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Toxicological Study of *Curcuma longa* Essential Oil Against the *Aedes aegypti* Mosquito Species

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Abstract

Mosquitoes are most vexing bloodsucking insects, transmitting vector-borne diseases. The present study aims to control mosquito species with the essential oil of *Curcuma longa*. Larvae were maintained at 27 ± 2°C and 60-70% RH under 14:10 h light and darcycles. 500ml of water containing 30 larvae late 3rd or early 4th instar was used to observe the mortality rate after 0, 1 h, 4 h, 20 h, 24 h, and 48 h of EO treatment using the solubilizer polysorbate 80. The control was set up with the same conditions, only with dechlorinated water. Serial dilutions like 100µL, 500µL, 1000µL, and 2000µL concentrations were used. 100% inhibition was observed for turmeric oil at 100µL at 20 h, 500µL at 20 h, 1000µL at 20 h and 2000µL at 20 h. Observations at 4 h time of 100µL show no mortality and increased concentrations like 500µL@15.67%, 1000µL@26.67%, 2000µL@70%. The Larvicidal potential of % inhibition increased with the increase of oil concentrations, 2000µL, and 70% Larvicidal activity was observed within 4 h. So, in formulations, higher concentrations like 2000µL equivalent will help potential inhibition within 4 h when compared other concentrations. If a low concentration, like 100µL, was sufficient for a gradual like time point and 100% inhibition within 20 h. Results showed that *C. longa* oil against *A. aegypti* has potential larvicidal properties, and can be used preventive aspect of vector-borne diseases. The phytochemical profiling carried out using GC–MS analysis confirmed the presence of major bioactive constituents, while FTIR analysis validated the characteristic functional groups responsible for the oil's biological and physicochemical properties. These analytical findings collectively support the therapeutic and larvicidal potential of the EO. This oil is further planned to be incorporated into mosquito-inhibitory sprays, pharmaceutical topical preparations, pharmaceutical skin creams, etc.

Major Findings: Essential oils of *C. longa* have significant larvicidal activity against *A. aegypti* mosquito species@2000µL concentration for 4 hours penetration with more than 70% larvicidal property under the above experimental conditions.

Keywords: *Aedes aegypti, Curcuma longa*, Larvicidal, Toxicological, Turmeric Oil

Abbreviations: ppm- Parts per million, n hexane - normal straight-chain hexane, g - grams, l - liter, m - meter, LC - Lethal concentration, h - hour, EO - Essential Oil, RH - Relative Humidity, µL - Micro liter

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1. Introduction

Mosquitoes kind of insect that can cause various vector-borne diseases to public health. Some of them, like Malaria, Dengue, and Chikungunya, are major diseases with high incidence of mortality around the world. These diseases are spread mainly by vectors like the *Aedes* and *Culex* genera. Insects like mosquitoes cause diseases in around 600 million cases, specifically newborn babies, children in public groups[1].

1.1 Current Scenario for Controlling Mosquitoes and Their Adverse Effects

Currently, control of mosquitoes is more challenging because of resistance to control agents[2]. Some of the vector-controlled methods include mosquito repellents, Mosquito coils with concentrations of Diethyltoluamide (DEET), pyrethrum, and sparing larvicidals in the water logging areas, etc[3].

Using these synthetic repellents, Larvicidals cause adverse effects like skin reactions, neurological, and cardiovascular

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disorders, which are the most common adverse effects of overexposure to synthetic coils and vaporous[4-6]. Some findings of the vapour phase of mosquito repellents lead to carbonyl compounds, mainly formaldehyde and acetaldehyde, which have a potent irritating effect on the upper respiratory tract system of the body[7,8].

1.2 Natural Larvicidal

At present, there is a need to concentrate on natural-based larvicidals and repellents to control various vector-borne diseases. The herbals, as well as herbal-based oils used in the ancient period and reported by the Indian system of medicines like *Ayurved*a and *Siddha* traditional systems[9].

These naturally available herbal plants have been used traditionally for various kinds of diseases because of their pharmacological benefits[10]. Phytoconstituents, individually or in combination, present in the plants, determine the therapeutic applications. Alkaloids, flavonoids, phenolics, and tannins are present in plants and have different kinds of pharmacological[11]. The potential benefits of herbs can be predicted by the knowledge of the phytochemicals available in them[12]. Currently, scientific Pharmacological and toxicological studies are needed to determine the phytochemicals that will be responsible for basic pharmacologically active properties[13].

