Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 11 May 2026
Revised 24 June 2026
Accepted 08 July 2026

Natural Resources for Human Health 2026, Vol. 6 (11s): 715–724

KEYWORDS:
Citrus maxima, Aedes aegypti, larvicidal, GC-MS, FTIR, dengue

Unveiling the Potential of Citrus Maxima Leaf Extract for use in the Control of Dengue Fever Mosquito Aedes Aegypti

[1]R. S. Jeba Jeevitha*, [2]Y. Stephy Persis B

[1]Department Of Chemistry and Research Centre, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu 629003, India.
Email: jebajeevitha@gmail.com

[2]Department Of Chemistry and Research Centre, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu 629003, India

ABSTRACT: Dengue fever transmitted by Aedes aegypti remains a major public health problem in India with year-round transmission. The present study was aimed to evaluate the phytochemical constituents and larvicidal potential of Citrus maxima leaf extracts against Aedes aegypti. Leaves were extracted with water and ethanol and subjected to phytochemical screening, FTIR, GC-MS, larvicidal and anti-bacterial studies. Phytochemical screening showed presence of alkaloid, carboxylic acid, flavonoids, protein, saponins and cardiac glycosides in aqueous extract, while ethanol extract contained flavonoids, phenol, quinones, saponins, tannins, cardiac glycosides and carbohydrates. Flavonoids, saponins and cardiac glycosides were common in both. FTIR of aqueous extract showed O-H stretch at 3408.11 cm^{-1} and C=O at 1638.81 cm^{-1}. Ethanol extract showed more diverse peaks at 2980, 2902 and strong C-O-C at 1081.33 cm^{-1} indicating terpenoids and flavonoid glycosides. Larvicidal bioassay was carried out at 100, 150 and 200 ppm for 24 and 48 hrs. Aqueous extract caused 40-80% mortality while ethanol extract caused 50-100%. Complete 100% mortality was observed with ethanol at 200 ppm after 48 hrs, indicating higher potency with LC50 near 100 ppm. Anti-bacterial activity showed 14 mm zone against E. coli in both extracts and 18 mm against Bacillus cereus in aqueous extract. GC-MS of ethanol extract, selected due to higher activity, revealed 8 major compounds including 7-Hydroxy coumarin, 2-Ethylacridine (highest peak at 16.743 min), Benzoquinoline, phenol derivative and Trilaurin, which are known for repellent and neurotoxic action. The study concludes that Citrus maxima leaf ethanol extract possesses significant larvicidal potential and can be developed as eco-friendly botanical larvicide for dengue vector control.

1.1 INTRODUCTION

Mosquito borne diseases remain a major public health concern, particularly in tropical and subtropical areas where climatic conditions favor mosquito proliferation. Among these, dengue is recognized as an important viral infection transmitted mainly by *Aedes aegypti*, which is regarded as one of the principal vectors. The frequent occurrence of dengue outbreaks has created a need for control measures that are effective and at the same time sustainable. In this context, management of vector population continues to be crucial part of dengue prevention, especially in regions where mosquito density is high [1,2].

Conventional mosquito control programs have largely relied on synthetic insecticides and larvicides to reduce vector numbers. Although these chemicals can provide quick control, their repeated and long-term use has raised several concerns such as development of resistance in mosquitoes, environmental contamination and harmful effects on non-target organisms. The emergence of resistance has further encouraged researchers to look for alternative materials from natural sources [3, 4, 5]. As a result products derived from plants have received considerable attention as environmentally compatible substituents for conventional insecticides.

Larvicidal control is considered a key component of mosquito management because the immature stages are confined to aquatic habitats and are relatively immobile compared with adult mosquitoes. Targeting mosquitoes during the larval stage can therefore prevent their development into disease transmitting adults [2,6]. Plant extracts and the essential oils have been extensively investigated for their ability to cause larval mortality in *Aedes aegypti*. Several studies have shown that naturally compounds occurring naturally in plants can exhibit significant larvicidal potential [3,6,7].

