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Mango ginger (*Curcuma amada* Roxb.) rhizome essential oils as source of environmental friendly biocides: Comparison of the chemical composition, antibacterial, insecticidal and larvicidal properties of essential oils extracted by different methods

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ABSTRACT

The essential oil isolated from plants is widely utilized as eco-friendly biocides and antibacterial agents. *Curcuma amada*, commonly known as mango ginger, is well-known for its applications in the food and aromatics industry for its significant mango-like aroma. The present study compared the different *C. amada* essential oils prepared by hydrodistillation (CHD), steam distillation (CSD), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), for their chemical composition, antibacterial, larvicidal and insecticidal properties. GC/MS analysis indicated the presence of compounds including α-pinene, β-myrcene, p-cymene, (Z)-β-ocimene, Camphor, linalyl acetate, safrole, ar-curcumene, and β-curcumene in the different *C. amada* essential oils. The antibacterial activity was observed against different strains of microbes, with a higher efficacy in the essential oils prepared by UAE and MAE methods. Apart from these, the MAE, UAE, CSD, and CHD were also shown to have significantly higher larvicidal activity against *Aedes, Culex*, and *Armigeres* species; however, no toxic effect was observed in non-targeted species like fishes and *Allium cepa* model of genotoxicity. Further, these essential oils were also found to have significant contact and fumigant toxicity as well as repellency against pests of stored grains (*Sitophilus* and *Tribolium*). Considering these results, the present study assumes that *Curcuma amada* essential oils may be a source of ecofriendly insecticides and antibacterial agents.

1. Introduction

Pests are important organisms that have a significant effect on the agriculture system, health systems, and economical status of the country (Farnsworth et al., 2017). Among the different classes of pests, the insects are the primary agents with significant pest status (Donatelli et al., 2017). They either attack the plants or their parts subsequently resulting in the reduced productivity of the same. The impact of pests is more severe on food crops, which often decreases the quantity of food grains that are produced. It has been reported that the pests can reduce the productivity by food grains between 20 and 40 % of the actual (Savary et al., 2019). Apart from the general pests, those acting on stored pulses and grains often intensity the damage by making the stored products useless. Often such damage leads to reduce the stored food product volume and subsequently to famine (Kumar and Kalita, 2017).

Conventional pesticides such as DDT, BHC another organochlorine, organophosphorus, and pyrethroids are reported to cause serious environmental issues including pollution, killing friendly organisms, destroying soil microbes, and altering the soil (Perrin et al., 2021). In addition, these pesticides are known to induce serious health damages in animals and humans (Piechowicz et al., 2021; Shearer et al., 2021). It leads to the quest for novel biological molecules that are environmentally safer for applications including antibacterial, larvicidal, and also as pesticides (Lengai et al., 2020); among these, plant-derived essential oils

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are the predominant ones (Palla et al., 2020). Spices especially those belonging to the Zingiberaceae have gained attention and the rhizomes of these plants are the major sources for essential oil extraction. The *Curcuma amada* (Mango ginger) is the less explored one for their beneficial effects of essential oils.

Curcuma amada is known for its mango flavor, which makes it more attractive as a fragrant and also for other industrial and medical applications. Compounds such as mangiferin (Padmapriya et al., 2012) and 2–3 pentanediol (Tiwari et al., 2014) had been isolated from the rhizome extract of the plant. Apart from these, the plant is reported to have antidiabetic (Sarkar et al., 2019), antitumor (Ramachandran et al., 2017), antibacterial (Divyashri et al., 2021), and antiobesity (Nissankara Rao et al., 2021) properties. Further, the essential oil prepared by hydrodistillation had been evaluated for the chemical composition and various biological activities. Reports indicated that the *C. amada* essential oil prepared by hydrodistillation is rich in pinene, ocimene, curcumene, and linalool (Padalia et al., 2013; Srivastava et al., 2001; Tamta et al., 2016). Antifungal activities of the essential oil had been reported against a variety of fungi including *Aspergillus* species (Singh et al., 2002). However, there are no further studies are available on the biological activities of essential oils of *C. amada*. It has been reported that the extraction methods, such as hydrodistillation, steam distillation, microwave-assisted extraction, and ultrasound-assisted extraction, result in the variation of volatile organic compounds in essential oils (Nora and Borges, 2017). Therefore, the present study aimed to evaluate the variation in the chemical composition of *C. amada* essential oil and its bioactivities prepared by different methods.