Some of the previous studies, like some of the toxicological studies, such as the Phasomkusolsil and Soonwera studies, tested essential oils from some of the plant species against *Ae. aegypti* adults, and reported that *Cymbopogon citratus* leaf and stem extracts showed the highest toxicological effect (100%)[14] reported that *Apium graveolens*, *Carum carvi*, *Curcuma zedoaria*, *Piper longum*, and *Illicium verum* had a toxicological effect against *Ae. aegypti* strains[15]. Chellappandian *et al.* reported *Sphaeranthus indicus* leaf essential oil against the vector[16]. Castillo *et al.* studied the adulticide action of *Lippia origanoides* oil against mosquitoes[17].

At present, there is a need to concentrate on natural-based larvicidals and repellents to protect ourselves from various vector-based diseases like Malaria, Dengue, and Chikungunya, etc.

1.3 *Curcuma longa* (Turmeric Oil)

In India, traditional systems of medicine like Siddha and Ayurveda used the *C. longa* for medicinal purposes[18-20]. In the southern part of India, turmeric (*Manjal kizhangu)* is used for food additives and medicinal preparations[21,22].

In India, *Curcuma* species are cultivated as *Manjal* in South India, in the Tamil Nadu state of the Erode zone. Curcumin and other phytoconstituents depend on the cultivation locations and the harvesting and storage methods. The *Manjal kizhanku*, called Rhizome, is a potential source of active constituents with various pharmacological properties[23,24].

Traditional systems of Indian Medicine, like Siddha and Ayurveda, suggest that turmeric has been used in various kinds of pharmacological actions, especially antimicrobial properties, as a potential use. In the Siddha system of medicine, the *Manjal* with various formulations and *Azakhu shathana Porutkal* (Cosmetics). *Manjal Kizhangu* is used for topical applications related to skin diseases like acne, etc[25,26].

This turmeric oil is a rich source of some key phytochemicals, including ar-turmerone, α-turmerone, curlone,β-sesquiphellandrene,α-zingiberene,terpinolene, and β-bisabolene, liable for treating several diseases and disorders. As previously reported, these turmeric oil phytochemicals show desirable biological activities by acting as antimicrobials, antifungals, antioxidants, anti-inflammatories, anti-mutagenics, hepatoprotectives, neuroprotectives, anticancer, and antidiabetic agents[27,28]. Moreover, some properties such as insecticidal, mosquito repellent, pest resistant, herbicide, antibiotics against eye-infecting pathogens, food emulsifier, and preservative in active food packaging materials, etc[29-31].

In Ayurveda, turmeric has been used as a stomachic, tonic, blood purifier, treatment of skin diseases, jaundice, and urinary tract diseases. *Curcuma*, a genus in the family *Zingiberaceae*, consists of many hundreds of varieties of species of rhizomatous herbs, grown extensively in East and Southeast Asia[32].

2. Materials and Methods

2.1 Authentication of Plant Material

The herbal plants are authenticated by Dr. Sunil Kumar KN, Research Officer, Siddha Central Research Institute, Chennai, Tamil Nadu. After identification, the plant material was processed for extraction.

2.2 Turmeric Oil Extraction from *Curcuma longa*

The aromatic substances of *Curcuma longa* rhizomes are due to its phytoconstituents present[33]. The plant of

medicinal parts, with all parts used for the isolation of Essential Oils (EO). The yield of EO is minimal in flowers and significant in the parts of the root as well as rhizomes[34-36]. Hydro distillation is one of the methods for the extraction of EO[37].

Plant Material Collection: The medicinal plant, *C. longa* Rhizome showed in Figure 1 was purchased from the Traditional Siddha store, Ramsey Chatty, Chennai, Tamil Nadu, India.

Extraction: The most important production process of EO, is the distillation method. EO depend on botanical conditions like the fresh or dried stage. Three types of distillation are used: Water, Water and Steam, and Direct steam. In this study, the hydrodistillation method was used to obtain the oil with an initial feed of 100g.

