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LARVICIDAL BIOASSAY OF FIVE TROPICAL PLANTS AGAINST *AEDES AEGYPTI*

Anju R Unnithan[1*] and Geetha Unnikrishnan[2]

[1]Department of Biotechnology, Birla College of Arts Science and Commerce, Mumbai University, Kalyan 421304, India.

[2]Department of Zoology, Birla College of Arts Science and Commerce, Mumbai University, Kalyan 421304, India.

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***Correspondence for Author**
Anju R Unnithan
Department of Biotechnology, Birla College of Arts Science and Commerce, Mumbai University, Kalyan 421304, India.

ABSTRACT

Background & Objective: Screening of plants for larvicidal activity against *Aedes aegypti* third instar larvae and chemical characterization of active fraction to identify a promising plant source for ecofriendly larvicides which may solve the problems related with injudicious and over application of synthetic insecticides in nature. **Method:** The larvicidal potential of Petroleum ether, n-butanol, ethyl acetate, acetone, methanol, ethanol, and water extracts of *Lantana camara, Terminalia catappa, Adhatoda vasica, Leucas aspera* & *Eupatorium odoratum* leaf extracts was evaluated against the third instar larvae of *Aedes aegypti*. Larval mortality was observed after 24 hr, 48hr and 72hr of exposure and LC_{50} for 24hr exposure time was determined. GC – MS analysis of active fraction was also done. **Results:** The highest larval mortality was found in *Lantana camara* petroleum ether leaf extract with an LC_{50} value of 48.851ppm. GC–MS analysis of *Lantana camara* petroleum ether leaf extract revealed the presence of around 62 compounds, of which the significant compounds were p-Cymene, Eucalyptol and Beta-Caryophyllene. **Conclusion:** The preliminary screening and chemical characterization provides a scientific rationale to highlight the importance of *Lantana camara* petroleum ether leaf extract as a source of larvicidal agents against *Aedes aegypti.*

KEY WORD: Larvicide; Aedes aegypti; LC_{50}; GC–MS; Lantana camara; Petroleum ether leaf extract;.

INTRODUCTION

Mosquitoes are medically significant pervasive insects transmitting many deadliest life threatening diseases affecting the health of humans and animals. *Aedes aegypti* is one of the most efficient mosquito vectors for arboviruses mainly for Dengue virus, because it is highly antropophilic and thrives in close proximity to humans preferring to live indoors.[1] Annoying populations of *Aedes* mosquitoes can occur anywhere in India because there are habitats favorable for mosquito species almost everywhere in the country. The effective management of mosquitoes involves many approaches, but larval control would seem to be an ideal approach as it eliminates mosquitoes before they reach the stage where they can transmit disease. The habitats of larvae are small, widely dispersed, and transient and thus the mobility of larvae is limited. Many larvicides are available in the market claim to be effective but the evidence for their efficacy with ecofriendly nature is generally weak and thus there is a critical need for more rigorous evaluation for new larvicides. India is a varietal emporium of plants enriched with phytochemicals having pharmacognostical and toxicological properties. The toxicological properties of phytochemicals reflect the potential of plants as a source of insecticidal agents. Prospection for new larvicidal molecules based on rich plant biodiversity is appreciable as compounds of plant derivatives are safer to use and leaves no residue in the environment.

The present study evaluated the larvicidal potential of five tropical plants including *Lantana camera, Terminalia catappa, Adhatoda vasica, Leucas aspera & Eupatorium odoratum. Lantana camera* belongs to the family verbenaceae, is a sturdy shrub with solid recurved prickles and a strong odor. *Lantana camara* is extensively used as a herbal medicine and as antimicrobial, fungicidal and nematicidal agents. Hot warter extracts of leaves can be used to relieve swellings and pain in the body. Flowers acts as nectar source for butterflies and moths.[2] *Adathoda vasica* belongs to the family Acanthaceae, is a small evergreen shrub. It grows to about 3 m, with leaves about 10-15 cm long and 5 cm wide, are opposite, entire, lanceolate, and shortly petiolate, tapering towards both apex and base and white or purple flowers and 4-seeded fruits. The leaves flowers, fruits and roots are extensively used for treating cold, cough, whooping cough, chronic bronchitis and asthma.[3] *Leucas aspera* species in the Lamiaceae family is commonly known as ‘Thumbai’. The leaves of the *Leucas aspera* can be obtuse, linear or linearly lanceolate or petiolate. *Leusas aspera* is reported to have antifungal, prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive and cytotoxic activities. It is also an antipyretic and can be inhaled to help treat nasal congestion,

