the production of synthetic pesticides and could eventually be a good substitute product for diseases caused by mosquitoes (Dahmana and Mediannikov, 2020) <sup>[2, 13]</sup>.

In the management of medically significant insect pests, plant products or plant-derived compounds hold promise as replacements for synthetic insecticides due to their low cost, biodegradability, environmental safety, potential for indigenous production, ability to control vectors, and ease of use by both individuals and communities (Tyagi, 2021) <sup>[14]</sup>. Some herbal products have been used as natural insecticides in the past, including nicotine from tobacco leaves, lupinine and anabasine, alkaloids from Russian weed *Anabasis aphylla*, rotenone from *Derris elliptica*, and pyrethrums from *Chrysanthemum cinerifolium* flowers (Rawani *et al.*, 2017) <sup>[15]</sup>.

In the north and northeastern parts of Brazil, the little annual herb *T. ulmifolia* L. (Turneraceae) is found and is regarded as a weed. It thrives best on hillsides and in sandy soils. As an expectorant, an anti-inflammatory, and a treatment for rheumatism, albuminuria, leukorrhea, furunculosis, and asthma, *T. ulmifolia* L. is already well known for its therapeutic benefits (Coutinho *et al.*, 2010) <sup>[16]</sup>. It is used to treat a variety of illnesses in traditional medicine, including diabetes, high blood pressure, chronic pain, and inflammation. Aphrodisiacs and anxiolytic qualities in plants belonging to the genus *Turnera* have also been reported by several writers (Teixeira *et al.*, 2024) <sup>[17]</sup>.

The most significant biological molecules of the genus *Turnera*, which have been linked to influencing biological processes in organisms, are phenolic compounds, flavonoids, alkaloids, and tannins. The antioxidant properties of extracts derived from *Turnera* species have been shown in several investigations. The biological characteristics and impacts of the leaf extract from *Turnera* spp are still unclear, nevertheless. Therefore, the larvicidal activity of *T. ulmifolia* plant extracts against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* larvae in their fourth instar was examined in the current study.

## Materials and Methods

### Selection of plant

*T. ulmifolia* was identified by Prof. P. Jayaraman of the Plant Anatomy Research Centre (PARC/2017/3531), West Tamabaram, Chennai-45, after being collected from the natural population in and around Ranipet, Vellore District,

Tamil Nadu, India. For roughly 20 days, the entire plant was allowed to dry at room temperature in the shade.



Fig 1: *Turnera Ulmifolia*

### Preparation of plant extract

Using an electric blender, the dried plant was ground into a fine powder and sieved. Using a Soxhlet extractor, 70g of the powder was placed in a thimble and extracted over the course of ten hours using ethanol, methanol, hexane, and chloroform. 50g of plant powder was steeped in 500ml of distilled water for 48 hours in order to prepare the aqueous extract. Using a rotary flash evaporator, all of the extracts were concentrated and stored in an airtight bottle at 5 °C until they were needed again (Babu *et al.*, 2018) <sup>[18]</sup>.

### Phytochemical screening

Standard protocol was used for phytochemical screening, and table 1 lists the phytocompounds present in the solvent extracts (Nweze *et al.*, 2004; Senthilkumar and Reetha, 2009) <sup>[19, 20]</sup>.

### Separation of bioactive compounds using TLC Preparation of extract

For TLC analysis, 10 mg/ml of the extract in methanol solvent was utilized. The aluminum sheets coated with silica gel 60 F 254 were cut to 1.5 x 5.5 cm. On a silica plate, the prepared methanol extract was loaded and allowed to air dry. The ratio of ethyl acetate to methanol (3; 1.5; 0.5) was determined by standardizing the extracts in ethyl acetate with hexane and then with chloroform.

### GC-MS analysis

The following conditions were used in gas chromatography interfaced to a mass spectrometer (GC-MS) equipment to further analyze the phytocompounds separation of study: 30 m x 250 m capillary column running in electron mode at 4.20 eV; helium was utilized as the carrier gas at a steady flow rate of 1.491 ml/min and injection volume of 1.0 ml; the injector was heated to 260 °C and the ion source was heated to 240 °C. The temperature of the oven was set to 60°C. At 4.2 eV, mass spectra were recorded.

