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Lavandula angustifolia Essential Oil Phyto-Compounds as Leads to Potential Mosquitocides

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Abstract: Mosquitocidal activities of *Lavandula angustifolia (L. angustifolia)* different concentration of essential oil (EO) and its major phytocompounds (MPCs) tested against three important human vector mosquitoes (HVMs). The quantitative analyses of EO phyto-chemical compositions (PCCs) were analyzed by using Gas Chromatography-Mass Spectrometry (GC-MS). The HVMswere exposed to various concentrations of EO and its MPCs tested under laboratory condition by using standard protocols. Vector mortalities were subjected to log-probit analysis. Chemical compositions (CCs) of 47 compounds were identified and the MPCs of EO were Terpinen (38.0339%) followed by Linalool (34.4992%), Caryophyllene (6.1480%), Octanone (2.3906%) and Camphene (2.0989%). The maximum larval mortality was found in Linalool against the larvae of *Aedes aegypti (Ae. aegypti)* followed by *Anopheles stephensi (An. stephensi)* and *Culexquinquefasciatus (Cx. quinquefasciatus)* with the LC_{50} values were 36.26, 36.81 and 37.49 ppm respectively. Ovicidal activity of the Linalool found most effective than other compounds. These results are clearly suggests that EO and its MPCs have the potential to be used as an ideal ecofriendly approach, traditional method for control of HVMs and this is the first report on the mosquitocidal activities of *L. angustifolia* EO of MPCs.

Keywords: GC-MS Analysis, Camphene, Octanone, Linalool, Terpinen, Caryophyllene

1. Introduction

Mosquito and mosquito-borne diseases (MBDs) are successfully spreading entire world with a disproportionate effect on children and adolescents, which are more responsible for significant global morbidity and mortality. Mosquitoes have been regarded as significant vectors for transmission of several diseases which bites cause skin irritation and allergic responses to humans and other blood yielding vertebrates. WHO has declared the mosquitoes as "number 1 enemy to public". MBDs are predominant in more than 100 countries which are worldwide infecting 70 million people annually and 40 million people suffered only in India [1-5]. *Aedes aegypti* (*Ae. aegypti*) is a very serious day biting vector which are proliferating fresh water bodies in and around home land. Recently estimated more than 50 million people are threatening worldwide by dengue virus. These mosquitoes are also known to spread chikunguniya, dengue and yellow fever in Central and South America, West Africa and many states in India [6-8]. *Anopheles stephensi* (*An. stephensi*) is the principal vector of malaria and one of the most predominant diseases in the tropical world which disturbs 350 to 500 million peoples and kills more than 1 million infants and young children annually [9-10]. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) vector of filariasis which created major public health hazard and challenging

socioeconomic problem in many parts of tropical countries [11-13]. HVMs control has been pushed to enter a new dispute to the public health practitioners as a result of amplified threat of synthetic chemicals insecticides grows rapidly resistance among the vector mosquitoes. Chemicals insecticides are non-biodegradable and causing many side effects to all living organisms [14, 15].

Moreover, mosquito coils, mat, liquid vaporizer and other repellent agents containing synthetic pyrethroids and other organophosphorus compounds cause so many side effects, such as breathing problem, asthma, itching, headache, eye irritation and sneezing to the users [16]. The recent negative impacts of chemical insecticides should be removed urgently and search efforts towards the development of new environment friendly, naturally available vector control methods by using realistic mediators. In search of new vector control approach, science has increased the investigations to plants in recent decades, so the plant kingdom is receiving renewed consideration as mosquitocides [17]. Recently, most of the researcher would like to search the new environmental friendly pesticides practices therefore working on phytochemical derivative pesticides. India is one of the vast plant diversity country as well as medicinal plants that have been used in traditional medicine for several thousand years and nearly 8000 plants species are documented for medicinal and insecticidal properties around 50% of all the medicinal plants are present in India [18, 19]. Phytochemicals are environmental and former friendly, target-specificity, non-development of resistance to pest, reduced number of applications, higher acceptability, suitability for rural areas, low cost, biodegradable, easy preparation and universally accepted. Botanicals pesticides are used for alternative to synthetic insecticides and phyto-compounds have been projected as a tool in future for pest control agents which are shown to function as general toxicant [20, 16]. Phyto-compounds are extracted from different parts of the variety of plants. Lamiaceae family plants are good odorous, medicinal properties flora and it consists of more than 252 genus and 7000 species. Lamiaceae are most abundant in Asian continents. It has been extremely cultivated in India, China, Pakistan, Malaysia and Indonesia for using of essential oil production [21]. In view of the recently increased interest in developing plant origin insecticides as an alternative to synthetic chemical insecticide for controlling HVMs, this present investigation was undertaken to assess the larvicidal and ovicidal potential of *L. angustifolia* EO MPCs against the medically important HVMs.