2. Materials and methods

2.1. Plant materials collection and essential oil extraction

The authenticated rhizomes of *Curcuma amada* weighing 17–25 g were collected from Kerala Agriculture University and planted in individual grow bags at a depth of 4–6 cm. The rhizomes were watered every day and nutrients were provided after germination in terms of Nitrogen: Phosphorus: Potassium at a ratio of 3:3:6. The plants were kept in a semi-shaded area with a temperature of 28 ± 2.5 °C and humidity of 92 %. The plants were allowed to grow for six months and harvested after that. The rhizomes of the *C. amada* were collected, washed to remove soil, and dried. The extraction of essential oil was done by different methods including hydrodistillation, steam distillation, microwave or ultrasound-assisted distillation, as follows. Each extraction was carried out in triplicate and compared the yield and composition for uniqueness.

2.1.1. Hydrodistillation

The protocol for hydrodistillation was derived from the method already described by Avanço et al. (2017). The powdered rhizome of *C. amada* (200 g) was added to a Clevenger type apparatus (Borosil, India) for hydrodistillation for 4 h. Then added sodium chloride (1 g) and dichloromethane (20 mL) to the aqueous distillate in a separating funnel. Further, continual shaking was performed for 40 min and allowed to stand for 20 min. After settling, the organic layer was extracted and concentrated. The oils existed in the organic layer were further dried over anhydrous sodium sulfate and collected in a vial then sealed and preserved at 4 °C until further analysis.

2.1.2. Steam distillation

Steam distillation is one among the commonly used method for essential oil extraction; the present study adopted the instructions provided by reports of Manzan et al. (2003). The *C. amada* powdered fresh rhizome was (200 g) was subjected to Steam distillation for 5 h in a steam distillation apparatus. About 2 g of NaCl and dichloromethane (50 mL) was mixed with the distillate. The organic layer was separated and concentrated to 5 mL and oil was stored in glass tubes at 4 °C.

2.1.3. Microwave-assisted extraction (hydrodistillation)

The extraction was set up in line with the previous descriptions of Moradi et al. (2018). The fresh and powdered rhizomes of *C. amada* were rehydrated in water and exposed to the irradiation frequency of 2450 MHz and then subjected to hydrodistillation. The finally extracted essential oil was stored under refrigeration.

2.1.4. Ultrasound-assisted extraction

The extraction was carried out according to the techniques and tools described in the studies of da Silva Moura et al. (2020) with certain amendments. The fresh powdered Phoenix Ultrasonic Cleaner (SGM Lab solutions, Bengaluru, India) at a frequency of 40 kHz for approximately 30 min (input power 50 W). In the end, the extract was collected and further subjected to hydrodistillation using Clevenger-type apparatus (Borosil, India) for another 5 h. The collected essential oil was then dried using sodium sulfate and preserved at 4 °C for usage.

2.2. Gas chromatography/mass spectrometry (GC/MS) analysis

The composition of *C. amada* rhizome essential oils was analyzed using Varian CP 3800 GC/MS. The GCMS analysis conditions were as described in the previously published works of Padalia et al. (2013). The column used was 30 m × 0.25 mm inner diameter with a thickness of 0.25 μm. The carrier gas used was Helium gas (flow rate of 1 mL/min). The injector temperature was set at 250 °C and the oven temperature was raised from 50 °C to 200 °C at a rate of 8 °C per minute. The identification was carried out using the NIST library according to the standard methods described in the previous reports of Padalia et al. (2013).

2.3. Antibacterial activity assay by disc diffusion methods

Antibacterial activity of the different essential oils was analyzed using an agar disc-diffusion assay as described by Walia et al. (2020). Briefly, Whatman No.1 filter paper discs of approximately 6 mm diameter were wet with different essential oils (10 μL) and immersed in the agarose plates containing different bacterial inoculum (10^7 CFU/mL). The plates were kept in incubation for another 24 h and the zone of inhibition was determined in mm for each bacterial colony.

2.4. Determination of minimum inhibitory concentration (MIC)

The determination of MIC was according to the standard protocols described by Standard methods (ESCMID, 2000). The bacterial cultures were maintained in the log phase of growth, and a loop of cells was transferred to lactose bile broth and incubated at 37 °C for 24 h in a bacteriological incubator. The bacterial density was set to 10^7 CFU/mL by spectrophotometric determination at 600 nm using appropriate dilution using fresh LB broth. Further, 25 μL of the inoculum was then added to microplates containing different concentrations of *C. amada* essential oils (0–10 mg/mL) and incubated for 24 h. The MIC was estimated as the lowest concentration of essential oil which had no visible growth after 24 h.