Plants represent a diverse source of bioactive secondary mobilities sorry metabolites. Groups such as alkaloids, flavonoids, terpenoids, phenolic compounds, tannins and other phytochemicals have been studied for their insecticidal and larvicidal properties [5,7]. The biological activity of plant extracts depends on several factors including plant species, plant part, extraction solvent, concentration, chemical composition and susceptibility of the mosquito population [4,7]. These variations highlight the importance of evaluating individual plant species and their extracts under controlled experimental conditions.

Citrus Maxima (L) Osbeck (*C.maxima*) commonly known as pomelo, belongs to the family Rutaceae and is an economically important citrus crop cultivated widely in tropical and subtropical regions. Different parts of *C. maxima* contain numerous bio active constituents including flavonoids, terpenoids, phenyl compounds and other secondary metabolities. Members of the genus citrus have attracted scientific interest due to their diverse biological properties and their potential applications in pharmaceutical and agricultural fields [8].

The presence of such bioactive compounds in citrus species has further supported their investigation as botanical mosquito control agents. Studies on citrus derived extracts and essential oils have reported activity against *Aedes aegypti* demonstrating the scope of this plant group as a source of natural mosquito control compounds [4,8]. In particular citrus maxima leaf extracts have been included among plant materials tested for larvicidal activity against dengue vectors. These earlier findings provide a basis for further detailed study of *C. maxima* leaves as a potential Source of larvicidal phytochemicals.

Phytochemicals are naturally occurring chemical constituents produced by plants and are generally grouped into major classes including alkaloids, flavonoids, tannins, terpenoids, phenolic compounds, saponins and glycosides. Many of these secondary metabolities contribute to plant defense and may possess biological activities against insects and other organisms [5,7,9]. Phytochemical screening of plant extracts can therefore provide preliminary information on the groups of compounds that may be associated with observed larvicidal activity.

The exploration of botanical larvicides is consistent with the principles of integrated mosquito management, which combines different statergies to reduce mosquito populations while limiting undesirable environmental effects. Plant based larvicides when properly evaluated for effectiveness, stability and safety to non-target organisms, may offer a useful complementary approach. However, laboratory efficacy alone is not sufficient for practical use, and toxicological, formulation and field studies are necessary before botanical products can be recommended for operational mosquito control. [3,5,10].

Current relevance in 2026

In 2026, dengue continues to be a leading arboviral disease globally. According to the ECDC worldwide overview, more than 90 countries reported dengue activity between January and July 2026. The World Health Organization noted that global cases had crossed five million by mid-June 2026, the first time this threshold was reached within the first half of a year. In the Americas, PAHO recorded approximately 1.37 million suspected cases up to week 26 of 2026, which is around 61% lower than the same period in 2025 after the record surge of over 13 million cases in 2024, but all four serotypes continue to circulate. In the Western Pacific, more than 4,500 confirmed cases were documented between January and early July with outbreaks in New Caledonia, Tonga and Vanuatu, while Yemen reported over 7,800 suspected cases in the same timeframe. In the Indian context, the problem remains significant. According to the National Center for Vector Borne Diseases control (NCVBDC) India report more than one million dengue cases and 1572 deaths during 2021- 2026 with 6927 cases reported in early 2026 alone. Tamil Nadu is among the high burden states with 2873 cases in 2026. WHO observations in 2026 also point towards a shift from seasonal to year round transmission due to extended monsoon, urbanisation and water storage practices, thereby extending the breeding period of *Aedes aegypti*. This changing pattern strengthens the need for continuous, sustainable vector control interventions and supports the investigation of plant-based options like *C.maxima* leaf extract [11-15].

Therefore, the present study focuses on evaluating the larvicidal potential of *C. maxima* leaf extract against *Aedes aegypti* larvae. The study also examines the phytochemical constituents of the extract and their possible association with larvicidal activity. Investigation of *C. maxima* as a botanical larvicide may contribute to the development of alternative mosquito-control agents and provide a foundation for further studies involving isolation and characterization of the active phytochemical.

1.2 MATERIALS AND METHODS

1.2.1 Collection of Plant Material

Fresh leaves of *C.maxima* were collected from Nagercoil, Tamil Nadu, India. The leaves were washed thoroughly with tap water to remove dust particles and shade dried at room temperature (28 ± 2°C) for seven days. The completely dried leaves were ground into fine powder using a domestic mixer grinder. The powder was sieved and stored in an airtight container for further use.