### Selection of mosquito species

The mosquito species selected for the present study were *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. *A. aegypti* (Linnaeus), the yellow fever mosquito spreads dengue fever, chikungunya, yellow fever and other diseases. *A. aegypti* is a vector for transmitting several tropical fevers only the female bites for blood, which she needs to mature her eggs. *A. stephensi* is the vector of malaria in India and the larvae

of this species is generally found in distinctly different habitat. These are nocturnal and crepuscular in nature and transmit the filarial worm causing filariasis (Babu *et al.*, 2018a) [21]. *C. quinquefasciatus* is the vector of West Nile,

which causes encephalitis or meningitis affecting the brain tissue, resulting in permanent neurological damage (Thomas *et al.*, 2023) [22].

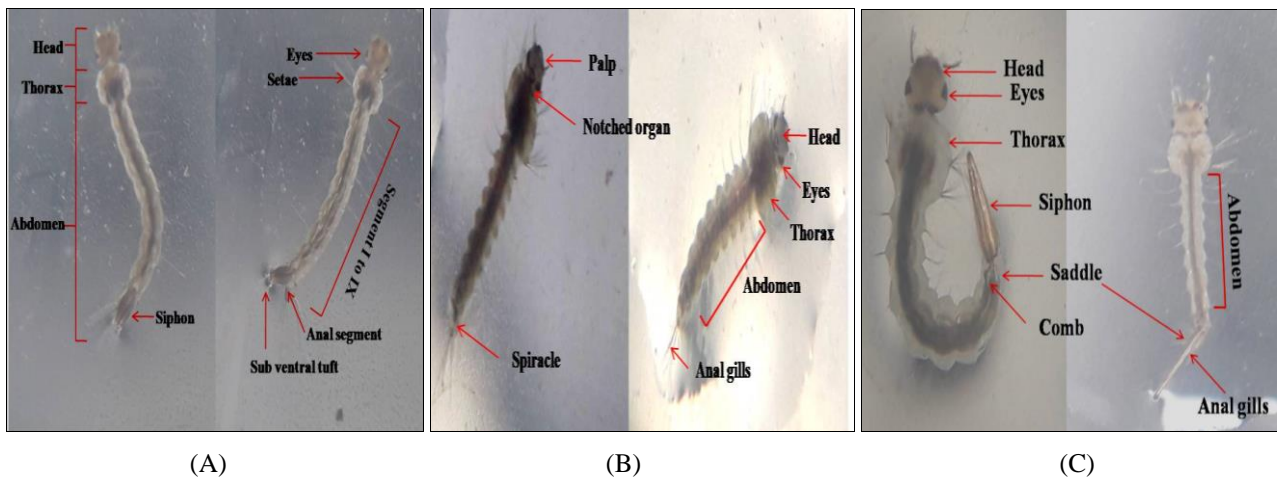


Fig 2 A, B and C: Fourth instar larvae of (A) *Aedes aegypti*, (B) *Anopheles stephensi* and (C) *Culex quinquefasciatus*

**Mosquito culture**

*A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* mosquito larvae in their fourth instar were obtained from the Entomological Research Institute (ERI) at Loyola College in Chennai. *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* raised in laboratories without exposure to pesticides or diseases were the subjects of all tests. In the insectariums, cyclic vector mosquito production was kept between 25 and 29 °C. Adult mosquitoes were fed a 10% glucose solution, while larvae were fed a larval diet consisting of a 3:1 mixture of yeast and powdered dog biscuit (Babu *et al.*, 2018) [18].

**Larvicidal Bioassay**

For the following bioassays, three trials were conducted against vector mosquitoes, each with five replicates. The larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* in their fourth instar were used in separate toxicity tests of the crude extract. 100 mg of crude extract was dissolved in 1 ml of hexane to create a stock solution (1000 ppm), which was then topped up with 100 ml of distilled water. Twenty fourth-instar larvae were released from various dilutions of 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm made in 200 ml of deionized water, and mortality was measured after 24 hours. The larvae were exposed to 200 ml of water containing 0.1 ml of hexane, which acted as a control, while the beakers were maintained at 28 °C ± 2 °C in a temperature control room. Five replications of each treatment were conducted (Babu *et al.*, 2018a) [31].

**Larval susceptibility tests**

The normal WHO method (2014) was followed while conducting the larval susceptibility tests. In order to observe the larvicidal property, larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* in their fourth instar were introduced in each test solution containing extracts of varying quantities. Twenty larvae per group were added to 200 milliliters of the extract solution. Parallel control tests were conducted without extract. Following a 24-hour exposure period, the number of dead larvae in each solution was counted, and the average of five replicates was used to

calculate the % mortality. Mortality was calculated using Abbott's (1925) formula and recorded when control mortality was between 5 and 20%.