2.3 Mosquito Culture

Larvae (*Aedes aegypti*) were collected from Chennai metropolitan city stagnant water areas and confirmed in the Central Malarial Research Centre (CMRC), Director of Medical and Rural Health Services (DMS) CAMPUS, Thenampet, Chennai. The collected larvae were impregnated with the medium of dechlorinated water for sustainability. The larvicidal study was performed at 27±2 degrees Celsius and 60-70% RH under 14:10 hours light and dark cycles. Brewer's yeast, dog biscuits, and algae collected from ponds were the diet for the larvae[38].



Figure 1. Dried rhizomes of *Curcuma longa*.

2.4 Larvicidal Bioassay

In the preliminary screening with the laboratory trial, the stock solution with different serial dilutions was prepared. The larvicidal activity was assessed by the procedure of the World Health Organisation (WHO) guidelines for testing of larvicides. The larva study was carried out using the method of the WHO protocol. The study was repeated 5 times for each oil concentration for accuracy and reproducibility data. The mortality was studied after 0, 1 hour, 4 hours, 20 hours, 24 hours, and 48 hours. The count of dead larvae was done by needles that were pricked in the neck or siphon. The control was set up with the same conditions, only with dechlorinated water (without adding any essential oil) and reagents. Solubiliser: Polysorbate 80, 2ml (Tween 80) was used for the study. Each contained 0.5L of de-chlorinated water and 30 larvae. 3rd Instar of *Aedes aegypti* mosquito species showed in Figure 2 used for the study. Stock concentrations were prepared by diluting the plant oil extracts with distilled water and drops of Tween 80 (Polyoxyethylene Sorbitan Monooleate) to obtain volumes v/v respectively. Serial dilutions of essential oils, (showed in Figure 3) like 100µL, 500µL, 1000 µL, and 2000µL, were used for the study. For each

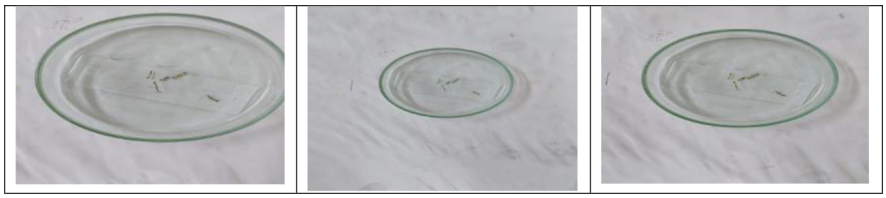


Figure 2. 3rd Instar of *Aedes aegypti* mosquito species.

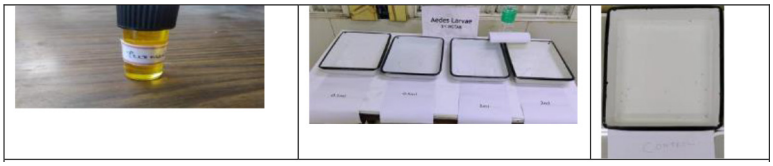


Figure 3. Toxicological bioassay of turmeric oil.

concentration, 5 replicates with a total of 150 larvae were utilised for the experiment. The number of dead larvae was counted after 0, 1 h, 4 h, 20 h, 24 h, and 48 h of exposure[39].

2.5 GC Fingerprint Studies of Turmeric Oil

Agilent Gas Chromatography (GC) instruments were used for the study. The initial temperature was set at 60°C for 2 minutes, then increased by 10°C per minute and gradually increased to 280°C hold. Internal temperature was 260°C, injection volume was 1µl at 1.5 ml flow per minute, and helium gas was used with a column of HP 560mm dimension.

2.6 FT-IR analysis of the Essential Oil of Turmeric Oil

Fourier Transform Infrared (FT-IR) spectra were collected using a Shimadzu Spectrum Two FT-IR Spectrometer using Attenuated Total Reflectance (ATR) mode in the 500–4000cm^{-1} region with 36 scans and 4cm^{-1} resolution with a data interval of 0.5cm.