2. Materials and Methods

2.1. Test Organisms

The mosquitoes were separately reared in laboratory, Department of Zoology, Govt. Arts College, Nandanam, Chennai –35, Tamilnadu, India. The larvae were fed 3:1 ratio of dog biscuits and yeast powder. Adults were provided with 10% sucrose solution with honey and one week old chick for blood meal. Mosquitoes were held at (28±2)°C, 70-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

2.2. Plant Material and Essential Oil Extraction

The fresh aerial parts (the collected plant parts were keenly watched and washed with dechlorinated water for 15min) of *L. angustifolia* were collected from a thick forest region of Malapuram District, Kerala, India. The plant materials were collected in the month of February 2018, which were allowed to more than 15 days for air and shade dried in room temperature and ground to a fine powder with the help of electric blender and sieved through kitchen strainer. Plant powder 500g was sequentially extracted through hydrodistilled in a Clevenger apparatus for 6 h. The distilled oil was dried, stored at refrigerator under 4°C and packed in air lock amphour bottle until further bioassay test. At the time of plants collection, two pressed voucher herbarium (NGAC: 3684) specimens were prepared and identified with the help of Plant Taxonomist, Department of Botany, Govt. Arts College, Nandanam, Chennai.

2.3. Gas Chromatography - Mass Spectrometry Analysis

GC-MS analysis of the *L. angustifolia* EOwas performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GCMS) equipped with an Elite-5MS (5% Diphenyl/95% Dimethyl poly siloxane), 30 x 0.25mm x 0.25m df. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.9%) was used as a carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 micro liter was employed (Split ratio of 1:10). Injector temperature of 250°C is maintained and Ion-source temperature was 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C /min to 200°C, then 5°C /min to 280°C, ending with isothermal for 9 min. at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. were measured. Total GC running time was 36 min and the relative percentage amount of each component was calculated by comparing its average peak area to the total areas with Software adopted to handle mass spectra and Chromatogram was Turbo mass ver. 5.2.0.

2.4. Larvicidal Activity

The larvicidal activity of EO MPCs was evaluated as per the globally acceptable protocol and previously described [22]. The larvicidal activity was determined, based on the wide and narrow range tests. The selected EO MPCs tested ranging from 10 to 70ppm which prepared and they were tested against the freshly moulted (0-6 hrs) third instar larvae of selected mosquitoes. The EO MPCs were dissolved in 1 ml DMSO and then diluted in 249 ml of dechlorinated tap water to obtain each of the desired concentrations. The control was prepared using 1ml of DMSO in 249 ml of dechlorinated water. The twenty five third instar larvae were

(previously monitored: disease free, hale and healthy and uniform sized) introduced carefully in 500ml of transparent, heat resistant and unbreakable plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water +1ml of DMSO) and the required amount of EO MPC was added. The larval mortality was keenly observed every 2 h interval and finally recorded after 24 h of post treatment. The entire bioassay experiment five replicates were maintained and the study of LC_{50} as well as LC_{90} value was calculated by using probit analysis [23].

2.5. Ovicidal Activity

The ovicidal activity was slightly modified and followed the method of Su and Mulla [24]. The eggs/egg rafts of selected mosquitoes were collected from vector control laboratory, Department of Zoology, Govt. Arts College, Nandanam, Chennai. The various concentrations EO MPCs ranging from 10 to 70 ppm and before treatment, the eggs/egg rafts of selected mosquitoes were counted individually with the help of hand lens. Freshly hatched eggs (100nos) were exposed to each concentration of chemical compositions until they hatched or died. Eggs exposed to DMSO in water served as control. After treatment, the eggs from each concentration were individually transferred into distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment.