**Statistical analysis**

In order to determine LC 50, LC 90, and other statistics at 95% confidence limits of upper and lower confidence limits, the average larval mortality data was subjected to probit analysis. SPSS 11.5 was used to calculate the chi-square values.

**Results**

**Preliminary phytochemical screening**

Table 1 displays the findings of *T. ulmifolia*'s phytochemical characterisation. Triterpenoids, saponins, flavonoids, alkaloids, and terpenoids were found to be highly present in aqueous, methanol, and ethanol extracts, according to the preliminary phytochemical screening. Steroids, glycosides, phenols, coumarins, and tannins were among the other phytochemicals found.

Table 1: Phytochemical screening of *Turnera ulmifolia* plant extracts

S. No.	Secondary Metabolism	Aqueous	Chloroform	Ethanol	Methanol	Hexane
1.	Carbohydrates	++	+++	++	+++	+
2.	Tannins	-	++	++	+++	+
3.	Saponins	-	+	++	+++	-
4.	Flavonoids	-	+	+++	+++	+
5.	Alkaloids	-	+++	++	++	++
6.	Betacyanin	-	+	+	+++	-
7.	Quinones	-	-	++	+++	+
8.	Glycosides	+	-	+	++	-
9.	Cardio Glycosides	+	+	+	+++	-
10.	Terpenoids	++	+	+++	++	-
11.	Triterpenoids	-	+++	+	++	+++
12.	Phenols	-	+	++	+++	+
13.	Coumarins	-	+++	++	+++	-
14.	Acids	-	-	-	+++	-
15.	Proteins	++	-	-	-	-
16.	Steroids	-	+	-	+++	-

+++ - Strongly Positive; ++ - Positive; -- Nil

**Mosquito larvicidal activity**

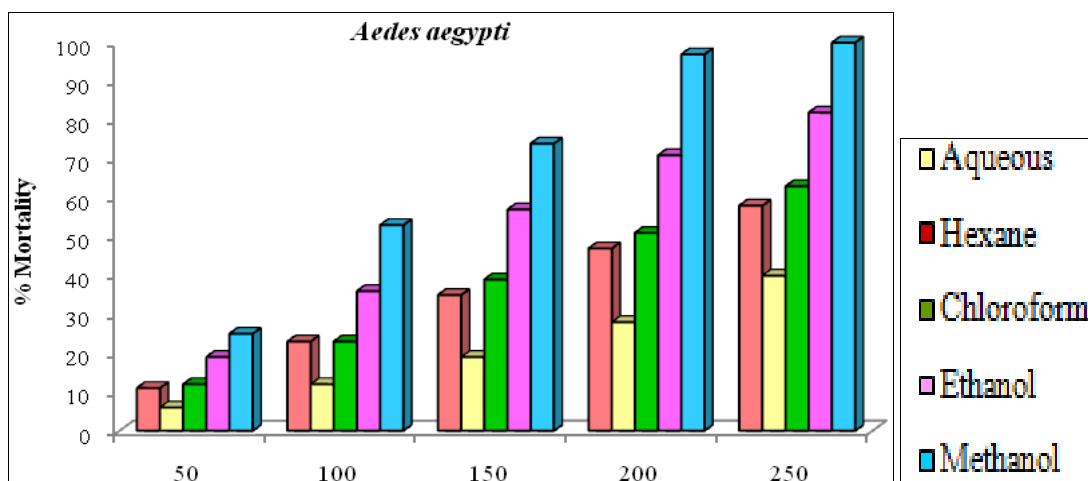
Tables 2, 3, and 4 represents the results of a probit analysis comparing the concentrations of plant extract against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* larvae in their fourth instar following a 24-hour exposure. The findings unequivocally show that *T. ulmifolia* plant extract was harmful to all three tested mosquito species at extremely low doses. With LC50 and LC90 values of 35.36 ppm and 107.42 ppm and 35.65 ppm and 106.95 ppm, respectively, the methanolic plant extract was shown to be more effective against *C. quinquefasciatus* and *A. stephensi* than *A. aegypti*, which had LC50 and LC90 values of 39.54 ppm and 118.62 ppm (Fig 1).