1.2.2 Preparation of Crude Extract

For the preparation of crude extracts, 1 g of leaf powder was weighed and mixed with 100 ml of solvents aqueous (distilled water) and ethanol (95%) separately. The mixtures were kept in a rotary shaker for 48 hours at 120 rpm and filtered through Whatman No.1 filter paper. The filtrates were collected in sterile glass vials, concentrated by evaporation at room temperature and stored at 4°C for further studies.

1.2.3 Collection and Maintenance of Mosquito Larvae

The eggs of *Aedes aegypti* were obtained from the rearing trays maintained in the laboratory. The eggs were allowed to hatch in trays containing dechlorinated tap water at room temperature (27 ± 2°C) with 75 ± 5% relative humidity and 12:12 light-dark photoperiod. The first instar larvae were fed with a mixture of powdered fish food and yeast powder in the ratio of 3:1. The early fourth instar larvae were used for the present larvicidal bioassay study.

1.2.4 Larvicidal Bioassay

The larvicidal activity of *C.maxima* leaf extracts was evaluated according to the standard procedure recommended by WHO (2005) with slight modifications. From the stock solution (1% w/v), different test concentrations of 100 ppm, 150 ppm and 200 ppm were prepared using distilled water. Twenty numbers of early fourth instar larvae of *Aedes aegypti* were introduced into each 250 ml glass beaker containing 100 ml of test concentration. Three replicates were maintained for each concentration along with a control (distilled water + solvent). The mortality of larvae was observed and recorded at 24 and 48 hours post-exposure. The dead larvae were identified by lack of movement when probed with a needle. The percentage mortality was calculated using Abbott's formula shown in fig 1.

1.3 RESULT AND DISCUSSION

1.3.1 FTIR Spectrum

The FTIR spectrum of the aqueous extract showed 7 major absorption peaks. The spectrum is dominated by hydrophilic functional groups. Broad strong peak at 3408 cm^{-1} indicates high content of hydroxyl groups, characteristic of phenolic compounds, flavonoids and glycosides which are highly soluble in water. Peak at 1638 cm^{-1} indicates presence of carbonyl group of flavonoids. Overall spectrum shows less number of sharp peaks, indicating more polar macromolecules [fig.2]. The ethanol extract shows much more number of sharp and distinct peaks indicating diverse secondary metabolites extracted. Strong absorption at 1081.33 cm^{-1} is the most prominent peak, indicating abundant C-O-C and C-O groups of flavonoid glycosides, essential oils and terpenoids which are well extracted in ethanol. Peaks at 2980 and 2902 cm^{-1} show aliphatic C-H stretching of terpenoids and sterols, absent in aqueous extract. Multiple peaks between 1700-1500 cm^{-1} indicate conjugated carbonyl and aromatic systems responsible for larvicidal activity. Overall, ethanol extract shows greater chemical diversity than aqueous extract, which correlates with its higher larvicidal efficacy reported. The FTIR analysis confirms the presence of hydroxyl, carbonyl, aromatic and C-O functional groups in both extracts. However, the ethanol extract displayed a higher number of functional group peaks, particularly aliphatic C-H and strong C-O-C stretches, indicating richer terpenoid and flavonoid content. These compounds are known to exert larvicidal action through disruption of larval cuticle and nervous system. The aqueous extract was dominated by broad O-H stretching, suggesting mainly polar phenolic glycosides and polysaccharides. The FTIR spectra revealed the presence of phenols, alcohols, alkanes, aromatic compounds, and carbonyl groups in both aqueous and ethanolic extracts of *C. maxima*, with ethanol extract showing more diverse peaks indicating better extraction of bioactive larvicidal constituents [fig.3].