coughing, cold, headache. etc.[4] *Eupatorium odoratum* is a tropical species of flowering shrub in the sunflower family, Asteraceae. It is a multi-stemmed shrub with hairy and glandular nature. The leaves are simple, opposite, decussate with acuminated base and toothed margin. The young leaves are crushed, and the resulting liquid can be used to treat skin wounds rashes and diabetes. *Terminalia catappa* belongs to the family combretaceae, is widely grown in tropical regions of the world as an ornamental tree. The leaves are large, long and broad, ovoid, glossy dark green and leathery. The leaves have many medicinal uses including diaphoretic, anti-indigestion, and anti-dysentery. Bark, leaves and unripe fruits are astringent haemostatic, digestive, antiseptic etc. Leaf is sudorific, antirheumatic, antileprotic and anticephalalgic.[5, 6, 7]

These plants are widely distributed in tropical and subtropical regions of the world. Leaves of these plants are easily available but are not involved many manufacturing processes. In the view of that a larvicide preparation from any of these plants shall not be causing any undesirable effect to environment, biodiversity or human health.

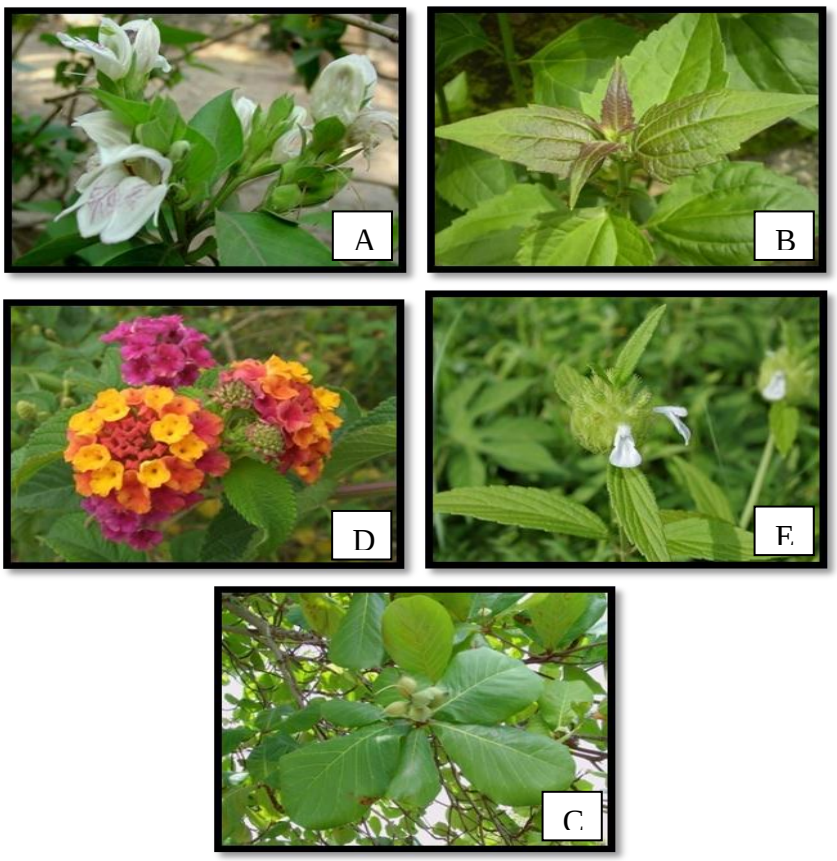

Figure 1: Plants Selected for the study A: *Adhatoda vasica* B: *Eupatorium odoratum* C: *Lantana camera* D: *Leucas aspera* E: *Terminalia catappa.*

MATERIALS AND METHODS

Plant Material Preparation

Fresh leaves of *Lantana camera, Terminalia catappa, Adhatoda vasica, Leucas aspera & Eupatorium odoratum* were collected from local regions of Kalyan, Thane. The leaves were washed and shade dried and pulverized to a coarse powder in a mechanical grinder and passed through a sieve. The powdered materials were stored in airtight, dark, glass container to prevent photochemical reactions.