2.6. Statistical Analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), and chi-square values were calculated using the SPSS 12.0 version (Statistical Package of Social Sciences) software. Results with $p<0.05$ were considered to be statistically significant.

3. Results

3.1. Chemical Analysis of L. angustifolia

The CCs of *L. angustifolia* aerial parts the retention indices and the percentage of the individual components are summarized in table 1 and figure 1. The essential oil was extracted by hydrodistilled in a clavenger apparatus and was analyzed by GC-MS. A total of 47 CCs were detected representing to 100%. The MPCs of EO were Camphene ($C_{10}H_{16}$) 2.0989%, Octanone ($C_8H_{16}O$) 2.3906%, Linalool ($C_{10}H_{18}O$) 34.4992%, Terpinen ($C_{10}H_{18}O$) 38.0339% and Caryophyllene ($C_{15}H_{24}$) 12.1480%. The percentage compositions of remaining 42 CCs ranged from 0.0072% to 1.6427%.

Table 1. *Chemical composition ofaerial parts of L. angustifolia EO.*

Peak	Compounds	MF	MW (g/mol)	RT	% Peak Area
1	Rhynchophorol	$C_8H_{16}O$	128.215	1.73	0.2020
2	Octanol	$C_8H_{18}O$	130.231	2.07	0.4838
3	Hexanal	$C_6H_{12}O$	100.161	4.23	0.0185
4	1-Hexanol	$C_6H_{14}O$	102.180	4.46	0.0293
5	Adamantane	$C_{10}H_{16}$	136.238	5.63	0.0364
6	α-Pinene	$C_{10}H_{16}$	136.238	5.83	0.1210
7	Camphene	$C_{10}H_{16}$	136.238	6.27	2.0989
8	β-Terpinene	$C_{10}H_{16}$	136.238	6.98	0.0181
9	β-Pinene	$C_{10}H_{16}$	136.238	7.09	0.0379
10	Octanone	$C_8H_{16}O$	128.215	7.52	2.3906
11	Eucalyptol	$C_{10}H_{18}O$	154.253	8.98	0.8920
12	β-Ocimene	$C_{10}H_{16}$	136.234	9.20	0.8131
13	Z-Ocimene	$C_{10}H_{16}$	136.234	9.58	0.2510
14	Epoxylinalool	$C_{10}H_{18}O_2$	170.252	10.68	0.0444
15	Linalool	$C_{10}H_{18}O$	154.249	12.99	34.499
16	d-camphor	$C_{10}H_{16}O$	152.233	14.48	0.8549
17	Grandisol	$C_{10}H_{18}O$	152.233	16.13	1.6427
18	Terpinen	$C_{10}H_{18}O$	154.249	20.16	38.033
19	Geranyl acetate	$C_{12}H_{20}O_2$	196.286	20.68	0.3035
20	Nopyl Acetate	$C_{12}H_{20}O_2$	208.301	20.81	1.3593
21	Geranyl acetate	$C_{12}H_{20}O_2$	196.286	21.05	0.0187
22	p-Cymen-7-ol	$C_{10}H_{14}O$	150.217	21.19	0.0158
23	Cyclobutanecarboxylic acid	$C_{11}H_20O_2$	100.117	21.94	0.0649
24	(Z)-8-Hydroxylinalol	$C_{10}H_{18}O_2$	170.252	22.75	0.4345
25	α-Terpinyl acetate	$C_{12}H_20O_3$	196.290	22.98	0.2875
26	Neryl acetate	$C_{12}H_{20}O_2$	196.290	23.33	0.5140
27	trans-Geranyl acetate	$C_{12}H_{20}O_2$	196.286	24.19	1.0596
28	Caryophyllene	$C_{15}H_{24}$	204.357	25.93	12.1480
29	α-Bergamotene	$C_{15}H_{24}$	204.351	26.10	0.0669
30	Isocaryophyllene	$C_{15}H_{24}$	204.351	26.47	0.0072
31	β-Farnesene	$C_{15}H_{24}$	204.351	27.00	0.2744
32	β-Cubebene	$C_{15}H_{24}$	204.351	28.04	0.1162
33	β-Bisabolene	$C_{15}H_{24}$	204.351	28.97	0.0438