At 150 ppm, *T. ulmifolia* methanol extract demonstrated 100% mortality against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* larvae in their fourth instar (1, 2, and 3). All three-mosquito species' fourth instar larvae were shown to be similarly susceptible to the effects of ethanol whole plant extracts. Comparing *C. quinquefasciatus* to *A. aegypti* (60.93 ppm and 181.99 ppm) and *A. stephensi* (79.03 ppm and 237.19 ppm), the LC50 and LC90 values were 50.17 ppm and 155.64 ppm, respectively. In comparison to methanol and ethanol plant extracts, all other examined extracts exhibited mosquito larvicidal action at a comparatively high concentration.

**Table 2:** Larvicidal activity of *T. ulmifolia* plant extracts against the fourth instar larvae of *A. aegypti*

Extract	Concentration (ppm)	24hr % Mortality	LC <sub>50</sub>	LC <sub>90</sub>	r <sup>2</sup>
			(UCL-LCL) (ppm)	(UCL-LCL) (ppm)	
Aqueous	250	58			0.918
	200	47			
	150	35	98.10	294.30	
	100	23	(123.58-77.87)	(319.42-276.29)	
	50	11			
Hexane	250	40			0.987
	200	28			
	150	19	60.64	184.72	
	100	12	(77.59-47.39)	(201.92-168.42)	
	50	6			
Chloroform	250	63			0.980
	200	51			
	150	39	86.91	260.73	
	100	23	(102.03-74.03)	(279.44-242.30)	
	50	12			
Ethanol	250	82			0.973
	200	71			
	150	57	60.93	181.99	
	100	36	(70.71-52.50)	(198.41-169.72)	
	50	19			
Methanol	250	100			0.975
	200	97			
	150	74	39.54	118.62	
	100	53	(53.46-29.25)	(127.07-104.39)	
	50	25			

Control - nil mortality  
 Significant at p < 0.05 level  
 LC<sub>50</sub> - Lethal concentration that kills 50% of the exposed larvae  
 LC<sub>90</sub> - Lethal concentration that kills 90% of the exposed larvae  
 UCL - Upper confidence limit  
 LCL - Lower confidence limit



**Fig 3:** Larvicidal activity of plant extracts of *T. ulmifolia* against *A. aegypti*

**Table 3:** Larvicidal activity of *T. ulmifolia* plant extracts against the fourth instar larvae of *A. stephensi*

Extract	Concentration (ppm)	24hr % Mortality	LC <sub>50</sub> (UCL–LCL) (ppm)	LC <sub>90</sub>	r <sup>2</sup>
				(UCL–LCL) (ppm)	
Aqueous	250	63			
	200	51	132.82	344.01	
	150	37	(154.70-114.30)	(361.19-327.88.)	0.984
	100	24			
	50	12			
Hexane	250	41			
	200	32	80.86	242.04	
	150	23	(98.80-66.12)	(261.42-227.09)	0.786
	100	15			
	50	8			
Chloroform	250	98			
	200	71	98.86	294.44	
	150	57	(116.55-83.86)	(311.42-274.09)	0.992
	100	38			
	50	18			
Ethanol	250	100			
	200	83	79.03	237.19	
	150	69	(89.86-69.50)	(246.07-214.59)	0.994
	100	47			
	50	24			
Methanol	250	100			
	200	100	35.65	106.95	
	150	83	(57.58-21.71)	(121.05-92.42)	0.949
	100	59			
	50	28			