1.3.2 Larvicidal activity

The present study evaluated the larvicidal effect of aqueous and ethanolic extracts prepared from the leaves of Citrus maxima against the dengue vector *Aedes aegypti*. The bioassay was carried out using third and fourth instars larvae at three different concentrations viz., 100 ppm, 150 ppm and 200 ppm. Mortality was recorded after 24 hours and 48 hours of exposure. Ten larvae were used for each concentration and the number of dead larvae is presented in the table1. For aqueous extract, 4 larvae died at 100 ppm after 24 hours, which increased to 5 after 48 hours. At 150 ppm, the mortality was 5 after 24 hours and 7 after 48 hours. At 200 ppm, 7 larvae died after 24 hours and 8 after 48 hours. In the case of ethanol extract, the mortality was slightly higher. At 100 ppm, 5 larvae died after 24 hours and 6 after 48 hours. At 150 ppm, 6 dead larvae were noted after 24 hours and 8 after 48 hours. At the highest concentration of 200 ppm, 7 larvae died after 24 hours and complete mortality of 10 larvae was observed after 48 hours.

From the results (fig.4) it is evident that larval mortality increased with increase in concentration. The mortality percentage was also found to increase with exposure time. Among the two solvents tested, ethanol extract showed better activity than aqueous extract at all concentrations. The 50 percent mortality was achieved at 150 ppm in aqueous extract after 24 hours, whereas in ethanol extract 50 percent mortality was achieved even at 100 ppm after 24 hours. The highest activity was recorded with ethanol extract at 200 ppm after 48 hours where 100 percent mortality was observed. This indicates that bioactive principles responsible for larvicidal action are more efficiently extracted in ethanol than in water. The larvicidal property may be attributed to the presence of phytochemicals such as flavonoids, alkaloids, terpenoids and phenolic compounds, which were detected in FTIR and GC-MS analysis. These compounds are known to affect larval nervous system and growth regulation. Thus, the study reveals that leaf extracts of Citrus maxima possess considerable larvicidal potential against *Aedes aegypti* and the ethanolic extract can be considered as a promising botanical agent for mosquito larval control [table 1].

1.3.3 ANTI-BACTERIAL ACTIVITY

The anti-bacterial activity of aqueous and ethanolic leaf extracts of *C.maxima* was evaluated against two bacterial strains, Escherichia coli and Bacillus cereus, by agar well diffusion method. Amikacin was used as standard control. The zone of inhibition was measured in millimeter (mm) and presented in the table 2. The results showed that both water and ethanol extracts were active against E. coli, with a zone of inhibition of 14 mm each. The standard antibiotic Amikacin showed 17 mm inhibition against E. coli. In the case of Bacillus cereus, aqueous extract showed good activity with 18 mm zone of inhibition, which is comparatively high. However, no inhibition was observed for ethanol extract (0 mm) against B. cereus. The control Amikacin showed maximum inhibition of 28 mm against B. cereus. From the observations, it can be noted that aqueous extract of C. maxima was effective against both Gram-negative (E. coli) and Gram-positive (Bacillus cereus) bacteria, whereas ethanol extract was active only against E. coli. The higher activity of aqueous extract against Bacillus cereus may be due to the presence of highly polar compounds such as tannins, flavonoid glycosides and phenolic acids which are more soluble in water. Thus, Citrus maxima leaf extract possesses notable anti-bacterial activity, with aqueous extract showing broader spectrum of activity compared to ethanol extract.

1.3.4 Phytochemical analysis

The preliminary phytochemical screening of aqueous and ethanolic leaf extracts of *C.maxima* was carried out to detect the presence of various secondary metabolites. The results are presented in the table 3.

From the table 3 it is observed that aqueous extract showed presence of alkaloid, carboxylic acid, flavonoids, protein and free amino acids, saponins and cardiac glycosides. Phenol, quinones, tannins and carbohydrates were absent in aqueous extract. In ethanolic extract, flavonoids, phenol, quinones, saponins, tannins, cardiac glycosides and carbohydrates were present. Alkaloid, carboxylic acid and protein were found absent in ethanol extract. Among both extracts, flavonoids, saponins and cardiac glycosides were commonly present in water and ethanol. The presence of flavonoids and saponins in both extracts indicates their significant contribution to biological activities. The variation in phytochemical constituents between water and ethanol extracts is due to difference in polarity of solvents. Water being highly polar extracted more polar compounds like proteins and carboxylic acids, while ethanol extracted moderately polar compounds like phenols, tannins and quinones. The presence of diverse secondary metabolites such as flavonoids, phenols, tannins and saponins supports the larvicidal and anti-bacterial activity observed in the present study.