Peak	Compounds	MF	MW (g/mol)	RT	% Peak Area
34	(S)-beta-bisabolene	$C_{15}H_{24}$	204.357	29.35	0.0755
35	T-Muurolol	$C_{15}H_{26}O$	222.372	29.73	0.0595
36	Ethyl Icosapentate	$C_{22}H_{34}O_2$	330.512	30.87	0.0509
37	Caryophyllene Oxide	$C_{15}H_{24}O$	220.356	32.37	0.2911
38	α-Bisabolol	$C_{15}H_{26}O$	222.366	33.20	0.0430
39	delta.-Cadinol	$C_{15}H_{26}O$	222.366	33.51	0.0220
40	τ-Cadinol	$C_{15}H_{26}O$	222.366	34.60	0.0460
41	Caryophyllene oxide	$C_{15}H_{24}O$	220.356	35.90	0.0642
42	Benzyl Benzoate	$C_{14}H_{12}O_2$	212.248	39.23	0.0459
43	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268.485	41.39	0.0136
44	Isoaromadendrene Epoxide	$C_{15}H_{24}O$	220.356	45.75	0.0094
45	Farnesyl acetone	$C_{18}H_{30}O$	262.437	49.17	0.0342
46	Nerolidol	$C_{15}H_{26}O$	222.366	49.74	0.0502
47	Cembrene A	$C_{20}H_{32}$	272.476	58.84	0.0119
Total percentage of chemical compositions					100.00

MF=Molecular Formula, MW=Molecular Weight, RT=Retention Time (min)

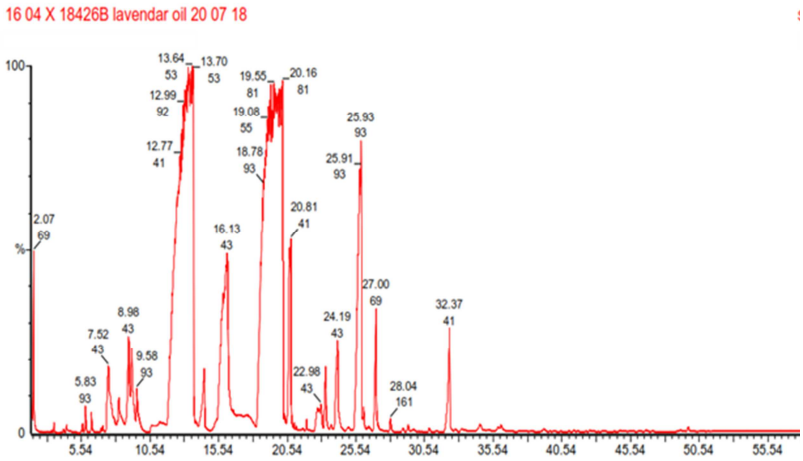

Figure 1. *GC-MS Chromatogram of L. angustifolia essential oil.*

3.2. Larvicidal Activity of L. angustifolia

The results of the larvicidal activity of *L. angustifolia* EO MPCs against the third instar larvae of three important HVMs*Ae.aegypti, An.stephensi* and *Cx.quinquefasciatus* are presented in table 2. Among five compounds tested, the maximum larvicidal potential was observed in Linalool against *Ae. aegypti*than *An.stephensi* and *Cx.quinquefasciatus* with the LC_{50} values were 36.26, 36.81 and 37.49 ppm respectively. The chi-square values are statically significant at $p \leq 0.05$ level. The chi-square values in the bioassays indicated probably the heterogeneity of the test population.