2.5. Larvicidal activity of C. amada essential oils prepared by different methods

The cultures of *Armigeres subalbatus*, *Aedes aegypti*, and *Culex tritaeniorhynchus* were maintained for 10 generations. The third instar larvae (50 Nos) was collected from the colony and placed in a 50 mL beaker and different concentrations of essential oils were added by dissolving in DMSO (0.5 mL) and made up to 50 mL with double distilled water. The mortality at the end of 24 and hours in each concentration of essential oil was determined by counting the dead larvae and the percentage of death and LC_{50} was estimated.

2.6. Non-targeted species toxicity in guppy fish (Poecilia reticulata)

The model organism for non-targeted toxicity was chosen as Guppy fish (*Poecilia reticulata*) (P Ferreira et al., 2019); approximately guppy fishes of average body length of 3.2 ± 0.2 cm and weight of 1.24 ± 0.12 g were chosen for the study. The concentration of essential oil tested was the same as that of those used for larvicidal studies; in each concentration six guppy fishes were placed and exposed for the respective concentrations over 48 h. The guppies were observed for any sign of toxicity or behavioral changes which were recorded immediately and also during 1, 6, 12, 24, and 48 h.

2.7. Efficacy of Curcuma amada essential oils in controlling Sitophilus oryzae and Tribolium castaneum

Beetle species such as *T. castaneum* and *S. oryzae* reared on wheat flour at a temperature of 25 °C and 65 % humidity were selected for the study. Adult insects of 12–14 days of both sexes were used.

2.7.1. Fumigant toxicity

Briefly, the essential oils were coated on a Whatman No.1 filter paper (2 mm diameter) in different concentrations (0–1000 μl/L air), and then it was placed in 50 mL bottles. About 10 adults were placed in each bottle and then properly screwed. It was placed in the bottle for 72 h and the death was calculated.

2.7.2. Repellant activity

The different concentrations of essential oils were dissolved in acetone (HPLC grade) and placed on one half of Whatman no. 1 filter paper disc (8 mm). The treated and untreated half of these filter papers were placed in a Petri dish at opposite ends. Then 20 adult insects were placed in the center of the dish and the lid was properly placed and sealed using parafilm. The number of insects present on either half (essential oil-treated and untreated) was observed for 24 h. The data was used for the estimation of percentage repellency and also for detecting the median repellent dose (RD 50).

2.7.3. Contact toxicity

The contact toxicity of essential oils was carried out according to the standard protocols. Briefly, the different concentrations (0–200 μg/mm^2) of essential oil prepared by different methods were diluted in acetone and coated on a Whatman no.1 filter paper disc (diameter of 70 mm) and placed in a glass chamber of 200 mL capacity. The filter papers were allowed to dry for 30 min and about 20 adult insects were placed in each bottle and closed properly. The toxicity was estimated at 48 h and the LD50 was calculated by comparing the mortality with control, treated with acetone alone.

2.8. Statistical analysis

The results of antimicrobial studies were expressed as Mean ± SD of three independent experiments, each carried out in duplicate. The biocide property was estimated using 10/20 adult insects and the experiment was repeated four times. The statistical analysis was carried out using ANOVA followed by Tukey- Kramer multiple comparison test.

3. Results

3.1. Yield of essential oil and its chemical composition analysis

The essential oil yield was dependent on the method that was employed for isolation. The conventional hydrodistillation had an average yield of 1.25 ± 0.12 %; whereas, the steam distillation yielded 1.44 ± 0.15%. In microwave-assisted hydrodistillation (MAE), there was a significantly higher average yield of 1.79 ± 0.16 % ($p < 0.05$), and in ultrasound-assisted hydrodistillation it was further increased to 1.84 ± 0.10 % ($p < 0.05$) compared to other conventional extraction methods (Fig. 1). The composition of individual essential oils is shown in Table 1; accordingly, the predominant compounds were significantly varied between the different modes of essential oil isolation.

3.2. Anti-bacterial activity

The antibacterial activities of *Curcuma amada* essential oils prepared by different methods were determined using disc diffusion and MIC dose methods. The zone of inhibition in disc diffusion methods is shown in Table 2; where, the essential oil prepared by microwave-assisted extraction had a significantly higher zone of inhibition against *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* ($p < 0.05$). On contrary, the essential oil prepared by ultrasound-assisted extraction method was found to have the highest zone of inhibition against *Salmonella enteritidis* ($p < 0.05$).