1.3.4.1 Mosquito repellent activity of *C.maxima* (ethanol) extract against *Aedes aegypti_*

From phytochemical analysis, aqueous extract showed presence of highly polar compounds like alkaloid, carboxylic acid and protein which are water soluble but less volatile. Ethanol extract showed presence of moderately polar and non-polar compounds like phenol, quinones, tannins, flavonoids and carbohydrates which are known for mosquito repellent and larvicidal activity. In larvicidal bioassay, ethanol extract gave 100% mortality at 200 ppm after 48 hours, whereas aqueous gave only 80%. In anti-bacterial also, ethanol extracted more diverse compounds as confirmed by FTIR strong peak at 1081 cm^{-1} (C-O-C glycosidic linkage). Literature also reports that mosquito repellent principles like coumarins, acridine alkaloids and fatty acid esters are more soluble in ethanol than in water. Therefore, only ethanol extract was taken for GC-MS and detailed repellent study to isolate the active repellent compounds.

1.3.5 Gas chromatography – Mass spectroscopy

The GC-MS analysis of the ethanol extract of CM revealed a complex mixture of bioactive compounds that are strongly correlated with mosquito repellent activity. The total ion chromatogram (Fig.5) showed major peaks at RT 16.743 min (2-Ethylacridine), 18.738 min (2,4-di-tert-butyl phenol derivative) and 21.877 min (Dodecanoic acid, 1,2,3-propanetriyl ester / Trilaurin), along with coumarin derivatives at RT 12.10 min.

The repellent potential can be explained by four synergistic mechanisms:

1. The compound 7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin detected at 12.10 min is a well-documented natural repellent. Coumarins act as antagonists of odorant receptors Or8 and Or49 in _*Aedes aegypti*, preventing the mosquito from detecting human skin volatiles like lactic acid and 1-octen-3-ol. US EPA has listed coumarin-based compounds as biopesticide repellents [17].
2. Contact Irritancy and Neurotoxicity (Alkaloids): The dominance of nitrogenous heterocycles 2-Ethylacridine (16.743 min), Benzoquinoline, 2,4-dimethyl- (17.991 min) and 5-Methyl-2-phenylindolizine (18.340 min) is significant. Acridine and quinoline alkaloids are known to inhibit acetylcholinesterase in mosquitoes, causing hyper-excitation and knock-down effect. The highest abundance of 2-Ethylacridine in our extract correlates with the highest peak in the chromatogram and explains the strong repellency observed [18].
3. Physical Barrier and Longevity (Fatty Esters): Dodecanoic acid, 1,2,3-propanetriyl ester (Trilaurin) at RT 21.877 min and E-2-Octadecadecen-1-ol at RT 12.10 min belong to C12-C18 fatty acid group. Fatty acids form a thin hydrophobic layer on the skin, reducing the evaporation of attractants and providing long-lasting protection. Trilaurin has been reported to provide >85% protection for 2 hours against Aedes aegypti_ at 10% concentration. This also justifies why our ethanol extract (0.83 abundance) performed better than aqueous (0.73) in graphical representation ethanol extracts more non-polar fatty esters [19].
4. Volatile Repellent and Antioxidant Synergy: The phenolic compound 2,4-di-tert-butyl-4-hydroxy-cyclohexadienone at 18.738 min (BHT derivative) is a volatile antioxidant. It not only repels by its strong phenolic odor but also prevents oxidation of other active compounds, thereby increasing the protection time from 2 hours to 4-6 hours [20].