3. Results

3.1 Determination of Percentage Yield

3.1.1 Estimation of Percentage Moisture Content and Oil Yield

Weighted plant material was kept in the oven at 105˚C for 4 hours. The weight loss indicates the percentage of moisture content.

$$\% \text{ moisture} = \frac{[\,Initial\ weight - final\ weight\,]}{Initial\ weight\ of\ sample} \times 100$$

3.1.2 Determination of the Percentage Yield of Turmeric Oil

The EO yield from rhizomes of turmeric was estimated by using the following expression.

$$\% \text{ Oil yield} = \frac{Essential\ oil\ obtained}{Fresh\ feed\ weight \times (1 - \%\ moisture\ content/100)} \times 100$$

3.2 Larvidal Results of *Curcuma longa* against *Aedes aegypti* Mosquito Species

3.2.1 Larvicidal Potential of Turmeric Oil (100µL)

The larvicidal potential was observed after the addition of 100µL turmeric oil to the culture. An inhibition of 0 ± 0 %, 0 ± 0 %, 100 ± 0%, 100 ± 0%, 100 ± 0% at 1 h, 4 h, 20 h, 24 h, 48 h, respectively, was observed. 100% mortality was observed at the 20th hour time point. Results showed Table 1 & Figure 4 for the Graphical Observations.

3.2.2 Larvicidal Potential of Turmeric Oil (500µL)

The larvicidal potential was observed after the addition of 500µL turmeric oil to the culture. An inhibition of 10 ± 2.36%, 16.67 ± 2.36 %, 100 ± 0%, 100 ± 0%, 100 ± 0% at 1 h, 4 h, 20 h, 24 h, 48 h, respectively, was observed. 100% mortality was observed at the 20th hour time point.

Table 1. *Larvicidal* potential of turmeric oil

Larvicidal Potential of Turmeric Oil									
S. No Time (hour) No of Larvae at Mortality (Average of 5 Replicates) ± SD		% inhibition ± SD	No of Larvae at Mortality (Average of 5 Replicates) ± SD	% inhibition ± SD	No of Larvae at Mortality (Average of 5 Replicates) ± SD	% inhibition± SD	No of Larvae at Mortality (Average of 5 Replicates) ± SD	% inhibition± SD	
		100 µL		500 µL		1000 µL		2000 µL	
1	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2	1	0 ± 0	0 ± 0	3 ± 0.71	10± 2.36	5.8 ± 0.84	19 ± 2.79	16.8 ± 0.84	56 ± 2.79
3	4	0 ± 0	0 ± 0	5 ± 0.71	16.67 ± 2.36	8 ± 0.71	26.67 ± 2.36	21 ± 0.71	70 ± 2.36
4	20	30 ± 0	100 ± 0	30 ±0	100 ± 0	30 ±0	100 ± 0	30 ±0	100 ± 0
5	24	30 ± 0	100 ± 0	30 ± 0	100 ± 0	30 ± 0	100 ± 0	30 ± 0	100 ± 0
6	48	30 ± 0	100 ± 0	30 ± 0	100 ± 0	30 ± 0	100 ± 0	30 ± 0	100 ± 0

Results showed Table 1 & Figure 5 for the Graphical Observations.

3.2.3 Larvicidal Potential of Turmeric Oil (1000µL)

The larvicidal potential was observed after the addition of 1000µL turmeric oil to the culture. An inhibition of 19 ± 2.79 %, 26.67 ± 2.36%, 100 ± 0 %, 100 ± 0%, 100 ± 0% at 1 hour, 4 h, 20 h, 24 h, 48 h, respectively, was observed. 100% mortality was observed at the 20th hour time point. Results showed Table 1 & Figure 6 for the Graphical Observations.

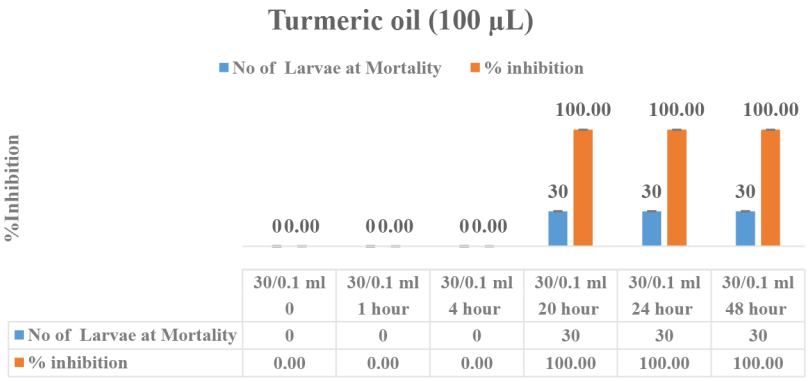
3.2.4 Larvicidal Potential of Turmeric Oil (2000µL)

The larvicidal potential was observed after the addition of 2000µL turmeric oil to the culture. An inhibition of 56 ± 2.79 %, 70 ± 2.36%, 100 ± 0%, 100 ± 0%, 100 ± 0% at 1 h, 4 h, 20 h, 24 h, 48 h, respectively, was observed. Within 1 h, 56% and 4 h, 70% mortality was observed. Results showed Table 1 & Figure 7 for the Graphical Observations.