Besides, the essential oils were also evaluated using MIC for their antibacterial properties. As shown in Table 3, the MIC values were significantly lower for the essential oils prepared by microwave-assisted and ultrasound-assisted methods against *Escherichia coli, Staphylococcus aureus, Salmonella enteritidis* ($p < 0.05$). However, against *Pseudomonas aeruginosa,* the *C. amada* essential oil prepared by microwave-assisted extraction was found to be more toxic with the lowest MIC value ($p < 0.05$).

3.3. Larvicidal properties of the essential oils

The larvicidal properties of essential oils also varied between the different preparations (Fig. 2). Against *Armigeres* species, the highest activity was observed in essential oils extracted by MAE, with an LC50 value of 75.22 ± 4.1 μg/mL, followed by essential oil prepared by the UAE method. Likewise, against *Aedes aegypti*, essential oils prepared by MAE and UAE were more toxic and found to have an LC50 value of 32.12 ± 3.2 and 30.42 ± 2.0 μg/mL. Against the *Culex* species, essential oils extracted by MAE (34.17 ± 2.4 μg/mL) and UAE methods (38.36 ± 3.1 μg/mL) had higher lethality.

3.4. Toxicity on the non-targeted organism (guppy fishes) and mutagenicity in Allium cepa

The different essential oils were also analyzed for their toxicity in the non-targeted organism, guppy fish. Up to a dose of 200 μg/mL (200 mg/L), there observed no significant toxic effect for any of the essential oils. Further, there was no lethality for these essential oils till the highest dose over 48 h (Supplementary material 1).

In the onion root cells, the *Curcuma amada* essential oils were treated up to a concentration of 10 mg/mL, however, there observed no

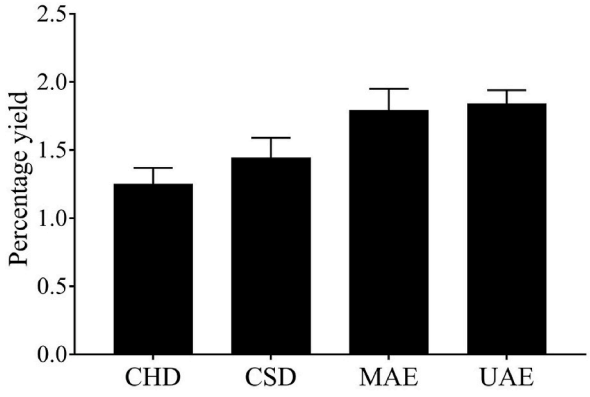

Fig. 1. Percentage yield of *Curcuma amada* essential oil extracted by hydrodistillation (CHD), steam distillation (CSD), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE).

Table 1
Important volatile organic compounds present in the *Curcuma amada* essential oil isolated by hydrodistillation, steam distillation, microwave assisted extraction, and ultrasound assisted extraction by GC-MS.

Compound	Retention Index	Compounds identified			
		Hydro-distillation	Steam distillation	Microwave assisted extraction	Ultrasound assisted extraction
Propanone	925		0.20		
α-pinene	935	0.50	0.50	0.30	5.40
Camphene	951	0.10		2.80	0.20
β-pinene	980	5.90	5.10	10.20	3.90
β-myrcene	990	37.40	43.20	25.50	30.80
Limonene	1031	0.27	0.40	0.20	
1,8-cineole	1035	0.70		4.20	
(Z)-β-ocimene	1036		9.30		
(E)-β-ocimene	1048	4.10	1.40	3.40	3.60
(E)-decahydro naphthalene	1060	2.60	2.10	4.10	5.40
Linalool	1098		8.30	1.60	9.20
Perillene	1101	2.80	0.90		
Camphor	1149	5.50	1.10	9.40	5.30
Isoboneol	1160			2.40	
Borneol	1168	0.70		2.20	0.90
terpinen-4-ol	1195		0.90		0.30
(E)-β-Farnesene	1244	3.20		1.10	1.70
ar-Curcumene	1282	7.90	15.60	6.10	9.20
δ-elemene	1397		2.40		
β-elemene	1397			4.40	2.10
β-(E)-caryophyllene	1418	1.20	1.80	0.30	
β-gurjunene	1436				
ar-Turmerone	1482	21.20		12.40	15.40
α-zingiberenne	1495	3.20		0.90	
Curzerenone	1605	0.90	3.56	6.20	0.30
β-Eudesmol	1628				1.20
β-bisabolol	1748	0.20		1.10	
Zerumbone	1729		0.10		0.50

Table 2
Antibacterial activity of *Curcuma amada* essential oils prepared by different methods.