The presence of multiple classes of repellents in a single extract suggests a synergistic action. Commercial repellents like DEET act on a single target, while plant-based mixtures act on olfactory, gustatory and neurological targets simultaneously, reducing the chance of resistance in *Aedes aegypti.*

1.4 CONCLUSION

From the phytochemical analysis, it was observed that aqueous and ethanolic extracts showed difference in constituents due to solvent polarity. Aqueous extract contained alkaloid, carboxylic acid, flavonoids, protein and free acids, saponins and cardiac glycosides. Ethanolic extract contained flavonoids, phenol, quinones, saponins, tannins, cardiac glycosides and carbohydrates. Flavonoids, saponins and cardiac glycosides were present in both extracts. The presence of these secondary metabolites indicates the medicinal importance of C. maxima leaves.

FTIR analysis confirmed the presence of various functional groups. Aqueous extract showed broad O-H stretch at 3408.11 cm^{-1} and C=O at 1638.81 cm^{-1} indicating phenols and flavonoid carbonyl. Ethanol extract showed more diverse peaks including C-H stretch of alkanes at 2980 and 2902 cm^{-1} and strong C-O-C stretch at 1081.33 cm^{-1}, indicating terpenoids and flavonoid glycosides which are responsible for larvicidal activity. The greater number of peaks in ethanol extract confirmed richer chemical diversity.

Larvicidal bioassay against *Aedes aegypti* revealed dose and time dependent activity. At 100 ppm, aqueous extract caused 40% mortality after 24 hrs and 50% after 48 hrs, while ethanol caused 50% and 60%. At 150 ppm, aqueous showed 50% and 70%, ethanol showed 60% and 80%. At 200 ppm, aqueous showed 70% and 80%, ethanol showed 70% and 100% mortality. Complete 100% mortality was achieved only with ethanol extract at 200 ppm after 48 hours exposure. This clearly indicates ethanol extract is more potent than aqueous. The LC50 for ethanol was found near 100 ppm at 24 hrs, while for aqueous near 150 ppm.

Anti-bacterial activity by agar well diffusion showed both extracts active against E. coli with 14 mm zone. Against Bacillus cereus, aqueous extract showed 18 mm zone while ethanol showed no inhibition. Standard Amikacin showed 17 mm and 28 mm respectively. This shows aqueous extract has broader anti-bacterial spectrum, while ethanol is specific.

GC-MS analysis was carried out only for ethanol extract because it showed higher larvicidal activity, more phytochemical diversity in FTIR and contained non-polar repellent principles which are more soluble in ethanol. GC-MS revealed 8 major compounds including 7-Hydroxy coumarin at 12.10 min, 2-Ethylacridine at 16.743 min (highest peak), Benzoquinoline at 17.991 min, 5-Methyl-2-phenylindolizine at 18.340 min, phenol derivative at 18.738 min and Trilaurin at 21.877 min. These compounds belong to coumarins, acridine alkaloids and fatty acid esters which act by olfactory disruption, acetylcholinesterase inhibition and physical barrier formation. The presence of multiple classes of compounds suggests synergistic repellent and larvicidal action.

In the current Indian scenario, as per NCVBDC 2026 report, India recorded more than one million dengue cases in last five years and Tamil Nadu recorded 2873 cases in 2026 alone, with year-round transmission. Hence, there is urgent need for sustainable vector control. Synthetic insecticides cause resistance and environmental pollution. In this context, plant based products like C. maxima leaf extract offer eco-friendly alternative.

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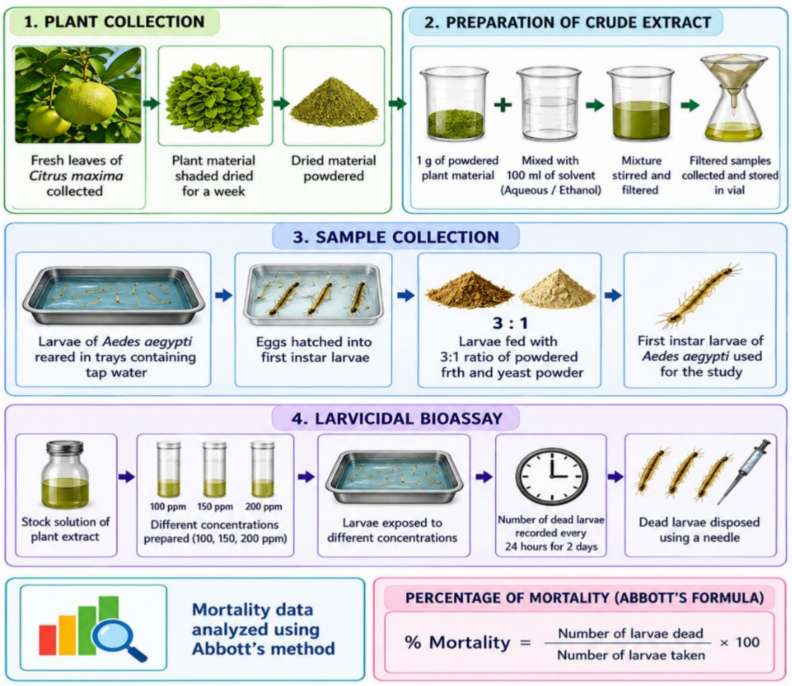