3.3 GC Fingerprint Studies of Turmeric Oil

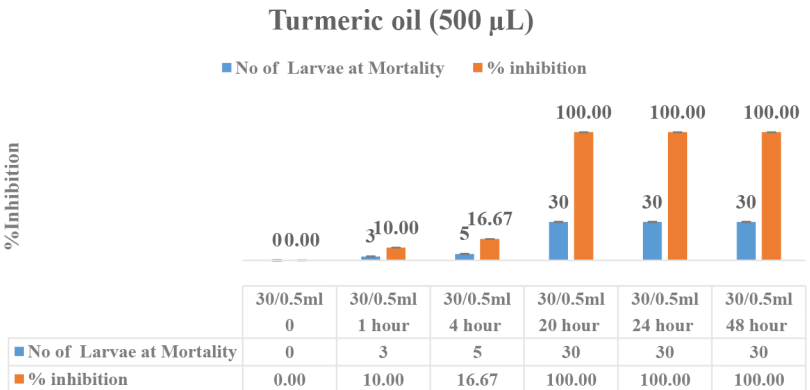
GC–MS analysis provided detailed insights into the chemical composition of the essential oil. The chromatographic profile revealed the presence of multiple volatile constituents, which were identified based on retention times and mass spectral fragmentation patterns in comparison with the National Institute of Standards and Technology (NIST) libraries.

Turmeric Oil: The GC–MS analysis of turmeric oil revealed (Figure 10) a chemically diverse profile comprising sesquiterpenes, aromatic hydrocarbons, nitrogen-containing compounds, and fatty acid derivatives, which collectively account for its well-known pharmacological and insect-repellent properties. The major constituent identified was 7-Isopropylidene-5-methyl-2,3-diazabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid, diethyl ester (RT 10.8 min), contributing the highest peak area (27.27%) and normalised percentage (100%), indicating its predominance in the oil. Another prominent component was benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl (RT 8.4 min; Area 6.95%; Norm



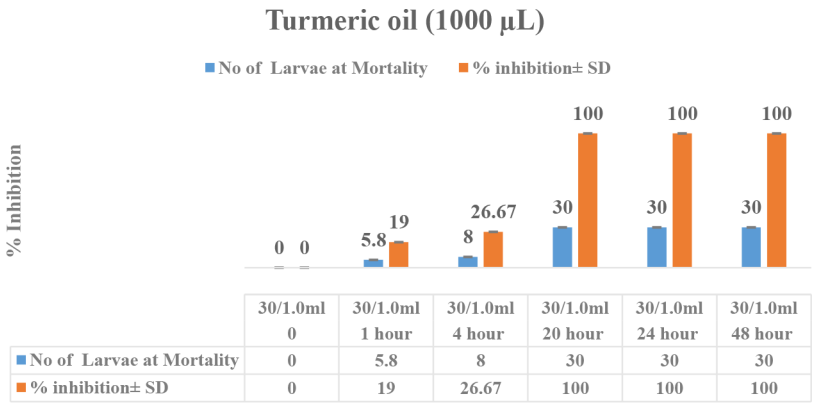
	30/0.1 ml 0	30/0.1 ml 1 hour	30/0.1 ml 4 hour	30/0.1 ml 20 hour	30/0.1 ml 24 hour	30/0.1 ml 48 hour
No of Larvae at Mortality	0	0	0	30	30	30
% inhibition	0.00	0.00	0.00	100.00	100.00	100.00

Figure 4. Larvicidal potential of turmeric oil (100µL).