Bacteria	Zone of inhibition (mm)			
	Hydro-distillation	Steam distillation	Microwave assisted extraction	Ultrasound assisted extraction
Escherichia coli	16.7 ± 0.3	15.3 ± 0.1	18.3 ± 0.2*	17.4 ± 0.2*
Staphylococcus aureus	14.4 ± 0.2	13.4 ± 0.1	19.2 ± 0.3*	18.4 ± 0.3*
Pseudomonas aeruginosa	14.1 ± 0.3	12.9 ± 0.2	18.0 ± 0.3*	17.6 ± 0.2
Salmonella enteritidis	12.4 ± 0.2	13.4 ± 0.1	15.7 ± 0.2	17.1 ± 0.4*

Table 3
Changes in the Minimum inhibitory concentrations (μg/mL) of *Curcuma amada* essential oil prepared by different methods.

Bacteria	MIC (μg/mL)			
	Hydro-distillation	Steam distillation	Microwave assisted extraction	Ultrasound assisted extraction
Escherichia coli	6.0 ± 0.2	6.5 ± 0.3	4.0 ± 0.2*	4.5 ± 0.3*
Staphylococcus aureus	5.5 ± 0.3	5.5 ± 0.4	3.5 ± 0.3*	4.3 ± 0.2*
Pseudomonas aeruginosa	4.0 ± 0.2	4.5 ± 0.3	3.0 ± 0.4*	4.0 ± 0.3
Salmonella enteritidis	6.5 ± 0.2	8.5 ± 0.2	4.8 ± 0.3*	5.0 ± 0.2*

significant changes in the chromosomal characteristics of the *Allium cepa*, neither there was any significant variation in the mitotic index in these cells (Supplementary material 2).

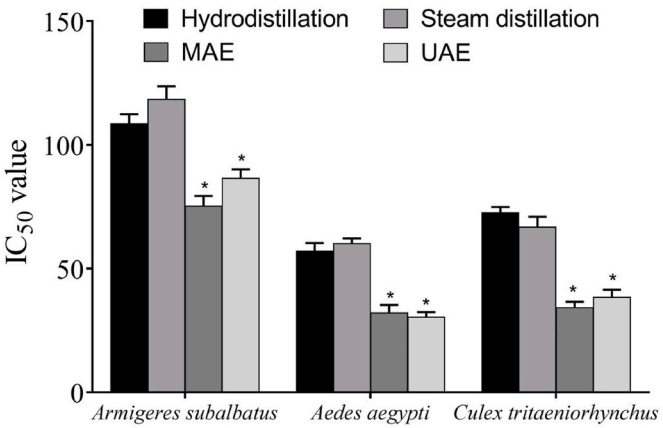

Fig. 2. Larvicidal activity of *Curcuma amada* essential oil extracted by hydro-distillation (CHD), steam distillation (CSD), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE).

3.5. Efficacy of C. amada essential oils against pests of stored foods

The toxic effects and repellant activities of *C. amada* essential oils were determined against two common pests, *Sitophilus oryzae* (lesser grain weevil) and *Tribolium castaneum* (red flour beetle). The fumigant toxicity potential was found to be higher in essential oil isolated by ultrasound-assisted methods, followed by the microwave-assisted extraction method (Table 4). On contrary, the repellent activity was higher in the essential oil extracted by MAE, followed by essential oil extraction by UAE ($p < 0.05$).

4. Discussion

The use of conventional chemical pesticides is known to have several environmental issues and health damages (Perrin et al., 2021).

Table 4
Efficacy of *Curcuma amada* essential oil isolated by different methods against different pests in terms of their repellent efficacy, contact toxicity, and fumigant toxicity.

Test	Unit	Assay	Hydro-distillation	Steam distillation	Microwave assisted extraction	Ultrasound assisted extraction
Fumigant toxicity	LC50 (μg/L of air)	*Sitophilus oryzae*	36.4 ± 1.12	32.7 ± 0.67	24.3 ± 0.44*	23.7 ± 1.02*
		Tribolium castaneum	26.2 ± 0.32	28.1 ± 0.83	16.6 ± 0.75*	15.8 ± 0.56*
Repellent activity	RC50 (μg/L of air)	*Sitophilus oryzae*	7.22 ± 0.32	6.17 ± 0.25	4.26 ± 0.27*	5.08 ± 0.22*
		Tribolium castaneum	9.54 ± 0.55	9.28 ± 0.39	6.12 ± 0.46*	6.58 ± 0.39*
Contact toxicity	LD50 ($\mu g/mm^2$)	*Sitophilus oryzae*	188.7 ± 4.11	165.2 ± 5.72	140.6 ± 5.49*	156.4 ± 5.36
		Tribolium castaneum	208.5 ± 6.03	199.4 ± 5.33	178.6 ± 6.37*	169.7 ± 6.10*