Control - nil mortality

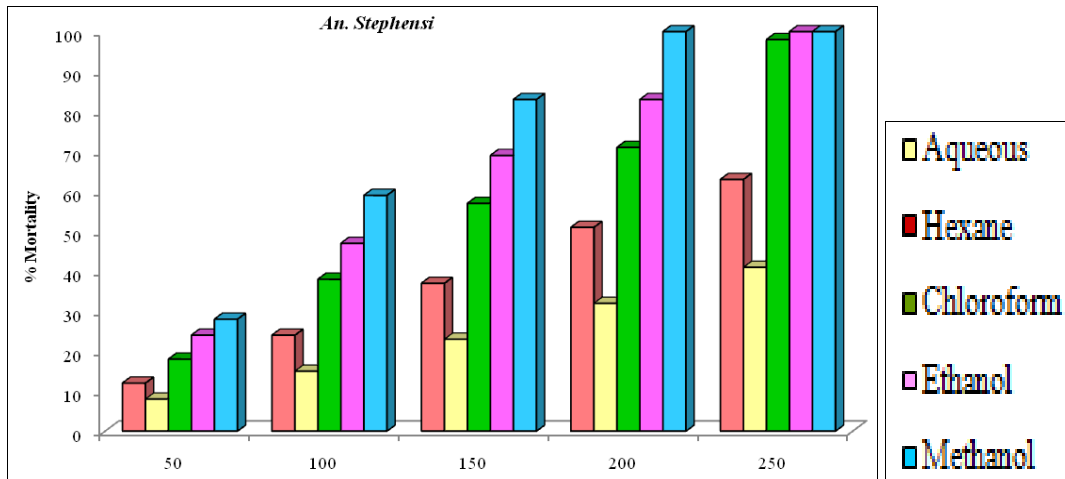
Significant at p < 0.05 level

LC<sub>50</sub> - Lethal concentration that kills 50% of the exposed larvae

LC<sub>90</sub> - Lethal concentration that kills 90% of the exposed larvae

UCL - Upper confidence limit

LCL - Lower confidence limit



**Fig 4:** Larvicidal activity of plant extracts of *T. ulmifolia* against *A. stephensi*

**Table 4:** Larvicidal activity of *T. ulmifolia* plant extracts against the fourth instar larvae of *C. quinquefasciatus*

Extract	Concentration (ppm)	24hr % Mortality	LC <sub>50</sub> (UCL–LCL) (ppm)	LC <sub>90</sub> (UCL–LCL) (ppm)	r <sup>2</sup>
Aqueous	250	61			
	200	47			
	150	36	95.55	244.11	0.929
	100	21	(112.20-81.37)	(258.77-231.09)	
	50	12			
Hexane	250	36			
	200	27	64.24	192.74	
	150	18	(72.66-56.81)	(209.45-166.33)	0.980
	100	12			
	50	7			

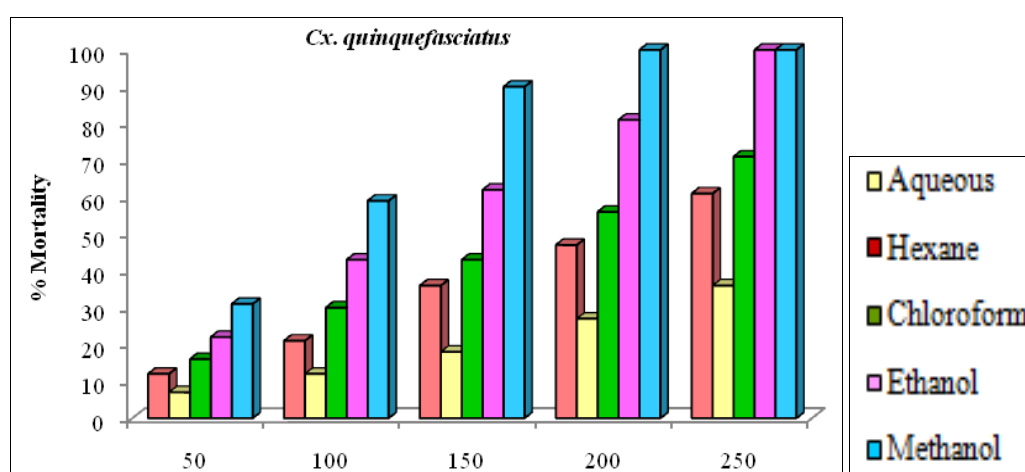
<b>Chloroform</b>	250	71			
	200	56	66.54	198.72	0.963
	150	43	(77.65-57.19)	(211.04-179.59)	
	100	30			
	50	16			
<b>Ethanol</b>	250	100			
	200	81	50.17	155.64	
	150	62	(67.06-42.15)	(172.22-132.74)	0.992
	100	43			
	50	22			
<b>Methanol</b>	250	100			
	200	100	35.36	107.42	
	150	90	(44.95- 27.81)	(120.09-91.47)	0.902
	100	59			
	50	31			

Control - nil mortality

Significant at  $p < 0.05$  levelLC<sub>50</sub> - Lethal concentration that kills 50% of the exposed larvaeLC<sub>90</sub> - Lethal concentration that kills 90% of the exposed larvae

UCL - Upper confidence limit

LCL - Lower confidence limit



**Fig 5:** Larvicidal activity of plant extracts of *T. ulmifolia* against *C. quinquefasciatus*

GC-MS analysis of methanol extract of *T. ulmifolia* Table 5 shows the GC-MS characterizations of *T. ulmifolia* methanolic extracts. Caryophyllene, lyxanthin, pentol,

cholestan, octadecanoic acid, cyclopropyl, hexadecanoic acid, and other chemicals were among the main substances found (Table 5 and Fig. 4).