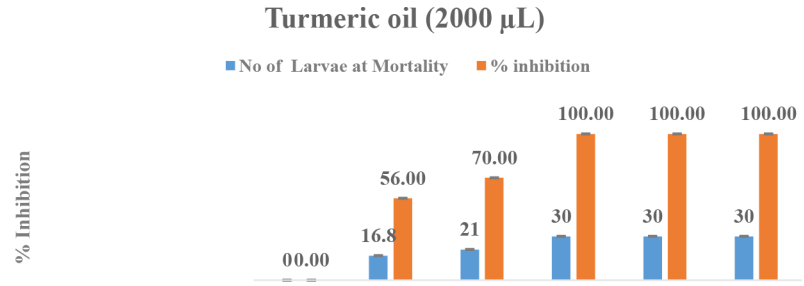
	30/0.5ml 0	30/0.5ml 1 hour	30/0.5ml 4 hour	30/0.5ml 20 hour	30/0.5ml 24 hour	30/0.5ml 48 hour
No of Larvae at Mortality	0	3	5	30	30	30
% inhibition	0.00	10.00	16.67	100.00	100.00	100.00

Figure 5. Larvicidal potential of turmeric oil (500µL).



	30/1.0ml 0	30/1.0ml 1 hour	30/1.0ml 4 hour	30/1.0ml 20 hour	30/1.0ml 24 hour	30/1.0ml 48 hour
No of Larvae at Mortality	0	5.8	8	30	30	30
% inhibition± SD	0	19	26.67	100	100	100

Figure 6. Larvicidal potential of turmeric oil (1000µL).



	30/2.0ml 0	30/2.0ml 1 hour	30/2.0ml 4 hour	30/2.0ml 20 hour	30/2.0ml 24 hour	30/2.0ml 48 hour
No of Larvae at Mortality	0	16.8	21	30	30	30
% inhibition	0.00	56.00	70.00	100.00	100.00	100.00

Figure 7. Larvicidal potential of turmeric oil (2000µL).

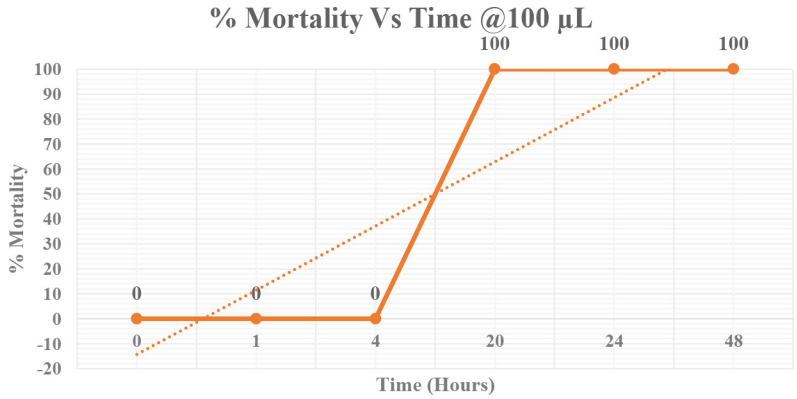
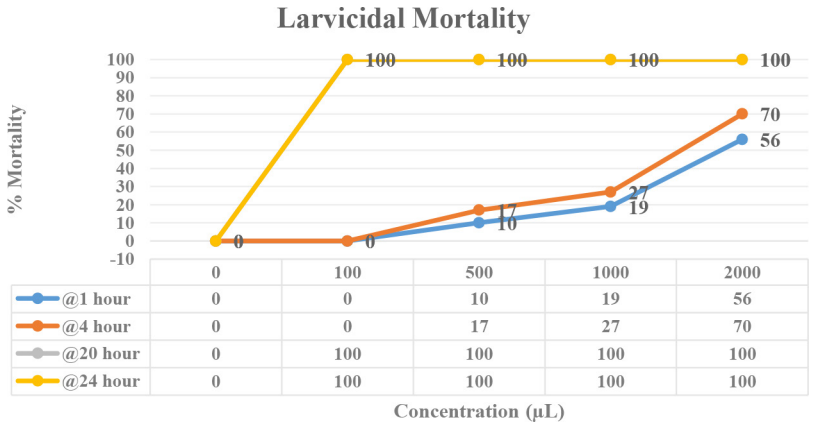


Figure 8. % mortality vs time @100µL.

25.50%), representing a sesquiterpene-like aromatic hydrocarbon commonly associated with bioactivity in turmeric essential oil.