Bioaccumulation and biomagnification of these pesticides are responsible for their ill effects on animals, plants, and humans (Shearer et al., 2021). In addition, several of the pests have gained resistance against a different spectrum of pesticides and therefore their use is also less effective (Piechowicz et al., 2021). Therefore, the search for environmentally friendly pesticides and antibacterial agents has been intensified for the past few decades (Lengai et al., 2020). Among the different possible compounds, plant-derived essential oils are the premier ones, which have been proven to be effective against an array of pests and vectors (Palla et al., 2020).

The results of the study indicated the antibacterial properties against different species of bacteria including *E. coli, S. aureus, P. aeruginosa,* and *S. enteritidis*. The most active *C. amada* essential oils are those prepared by microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE). It has been already reported that essential oils are important antibacterial agents and are having reduced resistance to these microbes (El-Tarabily et al., 2021; Mangalagiri et al., 2021; Varijakzhan et al., 2021).

Further, the essential oils are found to have repellent activity against the two pests of stored grains- *S. oryzae* and *T. castaneum*. These pests are associated with severe damage to the stored food grains and are also involved in significant economic damage (Ak, 2019; Khaliq et al., 2020). The *C. amada* essential oil prepared by MAE and UAE is highly effective and found to repel these pests significantly even at significantly lower concentrations than other essential oils preparations. Apart from the repellant properties, the *C. amada* essential oil prepared by MAE has significantly reduced the survival of both *S. oryzae* and *T. castaneum* by virtue of the fumigant and contact toxicity. The use of essential oils is highly preferred for the control of pests in stored grains because of their non-toxic nature and biocompatibility with food grains (Campolo et al., 2018; Lee, 2018). Hence, it is possible that the *C. amada* essential oil prepared by MAE and up to an extent UAE can be useful as potent biocides against the stored pests of food grains.

The essential oils of *C. amada,* especially the ones which is extracted by MAE, have also been found to kill the larvae of three different species of mosquitoes; includes *Aedes aegypti, Armigeres subalbatus,* and *Culex tritaeniorhynchus*. These mosquitoes are important vectors in spreading various diseases; the *A. aegypti* is associated with the spreading of dengue, Zika, yellow fever, and chikungunya (Powell, 2018; Souza-Neto et al., 2019), whereas, *Culex* is often associated with filariasis. The less known *A. subalbatus* is not considered an important vector, however, reports have indicated that these mosquitoes can carry filarial worms and Zika virus (Aliota et al., 2010; Li et al., 2020; Muslim et al., 2013). It is also to be noted that the population number of these mosquitoes is increasing in various countries including India (Manimegalai, 2010). Though these essential oils were toxic to the mosquito larvae, there observed no significant toxicity in the non-targeted guppy fishes. In addition, there is no evidence of genotoxic characteristics in the *Allium cepa* model studies up to a dose of 10 mg/mL.

The chromatographic profiling indicated the presence of various compounds such as β-myrcene, ar-Curcumene, ar-Turmerone, β-elemene, Camphor, and Linalool. It has been reported that these compounds possess strong larvicidal (Ajaiyeoba et al., 2008; Fujiwara et al., 2017; Liu et al., 2018; Shalaby et al., 2016) and antibacterial activities (Gao et al., 2019; Liu et al., 2020; Marliyana et al., 2019; Wang et al., 2020). Therefore, it is possible that the bioactivities of *C. amada* essential oil may be possibly due to their individual bioactive components. Hence, the present study concludes that the essential oils of *C. amada*, especially the one extracted by microwave-assisted extraction, may be a promising antibacterial, larvicidal, and insecticide agent. It is also observed to be environmentally safe in terms of toxicity to organisms as well as genotoxicity.

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Credit author statement

AN- Study design, Methodology approval, Data analysis, Review of the final draft; **AS-** Analysis, Initial draft preparation; **JTJ**- Analysis, Data collection, Initial draft preparation; **RR**- Data collection, Analysis, Review of Draft; **AA**- Design, Review of Final draft; **YOK-** Data analysis, Review of draft; **HJK-** Study design, Methodology approval, Review of final draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111718.

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