Preparation of Leaf Extracts

10gm of plant powders were suspended each in 100ml petroleum ether, Ethyl acetate, n-butanol, Acetone, Methanol, Ethanol, sterile water and mixed vigorously. The mixture was kept for 30 min in sonicator and then in rotary shaker for 24 hr. The extract was decanted, filtered with Whitman No. 1 filter paper and concentrated by evaporation.

Raring of *Aedes Aegypti* Larvae

The eggs of *A. aegypti* were procured from Haffkine Institute at Mumbai, India and authenticated at Department of Entomology, NIV, Pune. The egg rafts of *A. aegypti* were kept in the tray containing tap water at laboratory condition. After incubation, the eggs were observed to hatch out colonized and they were maintained continuously in the laboratory with controlled conditions of 28^0C - 30^0C temperature and 70-80% relative humidity. Larvae were fed on finely ground dog biscuit.Pupae were transferred from the trays to a cup containing tap water and placed in screened cages for adult emergence.The adults of *A. aegypti* were reared in the glass cage provided with 10% sucrose solution in cotton pads and it was periodically blood-fed on restrained rats.[8]

Bioassays and Larval Mortality

Larvicidal activity of plant extracts was assayed according to WHO protocol with modifications. The 3rd instar larvae of *Aedes aegypti* was treated with plant extracts of 500ppm concentration. The plant extracts were dissolved in DMSO to prepare the stock solution. Ten larvae of the early 3^{rd}instars were placed in 250 ml beakers containing 100ml of the test solution. The beakers were covered with muslin cloth and were kept in the growth room maintained at room temperature. A corresponding control was maintained. Experiments were done in triplicates. The effects of the extracts were monitored by counting the number of larvae surviving at the end of 24, 48 and 72 hrs and the percent mortality was calculated.[9]

Lethal Concentration

The LC50 of the plant extracts that showed 100% mortality was determined by a similar procedure as mentioned above. The plant extracts were dissolved in DMSO to prepare the stock solution. 1ppm, 5ppm, 10 ppm, 50ppm, 100ppm, 200ppm, 300ppm, 400 ppm concentrations were tested and the observation was recorded after 24 hrs of incubation. Based on the percent mortality values, LC_{50} of leaf extracts for an exposure time of 24 hrs were obtained separately by calculating the regression line employing Probit analysis.[10] using SPSS software.[11]

GC-MS analysis of *Lantana camara* petroleum ether extract

To identify the phytochemicals present in the petroleum ether extract of *Lantana camara* GC/MS analysis was performed on an Agilent gas chromatograph directly coupled to the mass spectrometer system (JoelAccuTOF GCV). Samples were injected using the split mode (split ratio 1:30). Helium was used as carrier gas with flow rate of 1ml/min. MS scan range was 50 to 500 a.m.u. The identification of volatile phytochemicals was achieved by comparing the mass spectra with the data system library (NIST) and other published spectra supported by retention index data, which were compared with available literature retention indices.

RESULTS

Plant Material Preparation and extraction

The plants *Lantana camara, Terminalia catappa, Adhatoda vasica, Leucas aspera & Eupatorium odoratum* were extracted with petroleum ether, n-butanol, ethyl acetate, acetone, methanol, ethanol, and water. The extractive value was maximum in water for all plants and was lesser in n-butanol and petroleum ether.