Moderate amounts of 9-borabicyclo[3.3.1]nonane derivatives, benzoic acid esters, and (E)- Atlantone were observed, reflecting the presence of oxygenated and bicyclic sesquiterpenes that contribute to antimicrobial, antioxidant, and repellent actions. Minor constituents such as 3-cyclohex-3-enyl-propionic acid, estragole, m-xylylenediamine derivatives, and cyclopropanecarboxamide derivatives were detected at lower retention times and area percentages, indicating volatile aromatic and nitrogen-containing



	0	100	500	1000	2000
@1 hour	0	0	10	19	56
@4 hour	0	0	17	27	70
@20 hour	0	100	100	100	100
@24 hour	0	100	100	100	100

Figure 9. Larvicidal mortality @ different drug concentrations with time.

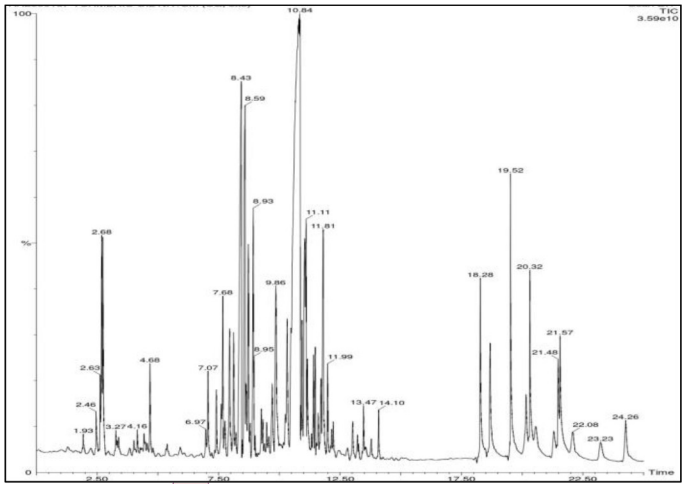


Figure 10. GC fingerprint of turmeric oil.

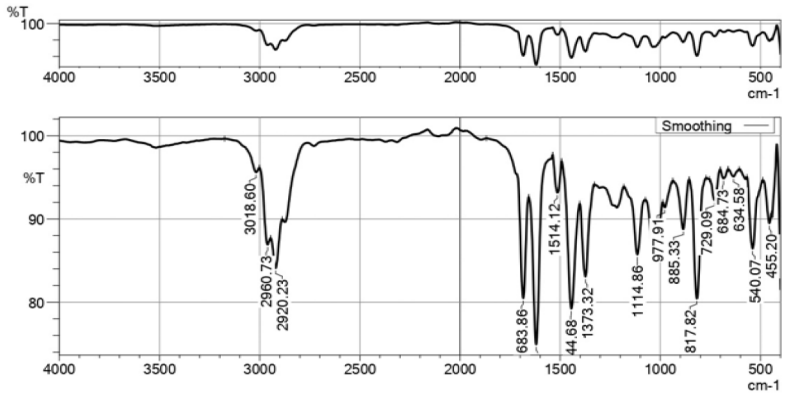


Figure 11. FTIR of turmeric oil.

compounds. Long-chain components, including 9-hexadecenoic acid esters and octadecanal derivatives, appeared at higher retention times, contributing to the stability and lipophilic nature of the oil.

3.4 Fourier Transform Infrared Study

FTIR analysis of EO confirmed the presence of characteristic functional groups responsible for its biological and physicochemical properties.

Turmeric Oil: The analysis revealed intense alkane C–H stretching (2960–2920cm^{-1}), C=C stretching (1683–1620cm^{-1}), C–O vibrations (1114cm^{-1}), and out-of-plane HC=CH bending (977–817cm^{-1}), characteristic of sesquiterpenes such as turmerones.

4. Discussions

4.1 Extractions of Oil

The percentage yield of Turmeric oil from *C. longa* was obtained by the hydrodistillation process, and preliminary phytochemical and GC fingerprints were performed to confirm the Turmeric oil, which was further utilised for toxicological studies. Similarly, the percentage yield of Turmeric oil obtained by the hydrodistillation Clevenger apparatus method was 2.8%.

4.2 GC Fingerprints of Turmeric Oil

The GC showed the chemical composition of Turmeric oil. Due to the complexity of essential oils, it is important to know the chemical composition and establish a fingerprint elution. It is characterised by the presence of dehydro Ar-turmerone, ar. Curcumene, Curcumem, Bisabolanes, αphellandrene, Arturmerone, Zingiberene, Cineole, Caryophyllene, a-Tumerone etc.