**Table 5:** GC-MS analysis of methanol extract of *T. ulmifolia*

S. No	Retention Time	Compounds	Molecular Formula	Molecular Weight
1	11.95	a-Caryophyllene	C <sub>15</sub> H <sub>24</sub>	204.36
2	13.72	2-[4-Methyl-6-[2,6,6-trimethylcyclohex-1-enyl] hexa-1,3,5- trinet] cyclohex-1-en-1-carboxaldehyded	C <sub>23</sub> H <sub>32</sub> O	324.49
3	14.05	17-[1,5-Dimethylhexyl]-10,13-dimethyl-3-styrylhexadecahydrocyclopenta[a]phenanthren-2-one	C <sub>35</sub> H <sub>52</sub> O	488.78
4	16.00	Lyxanthin	C <sub>40</sub> H <sub>56</sub> O	552.87
5	17.43	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.45
6	17.93	Ethaneperoxoic acid, 1-cyano-1- [2- [2 -phenyl-1,3-dioxolan-2 -yl] ethyl] pentyl ester	C <sub>19</sub> H <sub>25</sub> NO <sub>5</sub>	347.17
7	19.17	9-Octadecenoic acid [Z]-, methyl esters	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.48
8	19.35	1,2-15,16-Diepoxyhexadecane	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254.40
9	23.82	Dasycarpidan-1-methanol, acetate [ester]	C <sub>20</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub>	326.43
10	27.82	1H-Cyclopropra [3,4] benz [1,2-e] azulene-4a,5,7b, 9,9a(1aH)-pentol, 3-[(acetyloxy)methyl] -1b, 4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-,5,9,9a-triacetate, [1aR-(1aà,1bà,4aà,5à,7aà,7bà,8à,9a,9aà)]-	C <sub>28</sub> H <sub>83</sub> O <sub>10</sub>	534
11	28.72	Cholestan-3 -ol, 2-methylene- [3à,5 à]-	C <sub>27</sub> H <sub>48</sub> O	388.66
12	17.63	Benzenepropanoic acid, 3,5-bis [1,1, -dimethylethyl]-4-hydroxy-, methyl ester	C <sub>18</sub> H <sub>28</sub> O <sub>3</sub>	292.41
13	23.22	9-Octadecenoic acid (Z), hexyl ester	C <sub>24</sub> H <sub>46</sub> O <sub>6</sub>	366.63
14	12.88	1H-Cyclopropra [3,4] benz [1,2-e] azulene-4a,5,7b,9,9a(1aH)-pentol, 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-,5,9,9a-triacetate, [1aR-(1aà,1bà,4aà,5à,7aà,7bà,8à,9a,9aà)]-	C <sub>28</sub> H <sub>38</sub> O <sub>10</sub>	534

15	11.05	1,6,10-Dodecatriene,7,11, -dimethyl-3-methylene-, [Z]-	C <sub>15</sub> H <sub>24</sub>	204.35
----	-------	--	---------------------------------	--------

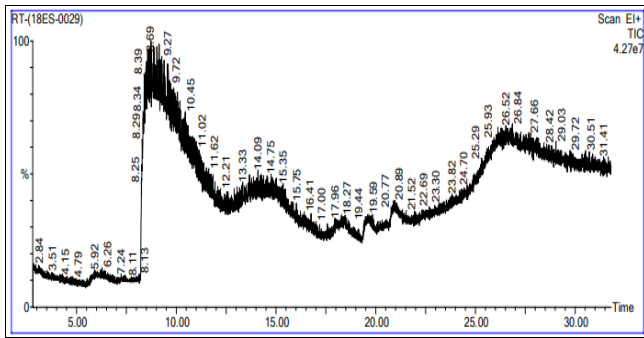


Fig 3: GC-MS analysis of methanol plant extract *Turnera ulmifolia*

**Thin layer Chromatography analysis**

TLC was used to further analyze the phytochemicals found in the plant extracts (Fig. 4). *T. ulmifolia* methanolic extract displayed seven bands when seen with iodine. Rf values of 0.96, 0.91, 0.78, 0.68, 0.46, 0.23, and 0.19 for the chemicals found in bands 1 through 7 indicated the presence of tannins, catechins, and steroids, respectively.