Raring of *Aedes aegypti*

The egg rafts immersed in declorinized water were hatched in lesser than 1 hr. The hatched larvae were observed using hand lens and handled using a 10ml pipette fitted with a rubber bulb. Larvae were fed with very small amount of dog biscuit everyday and transferred to fresh water in a gap of two days. The time required to complete larval development varies from 7-23 days depending on temperature, food and density of larvae. Generally pupation occurred in 6-7 days and were removed to mosquito cage and kept in water. Optimum temperature is for mass rearing of *Aedes aegypti* is 25^0C -30^0C and humidity between 70 – 80 %. Adults of both sexes require carbohydrate as food source and are fed with 10% sucrose

solution soaked cotton balls. The cotton balls were replaced daily. The ovarian development of female mosquitoes requires blood meal and was provided by placing mouse in the cage once in a day for 1 hr. The eggs of *Aedes* mosquitoes are usually deposited on the surface of the moist filter paper and can be collected and store for ~1 month at room temperature.

Larvicidal screening of Extracts

Preliminary bioassays evaluated the larvicidal potential of Petroleum ether, n-butanol, ethyl acetate, acetone, methanol, ethanol, and water extracts of *Lantana camara*, *Terminalia catappa*, *Adhatoda vasica*, *Leucas aspera* & *Eupatorium odoratum* leaf extracts (making a combination of 35 extracts) against the third instar larvae of *Aedes aegypti*. Percent mortality values of *Aedes aegypti* larvae exposed to the plant extracts at a concentration of 500ppm is given in the Table 1. Promising larvicidal activity was observed in the majority of the solvent extracts of all five plants, but as expected the toxicity was varying depending upon the plants and solvents used. The plant extracts showed 100 % mortality was preferred for the determination of LC_{50} values using SPSS for 24 hr time period and are represented in the Table 2.

Table 1: Larvicidal activity in mean percent mortality of plant extracts.

Plants/ solvents		*Lantana camara*	*Adhatoda vasica*	*Eupatorium odoratum*	*Leucas aspera*	*Terminalia catappa*
Petroleum ether	**24hr**	100	Nil	30	Nil	10
	48hr	-	Nil	50	Nil	30
	72hr	-	Nil	60	Nil	50
Ethyl acetate	**24hr**	40	20	10	60	30
	48hr	70	30	20	60	30
	72hr	100	30	70	90	40
n-butanol	**24hr**	100	60	10	70	70
	48hr	-	60	20	100	80
	72hr	-	70	70	-	100
Acetone	**24hr**	40	Nil	Nil	10	Nil
	48hr	70	Nil	Nil	10	Nil
	72hr	100	Nil	Nil	30	Nil
Methanol	**24hr**	100	10	90	10	10
	48hr	-	20	100	30	20
	72hr	-	30	--	50	20
Ethanol`	**24hr**	60	60	70	10	20
	48hr	100	90	80	40	50
	72hr		100		50	70
Water	**24hr**	Nil	Nil	Nil	Nil	Nil
	48hr	Nil	Nil	Nil	Nil	Nil
	72hr	Nil	Nil	Nil	Nil	Nil

Table 2: LC_{50} of plant extracts calculated using SPSS software at 24hr exposure period.

Plant extract	LC 50 value		LC 90 value	
	Concentration (ppm)	Log Concentration	Concentration (ppm)	Log Concentration
Lan-Petroleum ether	48.851	1.689	83.392	1.921
Lan- Ethyl Actetate	319.336	2.504	1582.55	3.199
Lan- Butanol	100.740	2.003	282.968	2.452
Lan- Acetone	393.898	2.595	7187.26	3.857
Lan-Ethanol	199.788	2.301	973.514	2.988
Lan - Methanol	97.885	1.991	281.634	2.450
Adh -Ethanol	293.605	2.468	951.233	2.978
Eup- Methanol	109.954	2.041	246.000	2.391
Leu - Butanol	239.814	2.380	918.102	2.963
Ter- Butanol	137.750	2.139	416.865	2.620

GC-MS analysis of petroleum ether extract.

GC–MS analysis of *Lantana camara* petroleum ether leaf extract revealed the presence of around 62 compounds, of which the significant compounds were p-Cymene, Eucalyptol Beta -Caryophyllene (Figure 2 & 3).