Table 2. *Larvicidal activity of L. angustifolia EO MPCs against freshly molted third instar larvae of selected mosquitoes.*

Oil Compounds	Test organisms	LC_{50} (ppm)	95% Fudicial Limit (LCL- UCL) (ppm)	LC_{90} (ppm)	95% Fudicial Limit (LCL- UCL) (ppm)	Slope	χ^2
	Ae. aegypti	38.47	(37.67 - 39.28)	45.28	(44.48 - 46.10)	3.0263	12.015
Camphene	*An. stephensi*	39.23	(38.44 - 40.04)	46.15	(45.35 - 46.96)	3.1231	15.563
	Cx. quinquefasciatus	39.78	(38.97 - 40.60)	46.88	(46.07 - 47.70)	3.2316	14.606
	Ae. aegypti	36.98	(36.25 - 37.71)	42.87	(42.14 - 43.60)	3.3600	14.761
Octanone	*An. stephensi*	37.31	(36.58 - 38.06)	43.34	(42.60 - 44.09)	3.2126	13.620
	Cx. quinquefasciatus	37.82	(37.09 - 38.57)	43.94	(43.20 - 44.69)	3.1715	13.128
	Ae. aegypti	36.26	(35.56 - 36.98)	41.85	(41.15 - 42.57)	3.3928	13.956
Linalool	*An. stephensi*	36.81	(36.10 - 37.53)	42.54	(41.83 - 43.26)	3.4138	12.860
	Cx. quinquefasciatus	37.49	(36.77 - 38.22)	43.38	(42.67 - 44.11)	3.1815	12.786
	Ae. aegypti	37.25	(36.50 - 38.02)	43.44	(42.69 - 44.21)	2.9656	15.524
Terpinen	*An. stephensi*	38.03	(37.27 - 38.80)	44.39	(43.63 - 45.16)	3.0225	14.495
	Cx. quinquefasciatus	38.74	(37.96 - 39.54)	45.45	(44.67 - 46.25)	3.0775	12.491
	Ae. aegypti	37.00	(36.26 - 37.77)	43.12	(42.37 - 43.88)	3.0140	13.070
Caryophyllene	*An. stephensi*	37.59	(36.85 - 38.35)	43.79	(43.04 - 44.55)	3.1048	14.558
	Cx. quinquefasciatus	38.23	(37.48 - 38.99)	44.51	(43.76 - 45.27)	3.1373	14.060

Mortality of the larvae observed after 24h of exposure period. * Significant at P <0.05 level. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90}=Lethal Concentration brings out 90% mortality. LCL=Lower Confidence Limit; UCL=Upper Confidence Limit.

3.3. Ovicidal activity of L. angustifolia

The % of egg hatchability of *Ae.aegypti, An. stephensi* and *Cx. quinquefasciatus* were tested with five different EO MPCs concentrations and the results are listed in table 3. The percent hatchability was inversely proportional to the concentrations of EO MPCs and directly proportional to the eggs. Among the five different EO MPCstested against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus*, the Linalool compound of EO exerted zero hatchability (100% mortality) at 40, 50, 60 and 70 ppm, respectively. The MPC Linalool compound found most effective than any other compounds against eggs of three HVMs and control eggs showed the 100% hatchability.

Table 3. *Ovicidal activity of L. angustifolia EO MPCs against the eggs of selected mosquitoes.*

Oil Component	Mosquitoes	Percentage of egg hatch ability, Concentration (ppm)							
		10 ppm	20 ppm	30 ppm	40 ppm	50 ppm	60 ppm	70 ppm	Control
Camphene	*Ae. aegypti*	60.3±1.4	51.3±1.8	44.3±2.2	36.6±1.8	22.6±1.8	11.2±1.6	NH	100±0.0
	An. stephensi	63.6±1.6	55.4±1.6	46.4±1.8	37.6±1.8	26.4±1.6	15.6±1.8	NH	100±0.0
	Cx. quinquefasciatus	68.4±1.2	57.8±1.2	48.3±1.4	39.7±1.2	28.2±1.8	17.4±1.2	NH	100±0.0
Octanone	*Ae. aegypti*	32.2±2.3	25.6±1.3	18.2±2.7	12.6±2.3	NH	NH	NH	100±0.0
	An. stephensi	38.5±1.4	29.6±1.4	21.2±1.3	15.4±1.8	NH	NH	NH	100±0.0
	Cx. quinquefasciatus	41.3±1.5	30.4±1.9	25.2±1.7	18.3±1.4	NH	NH	NH	100±0.0
Linalool	*Ae. aegypti*	28.3±1.4	21.3±1.8	10.3±2.2	NH	NH	NH	NH	100±0.0
	An. stephensi	34.6±1.6	21.4±1.6	15.4±1.8	NH	NH	NH	NH	100±0.0
	Cx. quinquefasciatus	36.6±1.8	25.0±2.2	18.3±1.8	NH	NH	NH	NH	100±0.0
Terpinen	*Ae. aegypti*	55.4±1.8	42.8±1.4	33.6±1.1	23.4±1.8	11.8±1.9	NH	NH	100±0.0
	An. stephensi	60.3±1.6	53.8±2.2	37.2±1.8	26.2±2.2	15.3±2.2	NH	NH	100±0.0
	Cx. quinquefasciatus	64.0±1.8	57.2±2.1	39.3±1.4	28.3±1.6	18.0±1.6	NH	NH	100±0.0
Caryophyllene	*Ae. aegypti*	43.8±1.8	31.6±1.8	22.4±2.4	14.6±2.2	NH	NH	NH	100±0.0
	An. stephensi	45.3±1.6	33.8±2.2	25.2±1.8	17.2±2.2	NH	NH	NH	100±0.0
	Cx. quinquefasciatus	49.0±1.8	35.2±2.1	28.3±1.4	18.3±1.6	NH	NH	NH	100±0.0