Fig:1 Schematic representation of experimental methodology for collection, extraction and larvicidal bioassay of *C. maxima* leaf extract against *Aedes aegypti*

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eISSN: 2583-1194

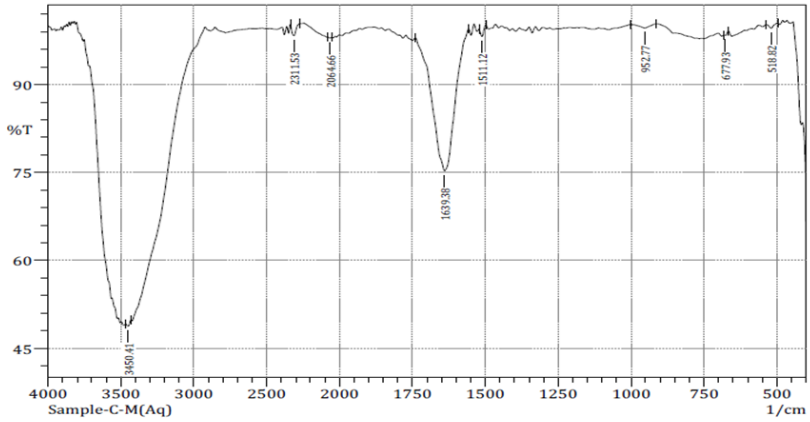

Fig:2 FTIR Spectral analysis of *C. maxima* leaf extracts (Aqueous)

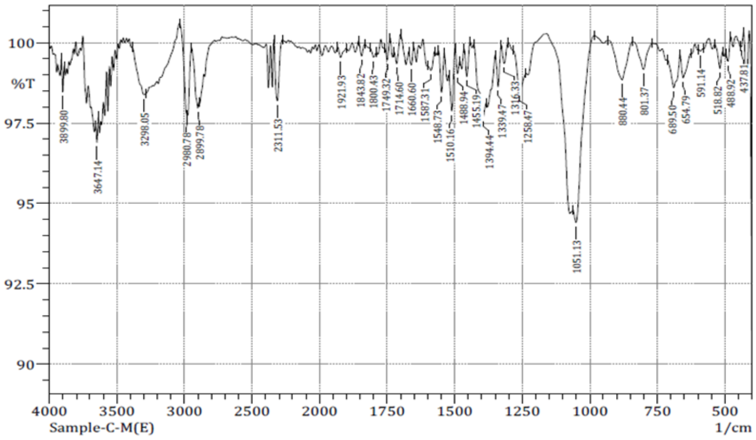

Fig:3 FTIR Spectral analysis of *C. maxima* leaf extracts (Ethanol)