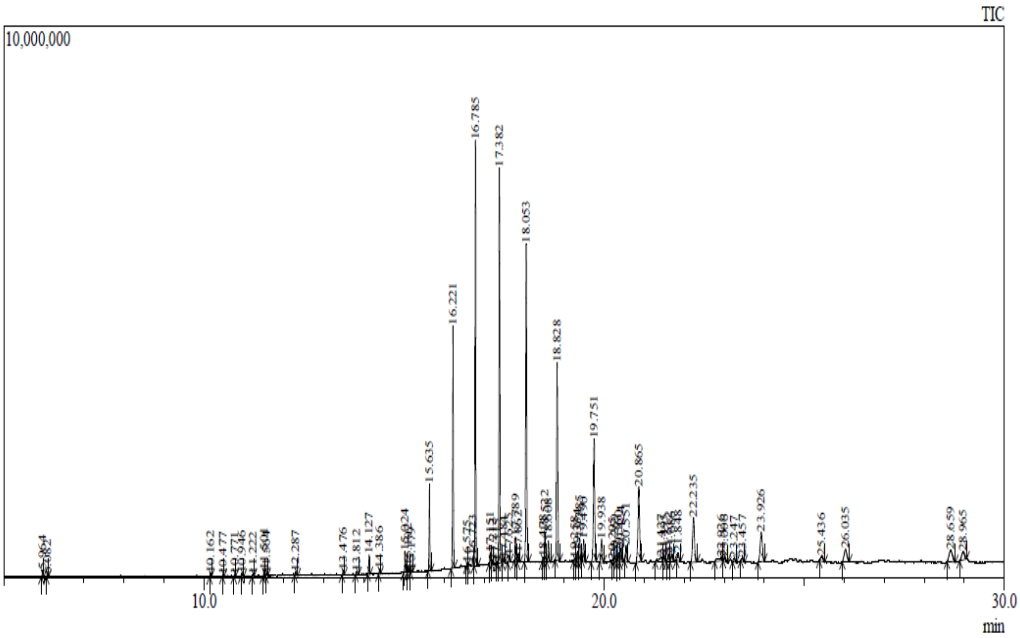

Figure 2: GC-MS analysis of *Lantana camara* Petroleum ether leaf extract.

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	5.964	153851	0.20	p-Cymene
2	6.082	43556	0.06	Eucalyptol
3	10.162	64717	0.09	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trim
4	10.477	32899	0.04	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-
5	10.771	43109	0.06	Butylated Hydroxytoluene
6	10.946	72694	0.10	Ethyl hydrogen fumarate
7	11.222	48681	0.06	Nerolidyl acetate
8	11.501	137427	0.18	(-)-Spathulenol
9	11.564	45110	0.06	Caryophyllene
10	12.287	90803	0.12	Heptadecane, 9-octyl-
11	13.476	103826	0.14	1,2-Benzenedicarboxylic acid, butyl 2-methylp
12	13.812	95001	0.13	2-Bromotetradecane
13	14.127	448966	0.59	Phthalic acid, butyl 2-chloropropyl ester
14	14.386	101828	0.13	Pentadecane, 7-methyl-
15	15.024	506822	0.67	OCTACOSANE
16	15.102	144427	0.19	Phytol
17	15.179	54268	0.07	Tridecane, 1-iodo-
18	15.635	1925335	2.54	OCTACOSANE
19	16.221	5597001	7.39	Octacosane
20	16.575	75036	0.10	Heptadecane, 2,6,10,15-tetramethyl-
21	16.723	252649	0.33	Hexanedioic acid, mono(2-ethylhexyl)ester
22	16.785	10091582	13.33	TETRATETRACONTANE
23	17.151	252366	0.33	2-methyloctacosane
24	17.213	81580	0.11	2-methyloctacosane
25	17.315	45835	0.06	Hexadecanoic acid, trimethylsilyl ester
26	17.382	10860205	14.34	Tetratetracontane
27	17.484	117440	0.16	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
28	17.625	187886	0.25	Phthalic acid, octyl 2-propylpentyl ester
29	17.789	640898	0.85	2-methyloctacosane
30	17.862	257808	0.34	2-methyloctacosane
31	18.053	9866408	13.03	TETRATETRACONTANE
32	18.478	151737	0.20	Heneicosane, 11-(1-ethylpropyl)-
33	18.522	899785	1.19	2-methyloctacosane
34	18.608	673319	0.89	2-methyloctacosane
35	18.828	6926005	9.15	TETRATETRACONTANE
36	19.258	94814	0.13	2-methyloctacosane
37	19.334	343244	0.45	Tetratetracontane
38	19.385	830904	1.10	2-methyloctacosane
39	19.490	747375	0.99	2-methyloctacosane
40	19.751	5051253	6.67	TETRATETRACONTANE
41	19.938	945043	1.25	trans-Geranylgeraniol
42	20.205	50202	0.07	Decane, 3,8-dimethyl-
43	20.269	165491	0.22	2-methyloctacosane
44	20.360	384719	0.51	Tetratetracontane
45	20.424	587261	0.78	2-methyloctacosane
46	20.551	653807	0.86	2-methyloctacosane
47	20.865	3816446	5.04	TETRATETRACONTANE
48	21.427	551440	0.73	Sulfurous acid, butyl tridecyl ester
49	21.495	277940	0.37	2-methyloctacosane
50	21.612	355234	0.47	Tetratetracontane
51	21.686	383894	0.51	2-methyloctacosane
52	21.848	421760	0.56	2-methyloctacosane
53	22.235	2664626	3.52	2-methyloctacosane
54	22.926	431736	0.57	Sulfurous acid, butyl dodecyl ester
55	23.006	166496	0.22	2-methyltetracosane
56	23.247	189027	0.25	2-methyloctacosane
57	23.457	177577	0.23	2-methyloctacosane
58	23.926	2022158	2.67	2-methyloctacosane
59	25.436	354662	0.47	2-methylhexacosane
60	26.035	1087428	1.44	2-methyloctacosane
61	28.659	1197612	1.58	2-methyloctacosane
62	28.965	668839	0.88	3.beta.-Hydroxy-5-cholen-24-oic acid
		75711848	100.00	