Values represent mean ± S. D. of five replications. Eggs in control groups were sprayed with no phytochemicals. NH - No hatchability (100% mortality)

4. Discussion

Plant products have been used as traditionally by human communities in many parts of the world against the vectors and the different species of insects. Phytochemicals explored from plant sources which has good mosquitocidal agents and transmission of MBDs at the individual as well as at the community level. Our results showed that the different compounds of *L. angustifolia* EO have significant larvicidal and ovicidal activities against *Ae. aegypti, An. stephensi* and *Cx. Quinquefasciatus* mosquitoes. The finding of the present investigation is comparable with earlier reports the larvicidal activities of the four MPCs D-terpinene, linalool, borneol and germacrene D tested against larvae of HVMs [25-28]. Similarly, Isoflavonoids from tubers of *N. mitis* had a larvicidal effect on malaria and filariasis vectors [29]. Saponin isolated from *A. aspera* against *Ae. Aegypti* and *Cx. quinquefasciatus* [30]. The presence of lantadene triterpenoids and furano naphtha quinones phytocompounds in *Lantana* species has the mosquito larvicidal properties [31]. A study on larvicidal effects of *B. mollis* EO against *Cx. quinquefasciatus* [32]. The EOs of *L. turbinata*and *L. polystachya*against the larvae of HVM against *Cx. quinquefasciatus* [33]. The oleic acid isolated from *T. javanicum*showed potential mosquitocidal agent tested against *Ae. aegypti* and *Cx. Quinquefasciatus* [34] reported that which compares well with present findings. The larvicidal activities of *A. hypogaea* MPC 9, 12, octadecadienoic acid ethyl ester showed strong mosquitocidal activity [35]. The sesquiterpene compound, Pytol isolated from *L. camera* and tested against the mosquitocidal activities of important HVMs [36]. The larvicidal activity of cinnamon and other EOs were recorded maximum activities [37] and the similar trend was also recorded in the study.

Plant-borne compounds and the fractions were tested as larvicides, ovicides, and repellency against *An. stephensi*. The larvicides activity was tested by 11- octadecenoic acid, methyl ester compound against *An. Stephensi* with LC_{50} values of 22.32ppm [16]. The phytochemicals like tannins, triterpenes, phenolics, and alkaloids of different medicinal plants showed different biological activities [38-43]. Several researchers reported phytochemical based experiments for exploring the insecticidal activity on mosquito vectors [44, 45]. Different parts of the *Citrus* plant (fruits, seeds, roots and leaves) have been tested for their use asmosquitocidal components [46, 47] which are similar to the present investigation. Since there is no previous record of literature available about the mosquitocidal activity of *L. angustifolia* EO MPCs of these present investigations serve as firsthand information. The finding of the present investigation revealed that the *L. angustifolia* EO MPCs possessed remarkable larvicidal and ovicidal activities against selected vector mosquitoes.

5. Conclusion

The finding of the present study reveal that *L. angustifolia* EO MPCs offers potential bio control agent for larvicidal and ovicidal against medically important vector mosquitoes *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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