4.3 FTIR Fingerprints of Turmeric Oil

The FTIR spectrum of turmeric essential oil exhibited distinct absorption bands corresponding to various functional groups present in the sample. Characteristic peaks were observed in the regions of 2960–2920cm^{-1}, 1683–1620cm^{-1}, 1114cm^{-1}, and 977–817cm^{-1}. These bands confirm the complex chemical composition of the oil. The spectral pattern was consistent with previously reported data for turmeric oil. Overall, the FTIR analysis provided supportive evidence regarding the chemical profile of the essential oil.

4.4 Dose-response Bio-assay

Previous studies of different kinds of plant extracts and essential oils of *Mentha piperita* and *Citrus sinensis* revealed the potential larvicidal mortality rate and inhibited the bites of mosquitoes, especially the *Ae. aegypti,* by Umashankar *et al*[40]. Osanloo *et al* studied the larvicides of *Callitris glaucophylla* and *Piper betle* against *Ae. aegypti*, *Tagetes minuta* against *Anopheles gambiae*, and *Cananga odorata* against *Culex quinquefasciatus* and *Anopheles dirus*[41]. Cheng *et al*. compared the EO from the leaves of eight provenances of indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh.) and reported an excellent inhibitory effect against the *Ae. aegypti*[42].

Sumitha KV *et al.,* revealed the larvicidal efficacy and chemical constituents of *Ocimum gratissimum* L (*Lamiaceae*) EO against *Aedes albopictus* species for the larvicidal property[43], and Pavela R revealed the acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against *C. quinquefasciatus* say larvae[44]. Ajaiyeoba *et al*., evaluated α-turmerone-rich essential oils of both rhizome and leaf of *C. longa* and found more toxicity in rhizome oil (Lethal Concentration LC50 = 0.017 mg/ml) than the leaf oil (LC50 = 0.029 mg/ml) against the malarial vector, *A. gambiae*[45].

The experimental larvicidal results showed 100% inhibition was observed after the addition of Turmeric Oil (100µL) at 20 h, 500µL at 20 h, 1000µL at 20 h and 2000µL at 20 h. The Larvicidal potential of % inhibition has been increased with the increase of oil concentrations, 2000µL, and 70% Larvicidal action was observed within 4 h. So higher concentrations, like 2000µL equivalent, will help potential inhibition within 4 h when compared to other concentrations like 100µL, 500µL, and 1000µL. If a low concentration, like 100µL, was sufficient for a gradual like time point and 100% inhibition within 20 h. The level of toxicity increased with exposure to the oil at higher concentrations (Figures 8 and 9). Several reports are available on other oils that reveal their efficacy against mosquito larvae.

Thus, the activity of the oil against mosquito larvae may be attributed to the additive or synergistic effect of the constituents of the oil. Such an effect has been previously observed with some essential oils, where the activity was due to the combination of the major constituents and not by individual compounds (Papachristos *et al*.,[46]).

5. Conclusions

This study concluded that the EO of *C. longa* demonstrated significant inhibitory activity against *Ae. aegypti*, indicating its potential application as a preventive agent against vector-borne diseases transmitted by this mosquito species. The phytochemical profiling carried out using GC–MS analysis confirmed

the presence of major bioactive constituents, while FTIR analysis validated the characteristic functional groups responsible for the oil's biological and physicochemical properties. These analytical findings collectively support the therapeutic and larvicidal potential of the EO. In future, it is proposed to incorporate this oil into mosquito inhibitory sprays and pharmaceutical topical formulations such as creams and gels for repellent applications. Furthermore, the documented larvicidal efficacy of herbal oils may assist public health departments and municipal corporations in adopting eco-friendly, plant-based alternatives to synthetic chemical pesticides for sustainable mosquito control and management of vector-borne diseases.

6. Acknowledgement

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Author Contributions

Kirubakaran N.: collected the data, extracted larvicidal experiments for writing the research under various headings. Umashankar M. S.: Corresponding author: Design, preparation of study protocol, technical guidance, review of results, and review of manuscript. Anuradha L.: Design of Toxicological study support. Shanthi S.: Experimental support for Larvicidal assay. All the authors have read and approved the final manuscript.

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