Figure 3: GC-MS analysis of *Lantana camara* Petroleum ether leaf extract.

DISCUSSION

Percent mortality values of *Aedes aegypti* larvae exposed to the plant extracts at a concentration of 500ppm showed 100 % mortality in the time period of 72 hrs in Lan-Petroleum Ether, Lan-ethyl acetate, Lan-Butanol, Lan- Acetone, Lan-Ethanol, Lan-Methanol, Adh –Ethanol, Eup- Methanol, Leu – Butanol, Ter- Butanol extracts exploring the presence of larvicidal compounds in these plants and their extraction to the selected solvents. Larvicidal mortality was not found in aqueous extract of any of the plants at the test concentration of 500ppm. This shows the least solubility of larvicidal compounds in aqueous solvent and their less polar nature. Previous studies of N S Oluah, V. Karthikeyan and Unnikrishnan G provides more evidences for the least larvicidal activity of aqueous extracts of *Lantana camara, Leucas aspera and Terminalia catappa* correspondingly even at higher concentrations,[6,12,13] As well in petroleum ether extract of *Leucas aspera* & acetone extracts of *Adhatoda vasica, Eupatorium odoratum* and *Terminalia catappa,* larvae were alive even after the exposure time of 72 hrs showing the inefficacy of these plant extracts as a larvicidal agent. The very low percentage of larvicidal activity of petroleum ether extract of *Leucas aspera* was observed by V. Karthikeyan validating the present observation.[12] But Kamaraj, C et al reported promising larvicidal activity of *Adhatoda vasica* acetone extract against *Culex quinquefasciatus*.[14] The observation in the present study might be for the reason that of change in the mosquito species and conditions. No behavioral changes were obtained in control group. Since the same solvent crude extracts from different plants showed promising difference in the toxicity, it can be pointed out that there is no association between the larvicidal activity and the polarity of the solvent used in the extraction.