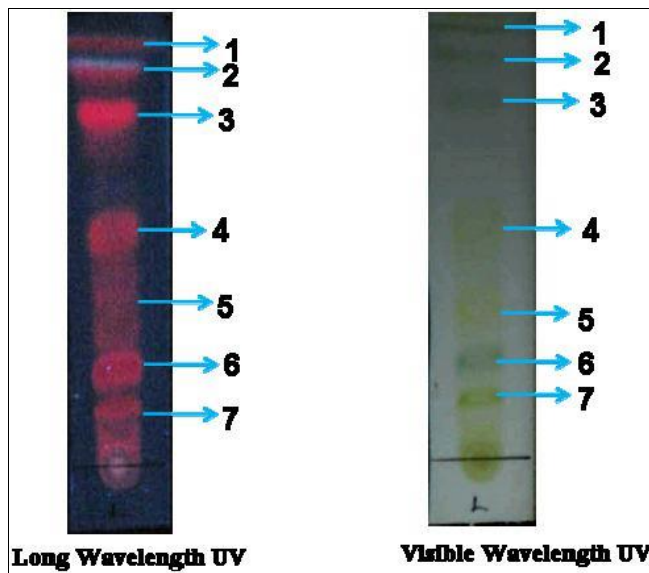


Fig 1: Separation of bioactive fraction of whole plant extracts of *T. ulmifolia* by TLC

**Discussion**

One of the most important aspects in the management of diseases caused by vectors is the use of larvicidal compounds to control mosquito larvae. At the community level, plants are thought to be a feasible and preferred choice for controlling mosquito species. Few plant products have demonstrated practical utility for mosquito control, despite the fact that several plant extracts have been reported to have mosquitocidal or repellent effects against mosquito vectors. When compared to *A. aegypti* and *A. stephensi*, the methanolic extract of *T. ulmifolia* shown 100% larvicidal activity against the fourth instar larvae of *C. quinquefasciatus* in the current investigation. Most people agree that triterpenoids have larvicidal properties against mosquitoes (Hemalatha *et al.*, 2015) [24]. Therefore, the presence of terpenoids and triterpenoids, which are hydrocarbons in the extract that hinder the developmental phases of insects, may be the cause of the

high mortality rate seen in this study (Mahajan *et al.*, 2022) [25].

Numerous studies have noted the various activities of phytochemicals produced from plants, which can serve as larvicides, insect development regulators, repellents, and ovipositor attractants (Mahajan *et al.*, 2022) [25]. Similarly, it has been observed that *Turnera sp.* contains furanonaphtha quinones and lantadene triterpenoids, which have larvicidal effects on mosquitoes. According to Karthika (2017) [26], *T. ulmifolia* contains antimicrobial, antioxidant, and anticancer properties as well as a mosquito-repelling effect.

**Conclusion**

It is clear that plant-based treatments are becoming a viable means of controlling mosquito larvae populations. Static water bodies, which are known to serve as mosquito breeding grounds, may benefit from the usage of crude extract or extracted bioactive substances from the plant *T. ulmifolia*. Extracts from the weed *T. ulmifolia* demonstrated encouraging results in controlling mosquitoes, and their commercial use is highly viable.

**References**

1. Khan MAHN A. Important vector-borne diseases with their zoonotic potential: present situation and future perspective. *Bangladesh J Vet Med*, 2015, 13(2).
2. Dahmana H, Mediannikov O. Mosquito-borne diseases emergence/resurgence and how to effectively control it biologically. *Pathogens*,2020;9(4):310.
3. Priya SS, Vasantha-Srinivasan P, Altemimi AB, Keerthana R, Radhakrishnan N, Senthil-Nathan S, *et al.* Bioactive molecules derived from plants in managing dengue vector *Aedes aegypti* (Linn.). *Molecules*,2023;28(5):2386.
4. World Health Organization. Vector-borne diseases. No. SEA-CD-300. WHO Regional Office for South-East Asia, 2014.
5. Senthil-Nathan S. A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes. *Front Physiol*,2020;10:1591.
6. Takken W, Lindsay S. Increased threat of urban malaria from *Anopheles stephensi* mosquitoes, Africa. *Emerg Infect Dis*,2019;25(7):1431.
7. Lima EP, Oliveira Filho AM, Lima JWO, Ramos-Junior AN, Cavalcanti LPG, Pontes RJS. Resistencia do *Aedes aegypti* ao temefos em municipios do Estado do ceara. *J Braz Soc Trop Med*,2006;39:259-263.
8. Ghayal N, Padhye A, Dhumal K. Larvicidal activity of invasive weeds *Cassia uniflora* and *Syndrella nodiflora*. *Int J Pharm Biol Sci*,2010;1:3.
9. Innocent E, Cosam CJ, Nicholus KG, Manien JM, Mayunga HH, Nkunya AH. Mosquito-larvicidal constituents from *Lantana Viburnoides varkisi* (*A. rich*) verde (Verbenaceae). *J Vector Borne Dis*,2008;45:240-244.
10. Wiseman Z, Chapagain BP. Larvicidal effects of aqueous extracts of *Balanties aegyptiaca* (desert date) against the larvae of *Culex pipens* mosquitoes. *Afr J Biotechnol*,2005;4(11):1351-1354.