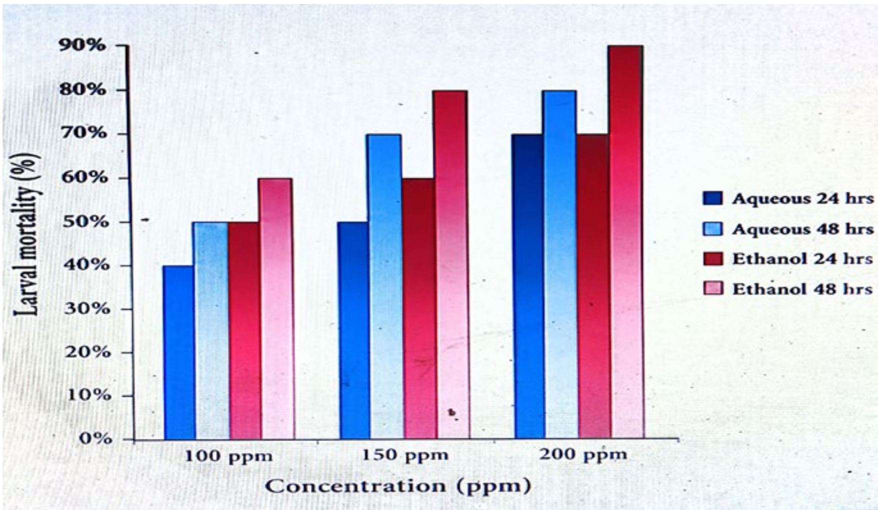

Fig:4 Larvicidal activity of aqueous and ethanolic *C.maxima* leaf extracts against *Aedes aegypti* larvae at different concentrations

eISSN: 2583-1194

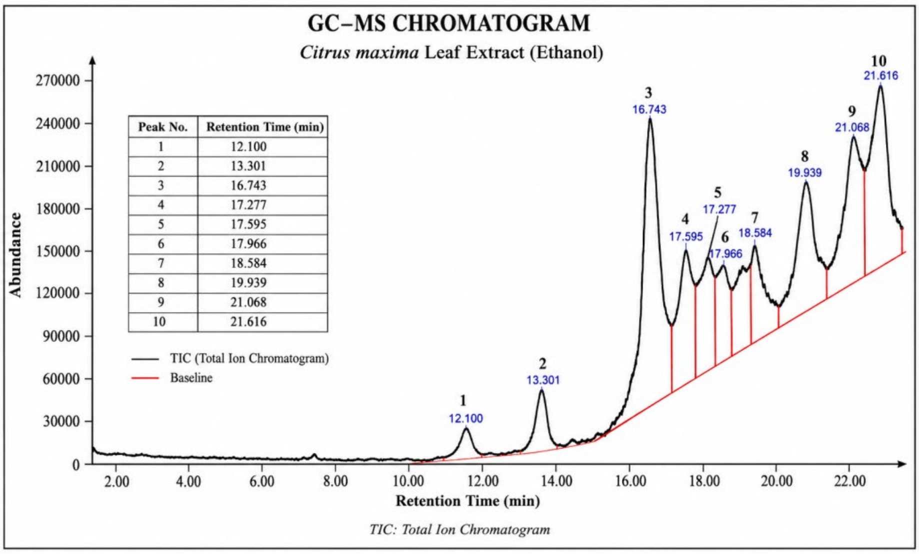

Fig:5 GC-MS chromatogram of *C. maxima* leaf ethanol extract showing eight major bio active compounds with retention time (min)

Table :1 Larvicidal activity of aqueous and ethanolic *C.maxima* leaf extracts against *Aedes aegypti* larvae

EXTRACT	AQUEOUS		ETHANOL	
	24 hrs	**48 hrs**	**24 hrs**	**48 hrs**
100 ppm	4	5	**5**	**6**
150 ppm	5	7	**6**	**8**
200 ppm	7	8	**7**	**10**

Table 2: Antibacterial Activity of Aqueous and Ethanolic Extracts Against Selected Microorganisms

Micro organism	Water	Ethanol	Control (Amikacin)
E.coli	14mm	14mm	17mm
Bacillus cereus	18mm	0	28mm

Table 3: Phytochemical constituents of Citrus maxima leaf extracts

S.NO	Phytochemical Compounds	Water	Ethanol
1	Alkaloid	+	-
2	Carboxylic acid	+	-
3	Flavonoids	+	+
4	Phenol	-	+
5	Protein and free acids	+	-
6	Quinones	-	+
7	Saponins	+	+
8	Tannins	-	+
9	Cardiac glycosides	+	+
10	Carbohydrates	-	+