Petroleum ether leaf extract of *Lantana camara* showed least LC_{50} and LC_{90} values of 48.851 and 83.392 at 95% confidence interval confirming the presence of bioactive compounds in the extract inducing highest larval mortality in a short period of time. As for *Lantana camara*, Butanol and methanol extracts also showed low LC_{50} values 100.740 and 97.885. But Ethyl acetate and Acetone extracts of *Lantana camara* showed maximum LC_{50} values (319.336ppm and 393. 898ppm respectively) showing the contribution of different solvents in extracting bioactive compounds from same plant. As well LC_{50} values of *Eupatorium odoratum* methanol extract and *Terminalia catappa* butanol extract (109.954 & 137.750) shows their promising larvicidal activity against *Aedes aegypti*. Preliminary screening of plant extracts for the larvicidal activity showed least LC_{50} value for *Lantana camara* petroleum ether leaf extract validating its importance as a source of larvicidal agents against *Aedes aegypti.*

GC- MS analysis provided preliminary information regarding the phytochemical constituents of the active fraction such as p-Cymene, Eucalyptol, and Beta caryophyllene showing the richness of terpenes in the active fraction.

CONCLUSION

The preliminary screening provides a scientific rationale to highlight the importance of *Lantana camara* petroleum ether extract as a source of larvicidal agents against *Aedes aegypti*. Chemical characterization of the active fraction provided preliminary information for further characterization of the extract. Advanced studies are needed for the isolation and identification of active principles present in the extract which could possibly be exploited to formulate a phytolarvicide.

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REFERENCE

1. Manorenjitha Malar, A P Sivanathan. The ecology and biology of *Aedes aegypti* (l.) and *Aedes albopictus* (skuse) (diptera: culicidae) and the resistance status of *Aedes albopictus* (field strain) against organophosphates in penang, Malaysia. Dissertation, 2006.
2. Neena P, P. K. Joshi. A review of *Lantana camara* studies in India. Int J of Scientific & Research Publications, 2013; 10(3): 1-11.
3. Sandeep D, Ramanjeet K, S. Ruhil, M. B, Seema D, A. K. A review on Justicia *adhatoda*: A potential source of natural medicine. African J of Plant Science, 2011; 5(11): 620-627.
4. Prajapati M S, Patel J B, Modi K, Shah M B. *Leucas aspera*: A review. Pharmacognosy Reviews, 2010; 4(7): 85-87.
5. Mallik J, Faruq A A, Banik R K. A comprehensive review on pharmacological activity of *Terminalia catappa* (combretaceae) - An update. Asian J of Pharmaceutical Research & Development, 2013; 1(2): 65–70.
6. Unnikrishnan G. Larvicidal and pupicidal activity of *Terminalia catappa* leaf extracts on *Aedes aegypti* mosquito: A Vector Intervention. IOSR J of Pharmacy and Biological Sciences (IOSR-JPBS), 2014; 9(2): 58-63.

7. Dasrao P, Pradeep H, Anil B. Determination of anthelmintic activity of *Terminalia catappa* extracts (red leaves). World Jof Pharmacy & Pharmaceutical Sciences, 2014; 4(1): 615-621.
8. Eugene j. G. Manual for mosquito rearing and experimental techniques. American Mosquito Control Ass. Inc., 1970; 5.
9. WHO. Guidelines for laboratory and field testing of mosquito larvicides, 2005.
10. Kim Vincent. Probit Analysis. http://userwww.sfsu.edu/efc/classes/biol710/probit., 2010.
11. IBM Corp. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp, 2011.
12. V. Karthikeyan, K. Sivakumar, Aishwarya Gokuldass, S.Mohanasundaram. Studies on larvicidal activity of *Leucas aspera*, *Vitex negundo* & *Eucalyptus* against *Culex quinquefasciatus* collected from coovum river of chennai, india. Asian J of Pharmaceutical and Clinical Research, 2012; 5(3): 1-4.
13. N S Oluah, O B Ezeabiakwa. Evaluation of Larvicidal Activity of Leaf Extract of *Lantana camara (Family: Verbenaceae)* against the *Aedes aegypti* Mosquito. African J online, 2012; 9(2): 1-5.
14. Kamaraj C, Abdul Rahuman A, Bagavan A, Abduz Zahir A, Elango G, Kandan P, Rajakumar, G, Marimuthu S, Santhoshkumar T. Larvicidal efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). Tropical Biomedicine, 2010; 27(2): 211–219.