11. Mazigo HD, Massawe IS, Rumisha SF, Kweka EJ, Mboera LE. Rice farmers' perceptions and acceptability in the use of a combination of biolarvicide (*Bacillus thuringiensis* var. *israeliensis*) and fertilizers application for malaria control and increase rice productivity in a rural district of central Tanzania. *Malar J*,2019;18:1-11.
12. Diaz JH. Chemical and plant-based insect repellents: efficacy, safety, and toxicity. *Wilderness Environ Med*,2016;27(1):153-163.
13. Dahmana H, Mediannikov O. Mosquito-borne diseases emergence/resurgence and how to effectively control it biologically. *Pathogens*,2020;9(4):310.
14. Tyagi BK. Arthropods of medical importance: need for genetic and other innovative vector control technologies, with emphasis on eco-biosocial and environmental considerations. In: *Genetically Modified and Other Innovative Vector Control Technologies: Eco-bio-social Considerations for Safe Application*, 2021, 1-20.
15. Rawani A, Ray AS, Ghosh A, Sakar M, Chandra G. Larvicidal activity of phytosteroid compounds from leaf extract of *Solanum nigrum* against *Culex vishnui* group and *Anopheles subpictus*. *BMC Res Notes*,2017;10:1-8.
16. Coutinho HD, Costa JG, Lima EO, Falcao-Silva VS, Siqueira-Júnior JP. Increasing of the aminoglycoside antibiotic activity against a multidrug-resistant *E. coli* by *Turnera Ulmifolia L.* and chlorpromazine. *Biol Res Nurs*,2010;11(4):332-335.
17. Teixeira TM, Boeff DD, de Oliveira Carvalho L, Ritter MR, Konrath EL. The traditional use of native Brazilian plants for male sexual dysfunction: evidence from ethnomedicinal applications, animal models, and possible mechanisms of action. *J Ethnopharmacol*,2024;318:116876.
18. Babu M, Kaleena PK, Janaki A, Velu K, Jeyanthi R. Phytochemical profiling and larvicidal potential of common grass *Pennisetum polystachion* against mosquito vectors. *Int J Res Anal Rev*,2018;5(4):103-116.
19. Nweze EI, Okafor JI, Njoku O. Antimicrobial activities of methanolic extracts of *Trema guineensis* (Schumm and Thorn) and *Morinda lucida* benth used in Nigerian. *Bio-research*,2004;2(1):39-46.
20. Senthilkumar PK, Reetha D. Screening of antimicrobial properties of certain Indian medicinal plants. *J Phytol*, 2009, 1(3).
21. Babu M, Kaleena PK, Janaki A, Velu K, Ravi S. Larvicidal activity and histopathological alterations effected by *Kyllinga nemoralis* grass weed extracts on the mosquito vectors. *Int J Res Anal Rev*,2018a;5:388-403.
22. Thomas SJ, Martinez LJ, Endy TP. Flaviviruses: yellow fever, Japanese B, West Nile, and others. In: *Viral Infections of Humans: Epidemiology and Control*. New York, NY: Springer US, 2023, 1-62.
23. Abbotts WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol*,1925;18:265-266.
24. Hemalatha P, Elumalai D, Janaki A, Babu M, Velu K, Velayutham K, Kaleena PK. Larvicidal activity of *Lantana camara aculeata* against three important mosquito species. *J Entomol Zool Stud*,2015;3(1):174-181.
25. Mahajan E, Singh S, Kaur S, Sohal SK. The genotoxic, cytotoxic and growth regulatory effects of plant secondary metabolite  $\beta$ -caryophyllene on polyphagous pest *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Toxicon*,2022;219:106930.
26. Karthika V, Arumugam A, Gopinath K, Kaleeswarran P, Govindarajan M, Alharbi NS, *et al.* *Guazuma ulmifolia* bark-synthesized Ag, Au and Ag/Au alloy nanoparticles: photocatalytic potential, DNA/protein interactions, anticancer activity and toxicity against 14 species of microbial pathogens. *J Photochem Photobiol B Biol*,2017;167:189-199.